2014 San Antonio Breast Cancer Symposium

Publication Number: P5-15-22
Average Grade: 0

Title: Evaluation of quality of life in patients with advanced and metastatic breast cancer proposed for palliative chemotherapy and best supportive care versus best supportive care

Anda-Natalia T Ciuhu¹, Gabriela I Rahnea Nita¹, Mihaela T Popescu² and Roxana Andreea D Rahnea Nita³. ¹Saint Luke Hospital, Bucharest, Romania; ²Colentina Clinical Hospital, Bucharest, Romania and ³Romanian Society of Palliatology and Thanatology, Bucharest, Romania.

Body: Background:
In Romania many patients neglect their symptoms and present to the doctor in advanced stages of cancer. In the case of patients with breast cancer who present with very advanced locoregional disease (ulcerated or hemorrhagic tumors) and symptomatic metastatic disease (visceral or skeletal pain, dyspnea), the choice between chemotherapy/hormonal therapy and best supportive care and only best supportive care became very difficult.

Objective:
Evaluation of quality of life in patients with advanced and metastatic breast cancer proposed for palliative chemotherapy and best supportive care versus best supportive care

Materials and methods:
In the last 6 month of the year 2013, 57 patients with advanced and metastatic breast cancer were randomized to either chemotherapy/hormonal therapy in addiction to best supportive care or to best supportive care. The patients were naive to chemotherapy, with performance status – ECOG between 2 and 3 and had symptomatic disease. The randomization of patients was made according to their choice. The performance status and the symptoms were evaluated at the baseline and, later, at each admission in the Oncology Palliative Care Department.

Results:
More patients in the chemotherapy group (92.68%, 38/41) had an improve quality of life compared to those in the best supportive care group (56.25 %, 9/16). The overall survival was longer in the chemotherapy group.

Conclusions:
The results show that chemotherapy/hormonal therapy improve the quality of life and overall survival in very advanced locoregional disease and symptomatic metastatic breast cancer. The number of patients who benefit from chemotherapy and best supportive care is higher versus patients who benefit only from best supportive care.

Key words: chemotherapy/hormonal therapy, best supportive care, breast cancer.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-10-19
Average Grade: 0

Title: Clinical outcome in breast cancer patients: What Epstein-Barr virus is doing?

Mohammed Habib¹, Gina Marrao², Dominique Bicout³, Artur Paiva², Carlos Freire de Oliveira⁴ and Emmanuel Drouet¹. ¹Université de Grenoble-Alpes, Unit for Virus Host-Cell Interactions, UMI 3265 UJF-CNRS-EMBL Grenoble1, Grenoble, Cedex 9, France; ²Coimbra University Hospital, Coimbra, Portugal; ³Team Environment and Health Prediction in Populations Unit – TIMC Laboratory, UMR CNRS 5525, Université Joseph Fourier, Grenoble1, Grenoble, Cedex 9, France; ⁴Coimbra University Hospital, Coimbra, Portugal; ⁵University Hospital, Coimbra, Portugal and ⁶Université de Grenoble-Alpes, Unit for Virus Host-Cell Interactions, UMI 3265 UJF-CNRS-EMBL Grenoble1, Grenoble, Cedex 9, France.

Body: In breast carcinomas (BC), the impact of Epstein-Barr Virus (EBV) infection on clinical outcomes has not been investigated yet. For nearly two decades, reports have suggested that Epstein–Barr virus (EBV), belonging to the gamma-herpes virus subfamily and infecting most people worldwide, may be a putative factor in the breast cancer natural history. In this paper, the overall survival of 85 patients with BC was assessed in a prospective study during an 87-month follow-up. The aim of our study was to evaluate the effect of EBV infection combined with the survival in all patients. We focused our study around three axis: i) The EBV DNA detection (measured by qPCR) both in breast cancer tissues and in peripheral blood mononuclear cells (PBMCs), ii) the IFN-γ and TNF-α intracellular immunostaining test combined with flow cytometry analysis iii) the clinical outcome of the patients and the pathological characteristics. These experiments were also completed by searching the EBV replicating form through the titration of serum anti-ZEBRA antibodies. No association could be found (i) between EBV detection in tumor/PBMCs and tumor histology and (ii) between EBV and other prognostic factors. The effect of the EBV load (PBMCs or/and tumor) on patients’ survival was analyzed by using both univariate and multivariate analysis combined with an analysis of covariance. The patients with anti-ZEBRA antibodies at high titers had a worse overall survival (p=0.002). According to the survival curves, patients who recovered from the disease had a measurable latent form of EBV DNA load, together with a high frequency of IFN-γ and TNF-α producing PBMCs (p=0.04), suggesting a type Th1- polarized immune response both in the tumor and in the periphery. These data point to a plausible beneficial role of EBV, which could occur through activation of nonspecific anti-tumoral immune responses. Our findings have revealed the following unexpected properties of this so-called “double faceted” EBV: (i) the latent form of this virus, measured and quantified by the tumor viral EBV DNA, confers a survival advantage to BC patients; (ii) there is an association between high anti-ZEBRA titers and poor outcome, though the high anti-ZEBRA response could be the result of late stage cancer and not the cause of poor outcome. Given that this study assessing the beneficial effects of the EBV was conducted over a long time period, these results are a relevant basis for future studies involving a larger patient population.
Title: Adenanthin, a new peroxiredoxin inhibitor, induces a switch between estrogen receptor alpha-mediated and Src/Akt-driven signaling in breast cancer cells

Malgorzata Bajor¹, Agata O Zych¹, Patrick C O'Leary²,³, Anna Czekalska¹, William M Gallagher³, Jakub Golab¹ and Radoslaw Zagozdzon¹. ¹Center of Biostructure Research, Medical University of Warsaw, Warsaw, Poland; ²Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA and ³Cancer Biology & Therapeutics Laboratory, UCD Conway Institute, UCD School of Biomolecular and BioMedical Science, Dublin, Ireland.

Body: Increasing evidence indicates that oxidative stress is involved in the progression of estrogen receptor (ER)-positive breast cancer. A moderate increase in cellular oxidants contributes to the genomic instability and to the change in cellular growth pattern, which in turn can facilitate progressive transformation of normal cells into cancer cells. Accordingly, the oxidative stress-related gene expression signature has been suggested to correlate with therapy resistance and poorer outcome in breast cancer. Therefore, it is crucial to determine the antioxidant defense mechanisms that are utilized by breast cancer cells to regulate oxidative stress.

Peroxiredoxin 1 (PRDX1) is one of the most prevalent hydrogen peroxide scavenging enzymes in mammalian cells. Our recent studies indicated that PRDX1 is an independent biomarker of favorable prognosis in ER-positive breast cancer. Our results indicate the mechanistic link between PRDX1 and ERα in breast cancer and suggest a role for PRDX1 in mammary carcinogenesis. We provide a molecular explanation for this phenomenon in the current project.

To evaluate the importance of PRDX1 activity in ER-positive breast cancer, we have used adenanthin, a newly described PRDX1/2 inhibitor. In our studies, we have shown that adenanthin strongly inhibits metabolism of exogenous hydrogen peroxide by breast cancer cells. This phenomenon is accompanied by a shift from H₂O₂-degrading PRDX1 dimers into enzymatically inactive monomers and by a dramatic decrease of ERα protein presence in the cells. Moreover, we have observed that incubation of ER-positive breast cancer cells with adenanthin leads to a marked increase in phosphorylation status of proteins associated with Src-Akt-driven signaling in breast cancer. Thus, our results suggest that PRDX1 can play an important role in controlling the switch between estrogen receptor- and growth factor-driven signaling in breast cancer.

In summary, in our studies we describe for the first time molecular consequences of rapid dysfunction of PRDX-related system in ER-positive breast cancer. The deeper knowledge on the mechanisms of PRDX1 functioning can change our understanding of the events leading to the progression of ER-positive breast cancer and provide new opportunities for pharmacological interventions in this disease, especially in the context of recent observations connecting the oxidative stress and resistance to endocrine therapy.
Title: CD59 expression is associated with MDA-MB-231-HM cells' metastasis and prognosis of breast cancer patients

qianwen Ouyang1,2,3,6, long Zhang4,6, peipei Ding4,5, weiguo Hu4,5, rongchen Luo1,2 and zhiming Shao3. 1Cancer Center, Guangzhou, China; 2Traditional Chinese Medicine-Integrated Hospital, Southern Medical University, Guangzhou, China; 3Breast Cancer Institute, Cancer Hospital/Cancer Institute, Shanghai Medical College, Institutes of BioMedical Sciences, Fudan University, Shanghai, China; 4Cancer Institute, Collaborative Innovation Center of Cancer Medicine, Fudan University Shanghai Cancer Center, Shanghai Medical College, Fudan University, Shanghai, China; 5Institutes of BioMedical Sciences, Fudan University, Shanghai, China and 6The Third Hospital of Nanchang, Nanchang, China.

Body: Breast cancer is the most frequent cancer among women so far and its metastasis remains predominant clinical challenge. CD59 is a membrane complement regulatory protein (mCRP), and is overexpressed in many types of solid tumors. It's been hypothesized that CD59 overexpression may facilitate tumor metastasis. To reveal the relationship between CD59 expression and metastasis of breast cancer, we transfected recombinant retrovirus encoding CD59 and shRNA targeting human CD59 into MDA-MB-231 and MDA-MB-231-HM cell lines respectively, followed by essential detection. CCK8 analysis, in vivo metastasis model construction were carried out to examine cell growth and metastasis in vitro and in vivo in both transfected cell lines. The results demonstrated that CD59 expression levels were functionally changed. Surprisingly, cell growth rates of the cell lines were altered both in vitro and in vivo accordingly, which was accompanied by reduced metastasis in the lungs in the MDA-MB-231-HM cell line xenograft model when CD59 was knocked down. Clinical data show that breast cancer patients with high expression of CD59 is more likely to suffer metastasis, shorter disease-free and overall survival. We show that CD59 expression is associated with tumor cell metastasis and outcome of breast cancer patients.
Title: Provista-001 a multi-center prospective study of protein signature used in the differentiation of benign breast lesions from invasive breast cancer in women under the age of 50 with a BI-RADS 3 or 4

David E Reese¹, Michael Silver¹, Susan Yeh², Sherri Borman² and Henderson C Meredith². ¹Provista Diagnostics, New York, NY and ²Provista Diagnostics, Scottsdale, AZ.

Body: Background: Over-diagnosis of breast lesions represents a significant problem in detection and screening of breast cancer, especially in women under the age of 50. Despite this issue, few new approaches have been developed to augment standard of care in the more precise detection of breast cancer. The combination of breast imaging, which provides anatomical evidence, with a robust protein signature that would detect biochemical cues of breast cancer offers an attractive approach to this problem. We have recently identified a protein signature composed of immune-regulatory cytokines, growth factors and tumor derived autoantibodies. Here, we test the hypothesis that a protein signature, combined with standard of care can greatly increase the precision of breast cancer diagnosis in women under the age of 50 in a randomized and blinded study.

Methods: Provista-001 enrolled 351 patients from 10 sites across the US and will follow patients for 6 months following the first blood draw under IRB approval. Patients were consented after first assessment of a BIRADS 3 or 4 and considered eligible if they were under the age of 50, no history of cancer, no prior breast biopsy, and were assessed as BIRADS 3 or 4 within 21 days of blood draw. Upon enrollment, patients were randomized to either training or validation groups. Clinical truth was set at equal to or greater than 80% sensitivity/specificity. Serum protein biomarkers and autoantibodies identified in prior proteomic screens were measured in serum samples collected prior to biopsy. Individual biomarker (22 serum protein and autoantibodies) concentrations, together with specific patient data were evaluated using various logistic regression models developed from prior retrospective studies. A training set, comprised of 200 patients, was used to develop and refine new models, which were then validated in the remaining 151 subjects. Clinical findings were compared to biopsy (largely BIRADS 4) or will be followed for 6 months and re-assessed (BIRADS 3).

Results: The novel algorithm utilizing patient data, serum protein and autoantibody concentrations combined with regression models was able to distinguish benign from breast cancer lesions in a statistically significant manner. Importantly, the serum protein biomarkers alone were unable to adequately distinguish benign lesions, consistent with prior work. However, the addition of tumor autoantibodies markedly increased both the sensitivity and specificity of the assay in this group of women. The use of the algorithm in conjunction with imaging was more accurate than imaging alone in this population.

Conclusions: Our current findings suggest that when used in combination, the protein signature developed here and breast imaging provides a more precise detection methodology than either alone. This is particularly important in women under the age of 50 where a low prevalence of disease makes detection difficult. The follow-up data at six months (BIRADS 3) will yield additional data in this understudied group of women. Additional studies utilizing the protein signature with women over the age of 50 are currently underway.
**Title:** Teasing out the PALB2 phenotype

Emily K Dalton¹, Rachel McFarland¹, Holly Laduca¹, Shuwei Li¹ and Chia-Ling Gau¹. ¹Ambry Genetics, Aliso Viejo, CA.

**Body:**

**Background:** Biallelic mutations in PALB2 (Partner and Localizer of BRCA2) are known to cause Fanconi Anemia Type N. Multiple reports have demonstrated an increased risk for cancer in individuals heterozygous for PALB2 mutations. For example, a recent study by Antoniou et al reported a 33-58% lifetime risk for breast cancer in PALB2 mutation carriers, with 30% of carriers reporting triple negative breast cancer (TNBC). Other studies have suggested associations between PALB2 heterozygosity and pancreatic cancer, ovarian cancer, male breast cancer, and prostate cancer as well. We aimed to better define PALB2 phenotypes by assessing clinical history of TNBC, pancreatic, ovarian, and prostate cancers amongst PALB2 mutation carriers identified via multigene panel testing.

**Methods:** We reviewed clinical histories of 11,007 individuals who underwent PALB2 sequence and deletion/duplication analysis as part of a multigene hereditary cancer panel. Descriptive statistics were utilized for clinical histories of PALB2 carriers, and chi square analysis was used to compare clinical histories of PALB2 mutation carriers to mutation-negative controls. Individuals with mutations in other cancer susceptibility genes were excluded from analysis.

**Results:** A total of 98 PALB2 mutation carriers identified among 9610 individuals were included in our analysis. The majority of mutation carriers were Caucasian (80%) and female (92.8%). All identified mutations were truncating (nonsense, frameshift, or gross deletions). No pathogenic missense mutations were identified in this cohort. 77.6% (n=76) of mutation carriers had breast cancer, diagnosed at a mean age of 48. Hormone receptor status was available for 48 mutation carriers and 2469 controls. 37.5% (18/48) of breast cancers in mutation carriers were reported as triple negative, compared to 17.1% (423/2469) of breast cancers in controls (OR: 2.9; p=0.0002). 7.8% (n=8) of PALB2 mutation carriers had ovarian cancer. There was no significant difference in the incidence of ovarian cancer between PALB2 mutation carriers and controls (OR: 0.65; p=0.25). Additionally, mutation carriers were significantly less likely to have a family history of ovarian cancer than controls (OR: 0.5; p=0.02). 5.9% (n=6) of mutation carriers had pancreatic cancer, diagnosed at a mean age of 57.8, compared to 61 for controls. PALB2 mutation carriers were 1.3 times more likely to have personal and/or family history of pancreatic cancer, although this was not statistically significant (p=0.22). Similarly, PALB2 mutation carriers were 1.5 times more likely to have a family history of prostate cancer, although this was not statistically significant (p=0.09).

**Conclusions:** Our data supports existing literature associating PALB2 mutations with TNBC. We did not observe significant associations between PALB2 carrier status and a clinical history of pancreatic, prostate, or ovarian cancers. However, this data should be interpreted with caution, as it is possible that unidentified genetic factors contributed to the clinical history of cancer in our mutation-negative controls. Investigation of PALB2-associated cancer risks in an unselected prospective cohort would help to further elucidate the PALB2 phenotype.
Title: Ki-67 and p53 are useful factors to predict long term survival in low-risk luminal A breast cancer patients

Ha Woo Yi¹, Se Kyung Lee¹, Soo Youn Bae¹, Jun Ho Lee¹, Hyun-Chul Lee¹, Won Ho Kil¹, Jeong Eon Lee¹, Seok Won Kim¹ and Seok Jin Nam¹. ¹Samsung Medical Center, Seoul, Korea.

Body: Overexpression of p53 is the most frequent genetic alteration in breast cancer. Recently, many studies have shown that the expression of mutant p53 differs for each subtype of breast cancer and is associated with different prognoses. In this study, we aimed to determine the suitable cut-off value to predict the clinical outcome of p53 overexpression and its usefulness as a prognostic factor in each subtype of breast cancer, especially in luminal A breast cancer. Approval was granted by the Institutional Review Board of Samsung Medical Center. We analyzed a total of 7739 patients who were surgically treated for invasive breast cancer at Samsung Medical Center between Dec 1995 and Apr 2013. Luminal A subtype was defined as ER&PR + and HER2- and was further subclassified according to Ki-67 and p53 expression as follows: luminal A (Ki-67-, p53-), luminal A (Ki-67+, p53-), luminal A (Ki-67-, p53+), luminal A (Ki-67+, p53+). Low-risk luminal A subtype was defined as negative for both Ki-67 and p53 (luminal A (Ki-67-, p53-)) and others subtypes were considered to be high-risk luminal A breast cancer. A cut-off value of 10% for p53 was a good predictor of clinical outcome in all patients and luminal A breast cancer patients. The prognostic role of p53 overexpression for OS and DFS was only significant in luminal A subtype. The combination of p53 and Ki-67 has been shown to have the best predictive power as calculated by the area under curve (AUC), especially for long-term overall survival.

Table 1. Factors associated with overall survival in luminal A subtype breast cancer patients (N=3918).

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Table 1. Factors associated with overall survival in luminal A subtype breast cancer patients (N=3918).
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<th></th>
<th>Positive</th>
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BCS, breast-conserving surgery; LVI, lymphovascular invasion; NG, nuclear grade; RM, resection margin; TM, total mastectomy; NG, LVI and total mastectomy were not significant in multivariable Cox analysis;* Cox-proportional hazard regression model.

In this study, we have shown that overexpression of p53 and Ki-67 could be used to discriminate low-risk luminal A subtype in breast cancer. Therefore, using the combination of p53 and Ki-67 expression in discriminating low-risk luminal A breast cancer may improve the prognostic power and provide the greatest clinical utility.
**Title:** Characterization of the constitutive and tumor microenvironment-mediated changes in the expression, localization and function of CREB in HER-2/neu-induced tumor cells

Barbara Seliger¹, Andre Steven¹, Sandra Leisz¹, Bernhard Hiebl², Claudia Wickenhauser³, Chiara Massa¹, Manuela Iezzi⁴, Davide Bedognetti⁵, Francesco Marincola⁵ and Rolf Kiessling⁶. ¹Institute of Medical Immunology, Halle, Germany; ²Center for Basic Medical Research, Halle, Germany; ³Institute of Pathology, Halle, Germany; ⁴Center for Aging Sciences, Chieti, Italy; ⁵Sidra Medical Research Center, Doha, Qatar and ⁶Karolinska Institutet, CCK, Stockholm, Sweden.

**Body:** Despite the cAMP responsive element-binding protein (CREB) is involved in tumorigenicity, a link between HER-2/neu-induced transformation, CREB activation and alterations of the tumor micromilieu and cellular localization has not yet been established, which might contribute to the pathogenesis of HER-2/neu-associated tumors including mammary carcinoma. Using in vitro models of HER-2/neu-overexpressing and HER-2/neu-negative/silenced tumor cells as well as human mammary carcinoma lesions with defined HER-2/neu status a correlation of HER-2/neu overexpression with an increased expression and activation of CREB accompanied by an enhanced signal transduction and increased angiogenesis was detected both in vitro and in vivo. Hypoxia revealed a HER-2/neu-dependent CREB activation, which was associated with an upregulation of HIF-1α, GLUT1 and VEGF expression and altered microenvironment such as enhanced cell migration and matrix metalloproteinase-mediated invasion as well as increased extracellular lactate concentrations. The CREB-dependent hypoxic response was further accompanied by ubiquitination and mitochondrial colocalization of CREB and an altered mitochondrial gene expression. Furthermore, CREB downregulation in HER-2/neu-transformed cells by shRNA and by the treatment with the inhibitors KG-501 and lapatinib caused morphologic changes, a reduced cell proliferation and G0/G1 cell cycle arrest, which could be rescued by CREB expression. This was accompanied by a reduced cell migration, wound healing, an increased fibronectin adherence, invasion and matrix metalloproteinase expression. In vivo shCREB HER-2/neu+ cells, but not control cells exerted a significantly decreased tumorigenicity associated with a decreased proliferative activity, an enhanced apoptosis rate and an increased frequency of T lymphocytes in peripheral blood mononuclear cells. Thus, CREB seems to play an important role in the HER-2/neu-mediated transformation process of (mammary) tumor cells by altering their in vitro and in vivo growth characteristics as well as their immunogenicity.
Novel oncogene CNOT2 regulates proliferation and tumor angiogenesis targeting VEGF in MDA-MB-231 breast cancer cells

Sunghoon Kim1, Eunjung Sohn1 and Deokbeom Jung1. 1Kyung Hee University, Seoul, Korea; 2Kyung Hee University, Seoul, Korea and 3Kyung Hee University, Seoul, Korea.

Body: CCR-NOT (CNOT2) complex plays an important role in regulating the mRNA decay and translation repression. Here, we investigated the role of CNOT2 in human breast cancers. We found that CNOT2 was overexpressed in breast, pancreas, or prostate cancer cell lines and tissues. Proliferation of MDA-MB231 cells was suppressed by siRNA and shRNA mediated silencing of CNOT2. Knockdown of CNOT2 by siRNA transfection in MDA-MB-231 breast cancer cells decreased cell motility of MDA-MB231 cells, while overexpression of CNOT2 enhanced motility. Inhibition of CNOT2 significantly reduced clonogenicity, while overexpression of CNOT2 enhanced. Furthermore, silencing of CNOT2 attenuated cancer related genes such as cyclin D1, VEGF, or IκB as well as ERK, AKT in MDA-MB-231 breast cancer cells. Overall, our findings suggest that novel oncogene CNOT2 promotes tumor proliferation and motility via enhancement of survival genes and VEGF in MDA-MB-231 breast cancer cells.
2014 San Antonio Breast Cancer Symposium

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Average Grade: 0

Title: Mammaglobin is a potent regulator of breast cancer processes leading to disease progression

Roxann Guerrette¹, Nadia Picot¹ and Gilles Robichaud¹. ¹Université de Moncton, Moncton, NB, Canada.

Body: Metastasis is the major cause of death in women suffering from breast cancer. To provide a better understand breast cancer progression, we have studied the role of mammaglobine-1 (MGB1) gene in breast cancer pathogenesis. MGB1 has been extensively studied as a diagnostic biomarker due to its abundant expression in mammary cancer cells. Yet, MGB1’s role in disease progression is still unknown. Our experimental results demonstrate for the first time that MGB1 in a pivotal regulator on breast cancer malignancy. More precisely, loss of MGB1 expression correlates with a decrease in proliferation, spheroid formation, migration, and invasion capacities of breast cancer cells. Concomitantly, we also observe that MGB1 expression activates pro-malignant signaling cascades such as MAPKs, focal adhesion kinase (FAK) and NFkB pathways. Moreover, MGB1 promote epithelial to mesenchymal (EMT) features which coincide with our findings. Our study provides the first evidence for MGB1 as regulator of breast cancer malignancy and disease progression.
Title: Loss of long non-coding RNA LOC285194 is associated with poor prognosis in triple-negative breast cancer by activating microRNA-24/TGF-β signaling

Xiaojian Ni\(^1\) and Zhimin Shao\(^1\). \(^1\)Shanghai Cancer Center and Cancer Institute, Shanghai Medical College, Fudan University, Shanghai, China.

Body: Background: Triple-negative breast cancer (TNBC) is a highly aggressive tumor subtype associated with poor prognosis. The lack of obvious targets is a major challenge in treating patients with triple-negative breast cancer. Long non-coding RNAs are reported to be strongly correlated with poor patient prognosis, suggesting a potential role in cancer progression. Thus, identification of novel targets of long non-coding RNA is urgent for improving outcomes in triple-negative breast cancer.

Methods and Results: Here we conducted HTA 2.0 microassay analyses in 200 triple-negative breast cancer patients, which include normal tissues and primary tumors. We identified for the first time that LOC285194 was significantly down-regulated in primary tumors than normal tissues in triple-negative breast cancer. Multivariate analysis showed that loss expression of LOC285194 was an independent predictor for poor disease-specific survival (DFS) in triple-negative breast cancer. Through in vitro assays, we confirmed that silencing of LOC285194 expression by shRNA in MDA-MB-231 and MDA-MB-468 breast cancer cells, can potentiate the proclivity to metastasize in transwell assay and enhance tumorigenesis in vivo by injection into the right flank of the nude mice. In a reverse-complimentary approach, we determined that elevated LOC285194 expression in highly metastatic breast cancer cell lines (MDA-MB-231Bo) can suppress the ability of invasion and metastasis in vitro and in vivo. Mechanistically, microassay reveals that the metastatic behavior strongly correlates with increased microRNA-24 levels and overexpression of TGF-β type II receptor (TGF-βR2). Dual luciferase reporter assay confirmed that long non-coding RNA LOC285194 was an target of microRNA-24. Conversely, restoration of LOC285194 can inhibit the activation of this pathway in MDA-MB-231Bo cells.

Conclusion: By utilizing a series of mammary tumor cell lines and animal models, we demonstrate that long non-coding RNA LOC285194 acts as a tumor suppressor in triple-negative breast cancer through regulating microRNA-24/TGF-β signaling pathway which might be a potential prognostic indicator and therapeutic target for triple-negative breast cancer.
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Title: Weight loss reversed the carcinogenic effect of obesity on basal-like breast cancer

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Body: Epidemiologic studies demonstrate that obesity is associated with a subtype of breast cancer called basal-like breast cancer (BBC), which is fast proliferating and aggressive, with no targeted therapies. Using the C3(1)-TAg murine model of BBC, we have previously shown that obese adult mice displayed significantly decreased tumor latency compared to lean mice with induction of the oncogenic HGF/c-Met pathway. Reducing adiposity is predicted to lower incidence of BBC in human population studies. Thus, the objective of this study was to investigate how a diet-switch to reduce obesity affected BBC onset and early progression. C3(1)-TAg mice were placed on a control low fat diet (LFD) and remained lean, high-fat diet (HFD) to induce obesity, and a group made obese by HFD that were then switched to LFD to induce weight loss. Just two weeks after weight loss, mice in the diet switch group had weights and adiposity similar to lean mice. Weight loss caused a 51.4% decrease in adiposity (P<0.0001) compared to obese mice. Importantly, mice that lost weight displayed significantly delayed tumor latency (17 week) compared to obese mice on HFD (15 week), with no differences detected in tumor burden or growth. Therefore the obese microenvironment that promotes early tumor onset can be reprogrammed with weight loss and restoration of a lean phenotype. Ongoing studies are examining HGF/cMet expression by immunohistochemistry in normal mammary glands and tumors to determine the contribution of this obesity-responsive pathway. Metabolomics and RNAseq analysis will identify novel obesity-dependent pathways relevant to BBC. In conclusion, we demonstrated that loss of adiposity protected against early BBC onset. Further research will identify important biomarkers associated with obesity and weight loss that can be compared through conserved biology approaches.
Title: Circulating methylated DNA of pregnancy-associated plasma protein A as a clinically useful biomarker in breast cancer

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Body: Background: A recent report suggests that Pregnancy-Associated Plasma Protein A (PAPPA) plays a pivotal role in normal cell division and is targeted early in breast cancer development. Epigenetic silencing of PAPPA is highly prevalent in precursor lesions and invasive breast cancer, correlating with loss of PAPPA protein expression. In normal breast tissue PAPPA promoter methylation was not observed (Journal of Pathology 344-356: 2014). We sought to test the hypothesis that PAPPA promoter hypermethylation can be detected in cell-free tumour specific DNA (ctDNA) in the bloodstream of patients with metastatic breast cancer.

Methods: Plasma derived from 12 patients with metastatic breast cancer was examined for ctDNA. A semi-quantitative real-time PCR based MethyLight assay was used for detection of PAPPA methylation. Spiking experiments with breast cancer cell lines were performed to develop the assay and confirm linearity of sodium bisulfite treatment.

Results: Percentage methylated reference (PMR) values from spiking experiments with PAPPA methylated and non-methylated DNA in plasma (matrix) demonstrate good linearity for the PAPPA gene and the endogenous control Col2A. PAPPA methylation was detected in 10 out of 12 (83%) of the metastatic breast cancer samples. In stark contrast, epigenetic silencing of PAPPA was not detected in any of the plasma samples from non-cancer bearing patients (n=10).

Conclusions: Given the potential clinical impact of our data, this study is being extended to include early stage breast cancer patients with poor prognostic clinic-pathological indicators. Our proof-of-concept findings indicate that testing for PAPPA promoter methylation in ctDNA may be used as a biomarker of disseminated disease, helping clinicians with treatment decisions and adjusting those choices as conditions change. By developing and refining more sensitive techniques, PAPPA methylation testing in ctDNA could also find practical utility in breast cancer screening.
Title: S0800: Nab-paclitaxel, doxorubicin, cyclophosphamide, and pegfilgrastim with or without bevacizumab in treating women with inflammatory or locally advanced breast cancer (NCI CDR0000636131)


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Body: Background. Locally advanced breast cancer (LABC) and inflammatory breast cancer (IBC) remain difficult challenges despite progress in multimodality treatment. The SWOG trial S0800 (clinicaltrial.gov NCT00856492) compared bevacizumab in combination with weekly nab-paclitaxel followed by dose-dense AC to nab-paclitaxel followed or preceded by AC as neoadjuvant treatment for HER2-negative IBC/LABC. The rationale was based on the proposed role of angiogenesis, potential role of improved flow and oxygenation in enhancing the delivery of chemotherapy agents, and the proapoptotic effect with certain chemotherapeutic agents, particularly taxanes.

Methods. This was a randomized open-label Phase II trial with an accrual goal of 200 patients equally allocated to either bevacizumab (Arm 1) or no bevacizumab. Two patients were ineligible and five withdrew consent leaving 208 for analysis. The no bevacizumab group was further randomized to two sequences (Arm 2: nab-paclitaxel - AC+PEG-G versus Arm 3: AC+PEG-G-nab-paclitaxel) with 50 patients expected in each sequence. The primary endpoint of this study was pathologic complete response (pCR) defined as no evidence of invasive tumor at the primary site and axillary lymph nodes in the surgical specimen. The power for the primary comparison of bevacizumab (bev) versus no bevacizumab (no bev) ignoring sequence was 80% with a 1-sided \( \alpha = 0.10 \). Randomization was stratified by hormone receptor status and type of disease (IBC or LABC).

Results 215 patients were accrued May 2010 - September 2012. Most had LABC (88%) versus IBC (12%) and most tumors were hormone-receptor (HR) positive (67%). Fourteen (7%) patients had no definitive surgery (included as no pCR); 135 (65%) had residual disease (no pCR) and 59 (28%) had pCR. The bevacizumab pCR rate was higher (35/96; 38%) than that in the non-bevacizumab arms (24/112; 21%) (exact \( p=0.021 \); stratified \( p=0.015 \)). In HR-positive disease there was slight improvement that was not statistically significant (bev 25% vs. non-bev 18%; \( p=0.41 \)) while the difference was larger in HR-negative disease (bev 59% vs. non-bev 28%; \( p=0.014 \)). In LABC the overall pCR rate was 29% with a higher rate in the bevacizumab patients (37% vs. 22%; \( p=0.035 \)). For IBC there was improvement (30% vs. 14%), but not statistically significant (\( p=0.61 \)) in a small sample. Overall, Grade 3 and 4 events were common in both (bev 67%; non-bev 65%), but did not differ by treatment. There were 21 deaths with 3-year overall survival (OS) of 87% and 83% for bevacizumab and non-bevacizumab, respectively (log-rank \( p=0.57 \)).

Conclusion Compared with combination anthracycline-taxane neoadjuvant chemotherapy, the Bev-Nab-paclitaxel-AC regimen significantly improved pCR rate overall, primarily for triple negative (TNBC) patients. This neoadjuvant regimen could be a good choice for TNBC/IBC. The observed pCR rate in ER negative disease (59%) suggests that the addition of bevacizumab to a standard chemotherapy backbone may improve outcome in this subset, and justifies further testing of such an approach. Correlative science studies to further delineate the biology of TNBC and the effects of bevacizumab are ongoing.
Title: Findings from the first prospective womb to breast cancer study: New gestational biomarkers support proof of concept that gestation is a window of susceptibility for the breast

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Body: Rationale. Here we provide the first prospective evidence for proof of concept that gestation is an important window of susceptibility for breast cancer. These findings could open the field to interdisciplinary investigation of mechanisms, and interventions via clinical and experimental science.

Objective. We tested the hypothesis that biomarkers in gestation predict early-onset breast cancer. Data were prospectively collected including maternal and paternal peri-conceptual body mass, tobacco and alcohol use, maternal pregnancy weight gain, pregnancy complications and outcomes, placental morphology assessed by a standardized examination at birth, and environmental chemicals recently assayed in archived maternal perinatal serum samples. This investigation was based on the observation of 20,000 pregnancies beginning in 1959, with surveillance for both maternal (F0) and offspring (F1) cancer in the Child Health and Development Studies pregnancy cohort. This report is based on the first 133 breast cancer cases in F1 that occurred from 1992-2012, diagnosed at ages 32 to 52.

Results. We observed gestational biomarkers of breast cancer risk which were independent of maternal history of breast cancer and race. Highlights of significant findings include independent, higher risk for women who: were born to mothers who lost weight during pregnancy (3-fold increase in risk, p<0.03), were growth retarded in utero (3-fold increased risk, p<0.01), were born with thick (p<0.01), but small diameter placentas (p<0.04), whose mothers had higher perinatal serum levels of environmental chemicals including o,p'-DDT (2.5-fold increase in risk for upper quartile, p<0.03), perfluorooctanesulfonic acid (PFOS) precursors (p<0.03) and total cholesterol (p<0.04). Maternal floor infarction of the placenta was a protective factor both for mothers and their daughters. In ancillary studies we observed that F1 breast density at mid-life is also impacted by placental morphology during F1 gestation.

Conclusions. Here we provide a high level of evidence for the existence of gestational biomarkers for breast cancer. Prospective design and direct clinical observation of pregnancies eliminates reporting and misclassification bias. Findings extend the discussion of gestational biomarkers beyond birthweight and pre-eclampsia which have been previously reported. The importance of the gestation window for breast cancer in humans is in line with toxicological evidence in animal models and strongly suggests the existence of opportunities for primary prevention beginning before birth.
Title: Efficacy of everolimus on multiple mechanisms of AI-resistance in vitro and xenograft, and characterization of their everolimus-resistance

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Body: Background: mTOR inhibitor, everolimus showed remarkable clinical efficacy and has been considered as a promising agent for breast cancer treatment especially among hormone receptor positive (HR+) postmenopausal advanced breast cancer. Now Affinitor (everolimus) is approved for HR+ breast cancer in Japan. However, definitive biomarker has been unclear so far. In addition, the subsequent treatment after refractory to everolimus has not been defined. In preclinical study, it has been reported there are several resistant models of endocrine therapy and we also reported several types of models established from MCF-7-E10, in which estrogen receptor (ER) activity could be monitored by transfected-ERE-GFP. Those EDR cells are characterized as the followings: 1) Type 1 shows constitutive ER overexpression without estrogen, and PI3K/Akt/mTOR pathway upregulated. 2) Type 2 shows low expression of ER (ERE-GFP negative) and IGF-1R/JNK signaling pathway upregulated (Fujiki, 2014, J Steroid Biochem Mol Biol). 3) Type4, which was cultured under the condition of estrogen depletion and addition of testosterone. It showed using androgen metabolite as ligand and both PI3K/Akt/mTOR and MAPK signaling pathway upregulated (Hanamura, 2013, Breast Cancer Res Tr). Methods: Efficacy of everolimus was analyzed in those cell lines in vitro and in vivo about inhibition of cell proliferation. In vivo study, ovarectomized mice were inoculated with Type1 or Type4. Treatments were performed as single agent letrozole, everolimus, or combination and tumor volume changes were compared. Then everolimus resistant cells were generated from those EDR cells after long term exposure to everolimus in vitro. Though we could not acquire everolimus resistant cell from Type4, Type1 and 2 showed its resistance. Those everolimus resistant (EVR) cells are equivalent to resistance to the combination therapy between AI and everolimus after the first line AI treatment failure. Using those cell lines, we investigated the different mechanism of resistance to everolimus. Results: Everolimus extremely inhibited cell proliferation in each EDR type in vitro and in vivo. Besides, Type4 was more remarkably sensitive than parental MCF-7-E10 under testosterone supplemented condition in vitro. Everolimus or combination treatments reached significant tumor volume reduction around 50 percent after 21 days treatment period, in contrast to increase of tumor volume in placebo or letrozole group. In vitro study of developing resistance to everolimus, Type1-EVR cells no longer responded to everolimus and surprisingly fulvestrant also lost sensitivity to EVR cell in spite of keeping ERE activity. Type1-EVR showed much higher expression of phosphorylation of MAP-kinase than parental cell, however, MEK inhibitor or PI3K inhibitor alone was not effective but combination with fulvestrant significantly effective more than parental cell. In type2, JNK inhibitor was effective for parental cell but EVR cell lost sensitivity to JNK inhibitor, however, Src inhibitor was much more effective in Type2-EVR than in parental cell. Conclusion: Everolimus showed remarkable efficacy to any types of EDR cells, however, the mechanism of acquired resistance to everolimus was likely to be different between each EVR cell. This finding implies that different mechanisms of AI resistance follow different types of everolimus resistance.
Title: Pilot study of a passive non-radioactive electromagnetic wave technology to localize non-palpable breast lesions

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Body: Background and Objectives: The standard preoperative technique for localizing non-palpable breast lesions is wire localization (WL). Radioactive seed localization (RSL) has been described as an alternative approach to address the number of clear disadvantages associated with WL. Yet, despite its proven advantages, the adoption of RSL has been impacted by considerable regulatory requirements for the handling of radioactive materials. To advance the progress made with RSL and eliminate the issues associated with radioactive components, the SAVI Scout® surgical guidance system has been developed. The SAVI Scout surgical guidance system is an FDA-cleared medical device that utilizes passive non-radioactive electromagnetic wave technology to provide real-time guidance during excisional breast procedures. The purpose of this pilot study is to determine the safety and efficacy of the SAVI Scout system in localizing and directing the removal of non-palpable breast lesions during excisional biopsy and lumpectomy procedures. The preliminary results from two institutions are reported.

Materials and Methods: Following a feasibility study using the SAVI Scout system in resected breast tissues ex vivo, Institutional Review Board approval was granted for both institutions for women with a non-palpable breast lesion requiring preoperative localization for excision. Participating patients underwent localization and excision with the SAVI Scout system, which consists of an electromagnetic wave reflective device (reflector), handpiece and console. Using mammographic or ultrasound guidance, the reflector was placed percutaneously up to 7 days prior to the scheduled excisional procedure. At surgical excision, the surgeon used the SAVI Scout handpiece to locate the reflector, which was removed along with the surrounding breast tissue. The console provides audible feedback of reflector proximity to the handpiece. Successful reflector placement, localization and retrieval were the primary endpoints.

Results: After the first training case (data not used), a total of 17 patients have participated in the study to date. The reflectors were successfully placed with mammographic guidance in 9/9 patients and with ultrasound guidance in 8/8 patients. Reflectors were placed an average of 1.7 days (range 0-6 days) before surgery. Five patients underwent excisional biopsy and 12 patients had a lumpectomy. The intended lesion and reflector were successfully removed in 17/17 patients. Reflector migration did not occur and no adverse events occurred. Of the 14 patients in which final pathology is currently available, 6 patients had no invasive or in situ carcinoma identified, 4 had no tumor in the excision, and 3/4 patients with tumor had clear margins. One patient had a focally positive margin and was recommended for re-excision.

Conclusions: The preliminary data show the SAVI Scout system to be a safe and effective tool for the localization of non-palpable breast lesions. Ongoing accrual to this pilot study will validate these findings with planned enrollment of a total of additional 50 patients in the next 60 days at up to 3 additional sites.
Title: Mature data (> 25 years) on 2 years adjuvant tamoxifen treatment in premenopausal women with breast cancer: Time to re-emphasize the progesterone receptor as predictive factor?

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Body: Background
In 2011 The Early Breast Cancer Trialists' Collaborative Group (EBCTCG) reported that five years of adjuvant tamoxifen (TAM) significantly reduces the 15-year risks of breast cancer recurrence and death. Moreover, the estrogen receptor (ER) was considered the clinically most relevant predictive factor for efficacy of TAM treatment. In premenopausal patients participating in a randomized trial of 2 years of adjuvant TAM, we have previously reported on increased recurrence free survival in patients with ER- and PR-positive disease and a trend for increased overall survival in patients with PR-positive tumors. The median follow-up time was 13.9 years for patients without events. Since the effect on mortality may increase beyond year 10, we investigated more mature of data.

Aims
To investigate the long-term effect of 2 years adjuvant TAM in premenopausal patients choosing cumulative mortality (CM) and cumulative breast cancer mortality (CBCM) as end-points.

Methods
Premenopausal patients (n=564) with stage II breast cancer were included in a Swedish randomized multicenter trial between 1986-1991 and allocated to 2 years of TAM (n=276) or no treatment (n=288). The hormone receptor status of the tumor was known for 541 (96%) of the patients included, determined by immunohistochemistry or cytosol-based methods. Less than 2% were treated with adjuvant chemotherapy and they were equally distributed between the two groups. Median follow-up time was 26.3 years (22.7-29.7). Mortality data was obtained from the Swedish Cause of Death Register.

Results
Death from any cause was recorded among 312 (55%) of the patients, and 265 (47%) of the deaths were due to breast cancer. Two years of TAM decreased CM and CBCM in all patients irrespective of hormonal receptor status (n=564) (CM: HR 0.81; 95% CI: 0.65-1.02, p=0.070; CBCM: HR 0.79; 95% CI: 0.61-1.01, p=0.058), as well as in patients with ER-positive disease (n=346) (CM: HR 0.77; 95% CI: 0.57-1.03, p=0.077; CBCM: HR 0.72; 95% CI: 0.52-1.01, p=0.051), however not strictly significant. Importantly, in patients with PR-positive tumors, TAM significantly reduced CM and CBCM (CM: HR 0.73; 95% CI: 0.55-0.98, p=0.037; CBCM: HR 0.68; 95% CI: 0.49-0.94, p=0.020). Moreover, in patients with ER- and PR positive tumors TAM decreased CM (HR 0.74; 95% CI: 0.55-1.01, p=0.057) and CBCM (HR 0.69; 95% CI: 0.49-0.97, p=0.033), whereas there was no effect of TAM in patients with ER-positive and PR-negative tumors (p=0.97 and p=0.99, respectively).

Conclusions
The present study demonstrates that 2 years of adjuvant TAM as monotherapy significantly reduced CM and CBCM in premenopausal women with PR-positive tumors as well as CBCM in ER- and PR-positive tumors, after > 25 years of follow-up. Five years of adjuvant TAM have been proven to reduce mortality rates at 15 years, but we herein show that also 2 years of TAM yields a survival benefit at 25 years.

In the latest meta-analysis from EBCTCG, PR did not add predictive information in patients with ER-positive tumors, but according to our results the significance of PR, as a predictive factor for TAM efficacy, should be re-emphasized in premenopausal women.
Title: High-resolution specimen-positron emission mammography (s-PEM) indicates the spread of cancer in breast-conserving surgery

Body: Background: Breast cancer surgery, including breast-conserving surgery or sentinel lymphnode biopsy, has become minimally invasive. Recent consensus guidelines on the margins for breast-conserving surgery have defined a negative margin as one with no ink on tumor, regardless of the distance from the tumor, whereas a positive margin has ink on the tumor. Therefore, the extent of required tumor resection will decrease for cosmeses and expand the indications for breast-conserving surgery. Therefore, there is a real need for a more accurate imaging of tumor spread. In particular, a functional imaging of breast cancer specimens will help determine negative margins in breast-conserving surgery. We collaborated with Sendai Medical Imaging Center (SMIC) who have high-resolution positron emission mammography (PEM) with the novel scintillator Pr3+-doped transparent ceramic Lu3Al5O12 (Pr:LuAG) for the imaging of breast cancer specimen with 18F-fluodeoxyglucose (FDG).

Methods: With the approval of the hospital ethics committee, positron emission tomography (PET) was conducted preoperatively on the day of surgery with 18F-FDG. After the PET results were explained to the patients, breast-conserving surgery was performed in 11 patients from February to July 2014. In the operating room, medical staff exposures were calculated with a portable dosimeter. All of the cases were carried out intraoperative frozen sections that were taken from all sides of the outside of the specimens. The specimens were serially sectioned in 5-mm slices for permanent histology. After the specimens were removed, they were sent to the SMIC and high-resolution specimen-PEM (s-PEM) was conducted. We evaluated the detection rate of s-PEM in in situ or invasive lesions (extent of locations were tolerated up to a 10% error), the predictive value of margin status between s-PEM and frozen section analysis and medical staff exposure.

Results: In 11 breast specimens, eight invasive lesions and 15 in situ lesions (excluding the foci that exist in less than three carcinoma ducts) were confirmed by permanent histology. s-PEM detected all of the invasive lesions and 14 out of 15 in situ lesions. Three breast specimens had positive margins in permanent histology. Two out of three cases with positive margin accumulated 18F-FDG at the edge of the specimens, which were considered positive margins with s-PEM, whereas positive margins were only one out of three of the frozen section analysis. In contrast, a false positive margin was found in only one case with s-PEM, which confirmed the preoperative core-needle biopsy that was diagnosed as an intraductal proliferative lesion. Medical staff exposure was determined for the operator, first assistant, second assistant, anesthesiologist, scrub nurse and circulating nurse as 31, 34, 25, 11, 21 and 6 µSV, respectively.

Conclusion: s-PEM detected not only invasive lesions but also in situ lesions with great accuracy. It might accelerate the decrease in the extent of resection required in breast-conserving surgery. However, further studies are needed to elucidate the characteristics of the false positive cases in s-PEM.
Title: Triple negative breast cancer targeting paramagnetic nanoparticle for non-invasive tumor imaging

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Body: Triple-negative breast cancer (TNBC) refers to a subtype of breast cancer that is negative for estrogen receptors (ER) and/or progesterone receptors (PR), and lacks HER2 overexpression. Therefore, this subtype of breast cancer lacks the benefits of specific therapies which target these receptors. TNBC has been characterized by an acidic extracellular environment. In human aggressive breast tumors, pH has been measured by microelectrode and the values are in good agreement with the values observed in animal systems i.e. that the pH is significantly acidic in the range from 6.2 to 7.0. However, tumor intracellular pH is either neutral or alkaline. Interestingly, a similar pH gradient is not observed in normal tissues. Therefore, this acidic extracellular pH within tumor tissues can be exploited for targeted delivery of drugs and imaging agents. Recently, a pH low insertion peptide (pHLIP) derived from the protein bacteriorhodopsin has been found to target tumor acidic pH. The peptide inserts across cell membranes as an α-helix when extracellular pH (pHe) is acidic, but does not form the helix at normal or alkaline pH. Since aggressive TNBC has an acidic environment, the pHLIP will insert into the cancer cell membrane, but the pHLIP will not insert into the cell membranes of normal tissues, providing excellent specificity for targeting TNBC. Here, we demonstrate that pHLIP-tagged nanoparticles bind to and are internalized by TNBC cells in vitro. Systemic delivery of the Gd-G5-pHLIP leads to accumulation of the nanoparticles in a flank mouse model of TNBC tumor that are detected by optical and MR imaging.

We have synthesized pH-responsive MRI nanoprobe, phosphonate G5-(GdDOTA-4AmP) by following our published synthetic method. The MW of the conjugated G5 dendrimer was estimated at 79,082 g/mole by maldi-tof analysis. This corresponds to a G5-dendrimer with an average of 44 chelated Gd³⁺ ions per dendrimer. Gd44-G5 dendrimer was reacted with heterobifunctional cross-linker (sulfo-SMCC) to form reactive maleimides and then maleimide- Gd44-G5 dendrimer was coupled with C-terminus cysteine group of biotinylated Bt-pHLIP (AEQNPIYWARYADWLFTTPLLLDLALLVDADEGTGC-pegBiotin). The HABA assay with biotin and avidin revealed that on average 3.1 molecules of biotin are present in Gd44-G5-Bt-pHLIP dendrimer. Finally, Rhodamine dye was conjugated to amines surface of preloaded Gd44-G5-Bt-pHLIP3 in order to achieve final conjugate Rhodamine-Gd44-G5-Bt-pHLIP3.

To study pH-dependent translocation of molecules across the cell membrane, we have added Rho-Gd-G5-Bt-pHLIP to the cells and incubated for 3 h at pH 7.4 and 6.5. The cellular uptake of Rho-Gd-G5-pHLIP was significantly higher at pH 6.5. When Rho-Gd-G5 was used, the cellular uptake was considerable lower at both, pH 6.5 and 7.4. Hence, we have shown the ability of pHLIP peptide for intracellular delivery of Gd-G5 nanoparticles in vitro at pH 6.5 but the same ability is attenuated significantly at neutral pH. We have created a mouse model of TNBC MDA-MB-231 tumor. The pharmacokinetics of Gd44-G5-pHLIP was visualized in the MDA-MB-231 tumor over the course of 105 min post-contrast administration.
Title: RAD1901, a novel tissue-selective estrogen receptor degrader (SERD) demonstrates estrogen receptor engagement in a phase 1 clinical study

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Body: Despite advances in the treatment of metastatic breast cancer through modulation of estrogen receptor (ER) activity, after initial efficacy, the use of hormonal therapies is frequently followed by the development of de novo or acquired endocrine resistance. There therefore exists a potentially significant opportunity for new agents that can overcome endocrine resistance. One mechanism by which this could be achieved is through degradation of the estrogen receptor and thereby elimination of estrogen receptor mediated signaling. RAD1901 is a novel, non-steroidal small molecule that selectively binds and degrades the ER that is currently being evaluated for the treatment of metastatic breast cancer. RAD1901 has demonstrated good tissue selectivity in preclinical models, its does not stimulate the uterine endometrium, and it protects against bone loss in an ovariectomy-induced osteopenia rat model. In addition, we believe that RAD1901 has the ability to cross the blood-brain barrier. In vitro, treatment of tamoxifen-sensitive and resistant human breast cancer cell lines with RAD1901 resulted in a potent degradation of the ER (IC50 1.6nM in MCF7 cells) and inhibition of both basal and estradiol-stimulated proliferation. In a mouse xenograft tumor model with MCF7, oral dosing with RAD1901 resulted in a significant decrease in estradiol-stimulated tumor growth. An ongoing phase 1 clinical study in healthy volunteers is being conducted to evaluate the tolerability, safety and pharmacokinetics of RAD1901 at escalating doses. This study is also using 18F-estradiol positron emission tomography (FES-PET) to provide a pharmacodynamic assessment of estrogen receptor engagement/turnover. Following 6-days of daily treatment with RAD1901, at doses that were well tolerated, a complete suppression of FES-PET signal was observed, with standardized uptake values (SUV) comparable to background tissues. The relationship between PK and PD will be presented. To date, the maximum tolerated dose of RAD1901 has not been determined. In conclusion, RAD1901 is a novel SERD that we believe has the potential to be used for the treatment of hormone driven and hormone resistant metastatic breast cancers, including breast cancer brain metastasis. The proposed phase 1b study design in metastatic breast cancer will be outlined.
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Title: Detrimental effects of sequential compared to concurrent treatment of pertuzumab plus T-DM1 in HER2+ breast cancer cell lines

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Body: Background. Pertuzumab and T-DM1 are two recently approved monoclonal antibody based therapies targeting HER2+ breast cancer. Pertuzumab interferes with dimerization of HER family members, while T-DM1 binds to HER2 and interferes with its oncogenic function while also specifically delivering a cytotoxic agent (emtansine). One arm of the I-SPY 2 clinical trial is to investigate the efficacy of a combination Pertuzumab plus T-DM1 in HER2+ breast cancer patients. Methods. We performed pre-clinical screening of response to each agent alone and in combination in a set of 21 HER2+ breast cancer cell lines, with an end goal of identifying markers of response to the therapies. There were five treatment regimens employed in the initial screen: i) pertuzumab alone for 72 h; ii) T-DM1 alone for 72h; iii) pertuzumab plus T-DM1 concurrently for 72h; iv) pertuzumab for 24h followed by addition of T-DM1 for 48h more; and iv) T-DM1 for 24h followed by addition of pertuzumab for 48h more. Response was assessed using the Cell Titer Glo assay as a measure of cell viability. To assess the effects of drug combinations, we used a stringent measure of synergy and antagonism employing the median effect method of Chou and Talalay that included 95% confidence intervals to determine significance. Results. Initial screens showed that concurrent treatment of cells with pertuzumab plus T-DM1 gave significant synergistic interactions in 15/21 cell lines as measured by the median effect method, with combination indices (CI) less than 0.5 (and 95% upper confidence levels less than 1.0) for at least one drug concentration. However, 24h pretreatment with pertuzumab followed by T-DM1 significantly diminished the response of cells to T-DM1, resulting in significant antagonism in 17/21 cell lines test (CI>1.5, lower confidence level greater than 1). Since this could be due to a shorter exposure time to T-DM1, and since patients are scheduled to be treated with pertuzumab first followed by T-DM1 one hour later, we repeated the experiment with one hour between pertuzumab and T-DM1 rather than 24h. While the inhibitory effect was diminished, this treatment regimen still resulted in significant antagonism when T-DM1 was given 1 hour after pertuzumab in 5/5 cell lines tested, in contrast to concurrent pertuzumab plus T-DM1 treatment, which showed synergy. Conclusions. Pertuzumab plus T-DM1 appears to be beneficial when given concurrently, but pretreatment with pertuzumab appears to blunt the efficacy of T-DM1. This has important potential ramifications for patient treatment, and may further elucidate mechanisms of action for both compounds. Further testing will be necessary to determine whether these timing effects are operational in vivo and whether immune effects mitigate the antagonism.
Title: Blocking a key region in the HER2 subdomain III inhibits the HER2-network in patients with resistance to HER2-targeted therapy

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Body: The HER2 receptor is over-expressed in about 25% of breast carcinomas and correlates with more aggressive breast cancer disease, poor prognosis as well as resistance to chemo- and endocrine therapies. The strong correlation between HER2 levels/activity and the malignancy of the disease made it a preferred target for anti-cancer therapy. For example, Trastuzumab, which targets HER2 homo-dimerization and Pertuzumab, which interferes with HER2 hetero-dimerization, are in clinical use. Unfortunately, Trastuzumab monotherapy is only partially effective and the majority of breast cancer patients who initially respond to Trastuzumab rapidly develop resistance to the drug. Thus, novel strategies/agents are in need, especially drugs that prevent hetero-dimerization with other HER family members.

Structural analyses revealed that sub-domain III in the extracellular domain (ECD) of the HER family members is responsible for dimerization. Therefore, we hypothesized that disabling the dimerization loop in the HER2-ECD sub-domain III would ultimately convert HER2 to a non-functional receptor. Therefore, we introduced a series of deletions in the HER2-ECD sub-domain III and determined their oncogenic properties compared to HER2wt expressing cells. Importantly, a small deletion (16 amino acids) of the HER2 extracellular domain (named HER2Δ6) abolished its homo- and hetero-dimerization and profoundly affected HER2-catalyzed activation of the HER network, both in the context of HER2 over-expression and ligand-induced trans-activation of HER2. Expression of the HER2Δ6 variant failed to promote anchorage-independent growth and interfered with the activation/Tyr phosphorylation of HER1, HER2 and HER3. Moreover, this mutant failed to promote resistance to Paclitaxel treatment in HER2-overexpressing breast cancer cells.

To determine the molecular mechanisms underlying this behavior, we assessed the mutant and wt expressing cells lines morphologically and biochemically and demonstrated that the HER2Δ6 is absent from the plasma membrane (PM), most likely due to a intracellular trafficking defect that mistargets the receptor to or traps it in an endomembrane compartment. Compared to HER2wt-expressing cells, the HER2Δ6 mutant proves to be even more effective in inhibiting the oncogenic properties of the receptor than the current drugs of choice such as Trastuzumab, Pertuzumab and Cetuximab alone or in combination as measured by anchorage-independent growth.

These findings reveal that the HER2-ECD bears an essential "activating" region that is indispensable for HER2 homo-and heterodimerization. Elimination of this "activating" element in HER2 seems to recapitulate and greatly improve the combined actions of Trastuzumab and Pertuzumab. These findings offer a strong rationale for developing this peptide sequence into a valuable anti-HER2 therapeutic drug.
Title: PI3K inhibitor LY294002 combined with RAD001, a mTOR specificity inhibitor, significantly reduced proliferation and induced apoptosis of triple negative breast cancer cell lines MDA-MB-231 through PI3K/Akt/mTOR pathway in vitro

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Body: Background and purpose Research of targeted therapies about PI3K/Akt/mTOR pathway have became a hotspot recently, and related researches have been extended from a single-target suppression to multiple-targets combined inhibition. In this study, by using LY294002 and RAD001, the specific inhibitors of PI3K and mTOR, to observe whether the anti-tumor effects on the proliferation, cycle distribution and apoptosis of combined inhibitors were a synergistic effect, and were differences between different molecular characteristics of human breast cancer cells alone and in combination in vitro. Materials and Methods Routinely cultured MCF-7, SK-BR-3 and MDA-MB-231 cells in vitro. Logarithmic phase cells among each cells were selected and divided into the blank control group, LY294002 group, RAD001 group and the combination group. MTT assay and flow cytometry were used to detect the cell proliferation, cell cycle distribution and cell apoptosis of different groups. Results (1) LY294002 and RAD001 could significantly inhibit the proliferations of MCF-7, SK-BR-3 and MDA-MB-231 cells with a dose-dependent manner respectively (P<0.05), and compared with other cells, MDA-MB-231 cells were more sensitive to both drugs (P<0.05). The anti-tumor effects were significantly increased in combination groups of different cells, and showed an additive effects. (2) MCF-7, SK-BR-3 and MDA-MB-231 cells could be arrest in G1 phase by IC50 of LY294002 and RAD001 respectively(P <0.05), and the effects of combination groups were more significantly compared with monotherapy groups(P <0.05). However, no significant differences among different cells. (3) LY294002 and RAD001 could significantly increase the apoptosis rates of human MCF-7, SK-BR-3 and MDA-MB-231 cells alone (P <0.05), but there were no differences between different cells. When two inhibitors were combined, the apoptosis rates of the different cells were significantly increased compared with single drug, especially in MDA-MB-231 cell. The apoptosis rates of MCF-7, SK-BR-3 and MDA-MB-231 cells were 17.58%, 44.28% and 52.67% respectively, and there were significantly different between different cell lines (P <0.05).

Effects of LY294002 and RAD001 on the apoptosis rates of human breast cancer cell lines MCF-7, SK-BR-3 and MDA-MB-231 alone or in combination in vitro

<table>
<thead>
<tr>
<th>CELL LINES</th>
<th>APOPTOSIS RATES</th>
<th>BLANK GROUP</th>
<th>COMBINATION GROUP</th>
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<tbody>
<tr>
<td></td>
<td>LY294002</td>
<td>RAD001</td>
<td></td>
</tr>
<tr>
<td>MCF-7</td>
<td>12.10%</td>
<td>9.22%</td>
<td>3.45%</td>
</tr>
<tr>
<td>SK-BR-3</td>
<td>9.64%</td>
<td>11.58%</td>
<td>4.20%</td>
</tr>
<tr>
<td>MDA-MB-231</td>
<td>10.11%</td>
<td>12.54%</td>
<td>2.41%</td>
</tr>
</tbody>
</table>

Conclusion Targeted therapy on P13K/Akt/mTOR pathway could significantly inhibit the proliferation of human breast cancer cell lines by inducing apoptosis and arresting cell cycle distribution. By using a combination of different inhibitors which were targeted on different related genes of this pathway such as PI3K and mTOR, the anti-tumor effects were more significant increased compared with monotherapy, especially in MDA-MB-231 cell line which were negative with ER, PR and HER-2. It was important to provide new research ideas for individualized treatment and translational medicine of breast cancer.
Title: Tumor infiltrating lymphocytes and correlation with outcome in the Cher-LOB study

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Body: Background: Tumor infiltrating lymphocytes (TIL) are emerging as a strong prognostic and predictive factor for breast cancer, especially for the HER2-positive and triple negative subtypes (Loi S, Ann Oncol 2014; Dieci MV, Ann Oncol 2014). Here we report the results of the TIL biomarker analysis performed in the CherLOB study.

Methods: The phase II neoadjuvant CherLOB study (Guarneri, J Clin Oncol 2012) randomized 121 HER2-positive, stage II-IIIA breast cancer patients to anthracyclines/taxane-based chemotherapy plus trastuzumab (arm A), lapatinib (arm B), or both (arm C). Primary endpoint was pathological complete response (pCR). Hematoxylin and eosin-stained slides from both pre-treatment biopsies and post-treatment surgical samples were centralized and evaluated for the % of intratumoral (It) and stromal (Str) TIL as previously described (Denkert C, J Clin Oncol 2010). Samples were classified as lymphocyte-predominant (LP) if ItTIL and/or StrTIL $\geq$60% and as non-LP if ItTIL and StrTIL <60%.

Results: Pre-treatment TIL evaluation was available for 105 of the 118 CherLOB patients who were assessable for pathological response. Both ItTIL and StrTIL as continuous variables (per 10% increase) were associated with a higher probability of achieving a pCR (adjusted OR: 2.64, 95%CI 1.46-4.79, $p=0.001$ and 1.32 95%CI 1.08-1.6, $p=0.006$ for ItTIL and StrTIL, respectively). pCR rates were significantly higher in LP compared to non-LP cases (59% vs 27%, $p=0.011$). According to treatment, TIL effect was more evident in patients treated with HER2 double-blockade (arm C). According to estrogen receptor (ER) status, no difference in pCR rates between LP and non-LP cases was observed in the ER-positive population, whereas pCR rate was more than doubled for ER-negative LP compared to ER-negative non-LP patients (table 1).

<table>
<thead>
<tr>
<th></th>
<th>$n$ tot</th>
<th>pCR rate %</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>overall</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>17</td>
<td>59</td>
<td></td>
</tr>
<tr>
<td>non-LP</td>
<td>88</td>
<td>27</td>
<td>0.011</td>
</tr>
<tr>
<td>Arm A</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LP</td>
<td>5</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>non-LP</td>
<td>27</td>
<td>26</td>
<td>0.52</td>
</tr>
<tr>
<td>Arm B</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>6</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>non-LP</td>
<td>28</td>
<td>21</td>
<td>0.15</td>
</tr>
<tr>
<td>Arm C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LP</td>
<td>6</td>
<td>83</td>
<td></td>
</tr>
<tr>
<td>non-LP</td>
<td>33</td>
<td>33</td>
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</tr>
<tr>
<td>ER+</td>
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<tr>
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<td>6</td>
<td>33</td>
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<tr>
<td>non-LP</td>
<td>59</td>
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<tr>
<td>ER-</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>LP</td>
<td>11</td>
<td>73</td>
<td></td>
</tr>
<tr>
<td>non-LP</td>
<td>29</td>
<td>31</td>
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</tr>
</tbody>
</table>

Overall, 71 of the 121 CherLOB patients had residual disease at surgery: for 54 of them, paired pre-treatment and post-treatment TIL were available. No significant changes in ItTIL and StrTIL levels were observed before and after treatment. However, six
cases presented a LP phenotype on the residual disease; all but one of them started from a non-LP pre-treatment phenotype and received lapatinib as part of the neoadjuvant treatment (4 arm B, 1 arm C).

Conclusions: In this analysis, TIL predicted the achievement of pCR for early HER2-positive patients undergoing neoadjuvant chemotherapy plus anti-HER2 agents. TIL predictive effect seems limited to ER-negative patients. Combinations of chemotherapy plus anti-HER2 agents containing lapatinib may be able to convert a non-LP into a LP tumor. Updating of follow-up is ongoing, correlations between TIL and survival will be presented at the meeting.
Title: Impact of tumor-infiltrating B-cell clonal diversity on response to neoadjuvant therapy in triple negative and HER2+ breast cancer treated on CALGB (Alliance) 40601 and 40603

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Body: Background: Tumor infiltrating lymphocytes (TILs) are associated with improved outcomes in breast (BrCa) and ovarian cancer (OvCa). This benefit is largely restricted to the basal-like and HER2-enriched subtypes of BrCa and the immunoreactive subtype of OvCa. It is not known whether TILs respond to a small subset of antigens, similar to an antiviral or antibacterial response, or if the response is nonspecific. We developed a novel method to assess B-cell population diversity by analyzing B-cell receptor (BCR) sequence complexity in mRNA-seq datasets derived from tumor biopsies. B-cells in a subset of basal-like and HER2-enriched BrCa showed high expression of immunoglobulins coinciding with reduced BCR diversity consistent with a restricted epitope-driven immune response. Analysis of DNA patterns from B-cells in basal-like and HER2-enriched BrCa showed a greater prevalence of BCR somatic hypermutation (SHM) suggestive of an antigen-restricted response (Iglesia et al, CCR 2014). Here, we studied the impact of this adaptive immune response on treatment response.

Methods: Using two neoadjuvant cooperative group trials in triple-negative (TNBC) and HER2-positive (HER2+) BrCa, we evaluated BCR diversity (as assessed by SHM diversity) as a continuous variable and as a binary variable (diverse/restricted) relative to BCR expression (the Restriction Index) in pre-treatment tumor samples from 265 patients with HER2+ BrCa treated on CALGB 40601, a randomized phase III trial of paclitaxel plus trastuzumab +/- lapatinib, and 443 patients with TNBC treated on CALGB 40603, a randomized phase II trial of standard chemotherapy +/- carboplatin and/or bevacizumab. We examined the relationship between a restricted immune response and pCR rate, the primary endpoint of both studies, overall and within molecular subtypes.

Results: In HER2+ BrCa, the combination of high immunoglobulin expression and lower sequence diversity (high Restriction Index) was observed in 28% of the pre-treatment biopsies, and varied by intrinsic subtype, with the greatest prevalence in the HER2-enriched subset (n=80, 46% vs 20% in all others). BCR restriction predicted improved pCR rates in all patients (67% versus 37%, p <0.0001). It remained significant in the HER2-enriched subset (n = 82, p=0.0086). The impact of Restriction Index on response to chemotherapy in TNBC is being analyzed and will be presented along with multivariate models to adjust for other patient and disease characteristics and explore potential interactions.

Conclusions: The presence of a restricted diversity B-cell response in HER2+ breast cancer correlates with improved response to neoadjuvant chemotherapy plus HER2-targeted therapy, which may in part explain its impact on prognosis. We will determine if a similar correlation exists with chemotherapy response in TNBC. This suggests that immunomodulatory therapies supporting a B-cell response may be a promising therapeutic approach to targeting these tumors.
Title: The significance of tumor infiltrating lymphocyte density, subset composition and organization in breast cancer

Karen Willard-Gallo1, Laurence Buisseret1, Soizic Garaud1, Chunyan Gu-Trantien1, Alexandre de Wind1, Sébastien Duquenne1, Denis Larsimont1, Christos Sotiriou1 and Martine Piccart1. 1Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium.

Body: The clinical relevance of tumor infiltrating lymphocytes (TIL) in breast cancer (BC) has been clearly established by recent large clinical trials (presented at SABCS 2013). The relationship between protective immunity, observed in some patients, and critical features of lymphoid subset composition and organization remain unknown. Our recent work revealed that tertiary lymphoid structures (TLS), principally composed of T cells, B cells and dendritic cells, are present in the peri-tumoral regions of breast tumors and associated with a CD4+ follicular helper T (Tfh) cell presence. Through retrospective analyses, we determined that TLS were associated with good clinical outcomes in both the neo-adjuvant and adjuvant settings. To gain insight into the immune components linked with a significant TIL and TLS presence, we initiated a prospective flow cytometric study. We systematically immunophenotyped T and B cell TIL in breast tissues from tumors (n=125), non-adjacent non-tumor tissues (NANT, n=115) and normal tissue from mammary reductions (n=26) on the day of surgery. TIL organization and spatial distribution was subsequently analyzed by immunohistochemistry (IHC) and immunofluorescence (IF) on paraffin sections for a subset of patients (n=78). The fresh tissue analyses revealed that TIL density was a continuum across the 125 patients analyzed. A cutoff for TIL-positive and -negative tumors was set at 58 CD45+ TIL per mm3 of tissue based on the number of CD45+ cells present in the remarkably similar normal and NANT tissues. Applying this threshold to BC, 65% were TIL-positive with approximately one-third considered extensively infiltrated. TIL-positive tumors are characterized by an increase in CD4+ T cells and CD19+/CD20+ B cells. The median CD4/CD8 ratio was >1 in TIL-positive compared to <1 in TIL-negative tumors and NANT. CD4+ and CD8+ T cells were predominantly CD45RO+ memory cells, with a significant proportion expressing PD-1. Infiltrating B cells were approximately 50% memory cells in contrast to <15% in normal and NANT tissues. Extensively infiltrated tumors were more frequently associated with Tfh and follicular B cells resident in TLS. IF analysis by confocal microscopy found that TLS resident cells included specific lymphocyte subsets: marginal B cells (CD20+CD27+IgD−), follicular mantle B cells (CD20+IgD+), germinal center B cells (CD20+Ki67+CD35+CD21+PD-1+) and germinal center Tfh cells (CD4+CD200+TIGIT+CXCL13+) in a B cell zone surrounded by a T cell zone containing CD4+ and CD8+ T cells. To determine whether the flow cytometry data was correlated with routine pathology, sections of the same tumors were stained by H&E and CD45 (total leukocytes) or CD3 plus CD19 (T cells + B cells; >95% of CD45+ cells) IHC and scored by trained pathologists. The best correlation was observed between CD3/CD20 flow cytometry and CD3/CD19 IHC, the latter also associated with lower inter-observer variability. TIL density was positively correlated with proliferation (Ki67 & histological grade) and hormone receptor negativity while TLS were more frequent in younger women. These data suggest that organized immune responses in TLS adjacent to the tumor bed provide an effective location for generating anti-tumor memory T and B cell responses.
Somatic leukemogenic mutations associated with infiltrating white blood cells in breast cancer patients

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Body: Background: In the last few decades, theoretical models of cancer growth and progression have long focused on the aberrations of cancer cells alone, such as the abnormal mitotic and invasive characteristics of cancer cells. More recent research across multiple solid tumors suggests a critical interplay between solid tumors and immune regulating cells. Mounting evidence suggests that the immune system can tip the scales of cancer progression, eliciting either an anti-tumor or pro-tumor immune response depending upon varying stimulating and inhibitory factors. Here, we are the first to demonstrate novel mutations including leukemogenic mutations among tumor infiltrating lymphocytes in breast cancer patients.

Methods: We obtained 17 primary breast cancer samples from patients who presented for either a lumpectomy or mastectomy as part of an IRB approved biospecimen protocol. Of the 17 patient samples, 13 had triple negative breast cancer, 2 had ER+, HER2+ disease, and 2 had ER+, HER2- disease. In the 17 samples, we used fluorescent activated cell sorting to separate CD45-positive hematopoietic cells from CD45-negative epithelial cells. We then performed exome sequencing of tumor-infiltrating hematopoietic cells to investigate for the presence of pathogenic mutations in tumor-associated leukocytes. In this first step, we identified candidate mutations in known cancer genes, including BCOR, NOTCH2, TET2, NF1, EZH2, and JAK1. As a validation step, we then performed capture-based sequencing of tumor-infiltrating leukocytes in 20 breast cancer samples matched to each patient’s germline DNA sample (buccal swab). In 10 of the 20 patients, we identified and validated somatic mutations. Of note, 6 of these patients harbored mutations known to be associated with leukemia, including DNTM3A, TET2, and BCOR. Most of these mutations were present in at least 5-20% of reads. This suggests that these mutations were present in enriched subclones and were not rare alleles occurring in a minority of hematopoietic stem cells. Lastly, we performed 454 deep sequencing analysis of microdissected tumor DNA samples and confirmed the absence of these mutations in breast cancer cells.

Conclusion: Our data demonstrate somatic mutations in tumor infiltrating leukocytes in breast tumors which were not identified in matched germline or tumor DNA samples. Notably, some of these mutations have been implicated in the pathogenesis of lymphoid and myeloid malignancies. This observation suggests a unique relationship between cancer cells and mutant infiltrating leukocytes. We are now investigating the functional interaction between cancer cells and hematopoietic cells. Our findings reframe our understanding of carcinogenesis and offer novel opportunities for cancer detection and treatment.
Title: Characterizing the Tumor Immune MicroEnvironment (TIME) in high-risk ductal carcinoma in situ

Michael J Campbell¹, Rita Mukhtar¹, Ekene Obi-Okoye¹, Booyeon Han¹, Vickram Tandon¹, Sarah Zheng¹, Zelos Zhu¹, Max Endicott¹, Max Wicha², Linda Lindstrom¹, Alfred Au¹, Frederick Baehner¹, Joe Gray³ and Laura Esserman¹. ¹University of California, San Francisco, CA; ²University of Michigan, Ann Arbor, MI and ³Oregon Health & Science University, Portland, OR.

Body: Background: Ductal carcinoma in situ (DCIS) of the breast is a premalignant condition. Although DCIS is treated as an obligate precursor of invasive ductal carcinoma, the rate and latency of progression from DCIS to invasive breast cancer (IBC) in the absence of treatment are unknown. DCIS is not one condition, but rather a spectrum of disease and although DCIS itself is not a lethal condition, women with DCIS are at higher risk of developing subsequent IBC over a time period of 1-20 years depending on DCIS subtype. Features of DCIS that are associated with high risk of recurrence include large size (> 5cm), high grade, comedo necrosis, palpable mass, hormone receptor (HR) negativity, and HER2 positivity. The objective of this study was to characterize the tumor immune microenvironment (TIME) of these high-risk DCIS lesions.

Methods: Forty-eight cases of high grade DCIS, enriched for large, confluent lesions and history of recurrence were age matched with 64 cases of non-high grade DCIS. IHC analyses were performed as single or two-color stains for the following antigens: CD68, CD8, CD4, CD20, HLA-DR, CD115, FoxP3, PCNA, Mac387, MRC1, ALDH, CD24, CD44, Ki-67, and HER2. HR status was determined from ER and PR staining results in pathology reports. A Nuance multispectral imaging system was used to image and spectrally unmix each stain. Protocols for automated image analysis were developed using CellProfiler software. Associations between immune cell populations and clinical parameters (tumor palpability, recurrence, HR status, HER2 status, and Van Nuys score [12-point scale: margins, age, size, grade]) were identified with non-parametric Spearman correlation tests.

Results: We found a high macrophage infiltrate associated with a high Van Nuys score, palpability, and high Ki-67. High CD115 (CSF-1 receptor) was associated with HER2+, high Ki-67, and recurrence. Mac387+ cells and FoxP3+ regulatory T cells (Treg) were associated with high Van Nuys score, comedo necrosis, high Ki-67, HR- and HER2+. Interestingly, both Mac387 and CD115 were expressed on tumor cells as well as macrophages and high CD115 staining on tumor cells was associated with recurrence. The presence of CD8+HLA-DR-negative T cells throughout a section was associated with high Van Nuys score, HR-, HER2+, and recurrence. In contrast, CD8+ T cells within the nests of tumor cells were negatively associated with Van Nuys score, palpability, and comedo necrosis. A tumor immune microenvironment score (TIME score) was developed based on the proportions of various immune cell populations. A high TIME score was significantly associated with high Van Nuys scores as well as with recurrence.

Summary: These results demonstrate that high risk DCIS features (palpability, high Van Nuys score, high proliferation, HR-, HER2+, and increased recurrence) are associated with a suppressed tumor immune micro-environment (high FoxP3+ cells, CD68+Mac387+ cells, CD8+HLA-DR-neg T cells, and upregulated CD115). These high risk lesions truly represent an opportunity to prevent cancer. Identifying these high risk lesions with the help of tumor immune microenvironment markers and manipulating the DCIS TIME via local or systemic immunotherapeutic strategies may represent an ideal preventative intervention.
Inhibition of PD-L1 by MPDL3280A leads to clinical activity in patients with metastatic triple-negative breast cancer

Leisha A Emens¹, Fadi S Braiteh², Philippe Cassier³, Jean-Pierre DeLord⁴, Joseph Paul Eder⁵, Xiaodong Shen⁶, Yuanyuan Xiao⁶, Yan Wang⁶, Priti S Hegde⁶, Daniel S Chen⁶ and Ian Krop⁷. ¹Johns Hopkins University, Baltimore, MD; ²Comprehensive Cancer Centers of Nevada, Las Vegas, NV; ³Centre Léon Bérard, Lyon, France; ⁴Institut Claudius Regaud, Toulouse, France; ⁵Yale School of Medicine, New Haven, CT; ⁶Genentech, San Francisco, CA and ⁷Dana-Farber Cancer Institute, Boston, MA.

Body: Background: Metastatic triple-negative breast cancer (TNBC) is associated with a poor prognosis and has no targeted therapy options. Programmed death-ligand 1 (PD-L1) expression is more prevalent in TNBC than in other breast cancer subtypes. PD-L1 may protect cancer cells from immune-mediated destruction by binding to its receptors PD-1 and B7.1. MPDL3280A, a human anti-PD-L1 monoclonal antibody with an engineered Fc-domain designed for optimized efficacy and safety, blocks PD-L1 activity and restores tumor-specific T-cell immunity.

Methods: This multicenter Phase I study selectively enrolled a cohort of patients with PD-L1-positive metastatic TNBC. PD-L1 positivity was centrally evaluated based on the PD-L1 immunohistochemistry (IHC) status of tumor-infiltrating immune cells (ICs). Eligible patients received MPDL3280A 15 or 20 mg/kg IV every 3 weeks for up to 1 year. The objective response rate (ORR) was assessed by RECIST v1.1. MPDL3280A immune correlates were evaluated for tumor and circulating biomarkers. The clinical data cutoff was January 1, 2014.

Results: Twelve patients were treated with MPDL3280A and evaluable for safety. Patients had ECOG PS 0-1 (33% ECOG PS 1), and the median age was 55 years (range, 29-72 years). All 4 patients (33%) with visceral metastases had liver metastases, and 1 patient (8%) had bone metastases. 92% of patients received ≥ 2 prior therapies. Prior chemotherapies included anthracycline (92%), taxane (75%) and platinum (42%). Grade 3-4 treatment-related adverse events (AEs) occurred in 8% of patients (1 event of Grade 3 adrenal insufficiency). One patient had an immune-related AE (Grade 2 pyrexia). No treatment-related deaths were observed. Nine patients were dosed by November 16, 2013 and evaluable for efficacy. The ORR was 33%, including 1 CR and 2 PRs. Responders included patients with visceral metastases at baseline. At the time of the clinical data cut-off, all responses occurred within 6 weeks of the first dosing of MPDL3280A, and all of the responses were ongoing. The median duration of response had not been reached. One patient achieved stable disease as best response. Two additional patients had tumor shrinkage (-43% and -44% change in target lesions, respectively) but were not counted as RECIST responders due to the appearance of new lesions, which is likely attributable to pseudoprogression. Preliminary pharmacodynamic biomarkers related to MPDL3280A activity, including circulating plasma markers and tumor immunomonitoring with CD8 IHC, will be presented along with updated clinical data.

Conclusions: MPDL3280A treatment was well tolerated and was associated with objective clinical activity in patients with pretreated metastatic TNBC. Further evaluation of the safety and clinical activity of MPDL3280A in both PD-L1-positive and PD-L1-negative metastatic TNBC is ongoing. Clinical trial information: NCT01375842.
Title: Differential expression of innate and adaptive immune responses in TNBC outcome

Yesim Gokmen-Polar1, Xianyin Lai1 and Sunil Badve1. 1Indiana University School of Medicine, Indianapolis, IN.

Body: Introduction:
Clinical management of patients with triple-negative breast cancers (TNBCs) presents a significant challenge. TNBCs tend to relapse early and exhibit poor prognosis compared to other breast cancer subtypes. Recent studies emphasize the influence of the immune system in prognosis of TNBCs. In particular, the percentage of tumor infiltrating lymphocytes (TILs) is associated with breast cancer prognosis and outcomes after conventional therapy in triple negative tumors. However, the biology underlying this immune response is unclear.

Methods:
To identify differential expression of key immune response markers in TNBC with clinical outcome, we have performed a three-step approach as follows: 1) quantitative LC-MS/MS proteomic analysis using 16 formalin-fixed paraffin-embedded (FFPE) triple negative tumors with prognostic outcome and Ingenuity Pathway Analysis (IPA) performed to determine the biological processes and networks, 2) in silico analysis of prognostic value (10 year- DFMS) with the expression levels of 96 immune response genes using large cohorts of publicly available gene expression datasets (11 Affymetrix datasets), and 3) integrating the quantitative LC-MS/MS proteomic analysis with the in silico analysis to further understand the relation between the type of immune response including innate and adaptive immune responses.

Results:
LC-MS/MS proteomic analysis identified and quantified a total of 1,560 proteins of which 254 were differentially expressed in TNBC tumors with poor prognosis compared to good prognosis (P≤0.05). IPA analysis revealed inflammatory response and immunological processes as top differentially regulated biological functions in these tumors [P ranging between 7.41E-10 - 8.05E-03 (53 proteins) and 2.59E-09 - 5.80E-03 (69 proteins), respectively)]. The top altered immunologic networks included "Immune cell trafficking" and "cell-mediated immune response". Among the differentially regulated markers from both analyses, osteopontin (OPN or SPP1), STAT1, and MX1 remained highly significant in both proteomic and in silico analyses (P<0.008). Interestingly, many components of the innate immunity (i.e TLRs) and adaptive immunity (Th2 response, Th17, T-reg, T cell activation, and related cytokines) did not reach statistical significance in combinatorial analysis.

Conclusion:
Integrated in silico and proteomic analysis provides clues regarding the relative importance of immune pathways associated with response in breast cancer. This will enable targeting of immune therapies to improve the functionality of the pathways and ultimately in choosing the right patient TNBC subgroup for potential therapeutics, thereby improving the clinical outcome.
2014 San Antonio Breast Cancer Symposium

Publication Number: PD2-1
Average Grade: 5.00

Title: The effect on overall and disease-free survival (OS & DFS) by adding bevacizumab and/or antimetabolites to standard neoadjuvant chemotherapy: NSABP Protocol B-40

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Body: Purpose
The NSABP B-40 study was designed to determine whether the addition of capecitabine (X) or gemcitabine (G) to neoadjuvant docetaxel (T) followed by AC increased pathologic complete response (pCR) rates and improved outcomes of women with operable, HER2-negative breast cancer. In addition, B-40 was to determine whether the addition of neoadjuvant plus adjuvant bevacizumab (Bev) to T-based regimens followed by AC would increase pCR and improve outcomes. The pCR results and toxicities have been reported previously. The DFS and OS results are reported here for the first time.

Methods
Patients (Pts) received one of three T-based regimens for 4 cycles: T 100 mg/m2 day 1; T 75 mg/m2 day 1 and X 825 mg/m2 BID days 1-14; or T 75 mg/m2 day 1 and G 1000 mg/m2 days 1 and 8. Pts then received preoperative AC x 4 cycles. Pts randomly assigned to the Bev groups received Bev at 15mg/kg, q3wks x 6 with the first 6 courses of neoadjuvant chemotherapy and were to resume Bev post-operatively for 10 doses. 1,206 pts were assigned and 1,163 of them were clinically eligible and had follow-up. The cutoff date for data presented here was March 31, 2014. The median follow-up was 4.7 years.

Results
Neither X nor G added to neoadjuvant chemotherapy increased DFS or OS. 50% of Bev pts for whom treatment data were available (577) completed 10 doses of post-op Bev; 26% did not start Bev post-op. Addition of Bev significantly increased OS (HR=0.70, p=0.01) and marginally increased DFS (HR=0.81, p=0.06). The effect of Bev was most notable in women with hormone receptor positive (HR+) breast cancers (DFS HR=0.71, p=0.04; OS HR=0.63, p=0.03).

Conclusions
Addition of G or X to neoadjuvant T + AC had no significant impact on DFS or OS. Bev added to neoadjuvant chemotherapy and continued post-operatively marginally increased DFS in the overall cohort, with a significant increase in DFS in the HR+ subset. The addition of bevacizumab significantly improved OS for women with operable HER-2-negative breast cancer. In agreement with previously reported results for pCR, the improvement in DFS and OS was seen preferentially in women with HR+ breast cancers.

Support
NCI PHS grants U10-CA-37377, -69974, -12027, -69651, -44066, and -44066-26, Genentech Inc, Roche Laboratories Inc, Eli Lilly, and Precision Therapeutics, Inc.
**Title:** Final efficacy and updated safety results of the randomized phase III BEATRICE trial evaluating adjuvant bevacizumab (BEV)-containing therapy for early triple-negative breast cancer (TNBC)

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**Body:**

**Background:** In the metastatic and neoadjuvant settings, BEV improves PFS and pCR rates, respectively, when combined with chemotherapy (CT) for breast cancer. However, in the adjuvant setting invasive disease-free survival (IDFS; primary outcome measure) was not improved by adding 1 year of BEV to CT in the open-label randomized phase III BEATRICE trial in early TNBC (stratified hazard ratio [HR] 0.87 [95% CI 0.72–1.07]). We report final efficacy and updated safety results.

**Methods:** Eligible patients (pts) had centrally confirmed triple-negative and/or basal-like operable primary invasive breast cancer (pT1a–pT3). Investigators selected anthracycline (anth)- and/or taxane (tax)-based CT for each pt. After definitive surgery, pts were randomized 1:1 to receive ≥4 cycles of either CT alone or the same CT + 1 year of BEV (5 mg/kg/wk equivalent). Stratification factors were nodal status (0 vs 1–3 vs ≥4 involved nodes), selected CT (anth vs tax vs both), hormone receptor status (negative vs low), and surgery (breast conserving vs mastectomy). Secondary outcome measures included overall survival (OS) and safety (CTCAE v3.0). Final OS analysis was prespecified after median follow-up of ∼5 years, ∼76 mo after the first pt was randomized, or after 340 deaths, whichever occurred first.

**Results:** At the data cut-off (June 30, 2014), median follow-up was 56 mo; 293 of 2591 randomized pts had died (86% of estimated events for the final analysis). There was no statistically significant difference in OS between treatment arms either overall or in prespecified subgroups (Table). 5-year OS rates were 88% (95% CI 85.7–89.6%) with CT alone and 88% (95% CI 86.0–89.8%) with CT–BEV. Updated IDFS results (exploratory analysis) were consistent with the primary IDFS analysis. 5-year IDFS rates were 77% (95% CI 74.4–79.4%) with CT alone vs 80% (95% CI 77.2–81.9%) with CT–BEV. New grade ≥3 AEs occurred in 4.6% and 4.5% of pts, respectively, in the period from 18 mo after first study dose to study end.

<table>
<thead>
<tr>
<th>Category</th>
<th>Subgroup</th>
<th>OS HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>All (N=2591)</td>
<td>0.94 (0.75–1.18)</td>
</tr>
<tr>
<td>Age, y</td>
<td>&lt;40 (N=484)</td>
<td>0.88 (0.51–1.54)</td>
</tr>
<tr>
<td></td>
<td>40–&lt;65 (N=1868)</td>
<td>0.89 (0.68–1.17)</td>
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<tr>
<td></td>
<td>≥65 (N=239)</td>
<td>1.50 (0.73–3.05)</td>
</tr>
<tr>
<td>ECOG PS</td>
<td>0 (N=2388)</td>
<td>0.98 (0.77–1.25)</td>
</tr>
<tr>
<td></td>
<td>1 (N=192)</td>
<td>0.68 (0.31–1.49)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td>Post (N=1250)</td>
<td>1.16 (0.83–1.62)</td>
</tr>
<tr>
<td></td>
<td>Pre (N=1341)</td>
<td>0.79 (0.58–1.08)</td>
</tr>
<tr>
<td>Tumor size, cm</td>
<td>0–&lt;2 (N=939)</td>
<td>1.17 (0.69–1.98)</td>
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<td>---------------------</td>
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<tr>
<td></td>
<td>≥2–5 (N=1514)</td>
<td>0.93 (0.70–1.23)</td>
</tr>
<tr>
<td></td>
<td>≥5 (N=132)</td>
<td>0.84 (0.45–1.58)</td>
</tr>
<tr>
<td>No. of positive lymph nodes</td>
<td>0 (N=1640)</td>
<td>0.79 (0.56–1.13)</td>
</tr>
<tr>
<td></td>
<td>1–3 (N=638)</td>
<td>1.01 (0.65–1.56)</td>
</tr>
<tr>
<td></td>
<td>≥4 (N=313)</td>
<td>1.14 (0.75–1.73)</td>
</tr>
<tr>
<td>Adjuvant CT</td>
<td>Anth (N=947)</td>
<td>0.83 (0.54–1.28)</td>
</tr>
<tr>
<td></td>
<td>Anth + tax (N=1508)</td>
<td>1.03 (0.78–1.37)</td>
</tr>
<tr>
<td></td>
<td>Tax (N=136)</td>
<td>0.63 (0.24–1.66)</td>
</tr>
<tr>
<td>Hormone receptor status</td>
<td>ER and PgR negative (N=2453)</td>
<td>0.93 (0.73–1.17)</td>
</tr>
<tr>
<td></td>
<td>ER and/or PgR low (N=138)</td>
<td>1.42 (0.46–4.34)</td>
</tr>
<tr>
<td>Surgery</td>
<td>Breast conserving (N=1644)</td>
<td>0.93 (0.67–1.30)</td>
</tr>
<tr>
<td></td>
<td>Mastectomy (N=947)</td>
<td>0.95 (0.69–1.31)</td>
</tr>
</tbody>
</table>

**Conclusions:** The final OS analysis after 293 deaths showed no significant benefit from the addition of BEV to standard adjuvant CT for early TNBC. Updated IDFS results were similar to the primary IDFS results, showing no difference between treatments. Late-onset AEs were rare in both groups. 5-year IDFS and OS rates suggest that the prognosis for pts with TNBC is better than previously thought.
**2014 San Antonio Breast Cancer Symposium**

**Title:** ARTemis: A randomised trial of bevacizumab with neo-adjuvant chemotherapy for patients with HER2-negative early breast cancer

Helena M Earl¹,², Louise Hiller³, Janet A Dunn³, Clare Blenkinsop³, Louise Grybowicz⁴, Anne-Laure Vallier⁴, Jean Abraham¹,²,⁵, Jeremy Thomas⁴, Elena Provenzano²,³, Luke Hughes-Davies⁴, Karen MacAdam⁴, Stephen Chan⁴, Rizvana Ahmad⁴, Tamas Hickish⁶, Stephen Houston⁶, Daniel Rea⁷, John Bartlett¹,³,¹⁴, Carlos Caldas¹,²,⁵, David Cameron¹ and Larry Hayward⁶.

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**Body:** Background: Bevacizumab (bev) has been used with neo-adjuvant chemotherapy (NACT) in breast cancer trials. Geparquinto reported benefit for bev in triple negative (neg) patients (pts) (pathological complete response (pCR) 36.4% vs 27.8% p=0.02), as did CALGB 40603 (pCR 52% vs 44%, p=0.057), although NSABP-B40 showed benefit in ER positive (pos) pts (pCR 23.3% vs 15.2%, p=0.008).

Methods: ARTemis is a randomised phase 3 trial adding bev to NACT (docetaxel (D)-FEC). Pts with HER2-neg invasive breast cancer were eligible. Stratification was by age, ER status (neg:weak pos:strong pos), tumour size (T2:T3/4), clinical involvement of axillary nodes and inflammatory/locally advanced disease. Pts were randomised (1:1) to bev+D-FEC or D-FEC. The primary endpoint was pCR, defined as no residual invasive cancer in the breast or axillary lymph nodes after NACT. 800 pts were required to detect 10% differences in pCR rates; 85% power, 5% alpha level.

Results: 800 pts were randomised from 66 UK centres (May 2009 to Jan 2013). 68% were <50 years old, 19% had inflammatory and/or locally advanced disease, 79% of tumours <50mm, 52% clinical node pos and 33% ER-neg. A 2-reader independent review of pathology reports determined whether pCR had been achieved or, at least, minimal residual disease (MRD) status. Significantly more pts on bev+D-FEC had a pCR (22% vs 17%; adjusted p=0.03) (see table). pCR rates differed significantly across ER groups (neg 38%, weak pos 39%, strong pos 7%; p<0.0001). Treatment effect of bev remained significant after adjustment for ER (p=0.03). Similarly significantly more pts on bev+D-FEC had a pCR or MRD (36% vs 29%; adjusted p=0.035). Rates differed significantly across ER groups (neg 51%, weak pos 58%, strong pos 18%; p<0.0001). Treatment effect of bev remained significant after adjustment for ER (p=0.03).

<table>
<thead>
<tr>
<th></th>
<th>D→FEC % (95%CI)</th>
<th>Bev+D→FEC % (95%CI)</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>pCR in all breast tumours AND absence of disease in ax LNs in all breast tumours</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=66/393)</td>
<td>(n=87/388)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER neg (Allred 0-2) (n=253)</td>
<td>17% (13-21%)</td>
<td>22% (18-27%)</td>
<td>0.03</td>
</tr>
<tr>
<td>ER weak pos (Allred 3-5) (n=67)</td>
<td>32% (24-41)</td>
<td>44% (36-54)</td>
<td></td>
</tr>
<tr>
<td>ER strong pos (Allred 6-8) (n=461)</td>
<td>7% (4-11)</td>
<td>6% (3-10)</td>
<td></td>
</tr>
<tr>
<td><strong>pCR or MRD in all breast tumours</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n=114/394)</td>
<td>(n=138/388)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>29% (25-34%)</td>
<td>36% (31-41%)</td>
<td>0.035</td>
<td></td>
</tr>
</tbody>
</table>
Conclusions: ARTemis showed a significant improvement in both pCR and MRD rates with the addition of bev to D-FEC. ER-neg and ER-weak pos / HER2-neg breast cancer pts appeared to benefit most from bev, whilst pCR and MRD rates in ER-strong pos pts were lower and did not appear to benefit from bev. Our results are similar to those reported in Geparquinto and CALGB 40603.
Title: Subgroup efficacy analyses of the randomized phase III TANIA trial evaluating continued or reintroduced bevacizumab (BEV) after 1st-line BEV for HER2-negative locally recurrent/metastatic breast cancer (LR/mBC)

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Body: BACKGROUND: The open-label randomized phase III TANIA trial demonstrated statistically significantly improved progression-free survival (PFS; primary endpoint) with the addition of BEV to 2nd-line chemotherapy (CT) in patients (pts) with HER2-negative LR/mBC progressing after 1st-line BEV-containing therapy. We describe efficacy in clinically relevant subgroups to assess consistency of the BEV treatment effect.

METHODS: Pts whose HER2-negative LR/mBC had progressed during/after 1st-line BEV–CT were randomized 1:1 to investigator's chosen 2nd-line single-agent CT given either alone or with BEV (15 mg/kg q3w or 10 mg/kg q2w). 2nd-line therapy was continued until disease progression (PD), unacceptable toxicity or consent withdrawal. At PD, BEV was continued with 3rd-line CT (investigator's choice) in pts initially randomized to BEV–CT but was not permitted in pts randomized to CT alone. The primary endpoint was PFS from randomization to 2nd-line PD/death. Sample size was calculated based on a log-rank test assuming median PFS of 7–9.3 mo with a corresponding hazard ratio (HR) of 0.75. PFS events were required in 384 of 488 pts for 80% power at 2-sided α=0.05. Subgroup analyses of the primary endpoint were prespecified and included subgroups defined by stratification factors.

RESULTS: From Jan 2011 to Apr 2013, 494 pts were enrolled. The data cut-off for the primary analysis was Dec 20, 2013 (median follow-up: CT 15.9 mo; BEV–CT 16.1 mo). The PFS benefit seen in the overall population was observed consistently in most subgroups.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Pts with PFS events/Total No. of pts (%)</th>
<th>Median 2nd-line PFS, mo</th>
<th>Unstratified HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CT</td>
<td>BEV–CT</td>
<td>CT</td>
</tr>
<tr>
<td>All</td>
<td>203/247 (82)</td>
<td>204/247 (83)</td>
<td>4.2</td>
</tr>
<tr>
<td>Hormone receptor status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triple negative</td>
<td>56/60 (93)</td>
<td>45/56 (80)</td>
<td>2.1</td>
</tr>
<tr>
<td>Positive, HER2 negative</td>
<td>147/187 (79)</td>
<td>159/191 (83)</td>
<td>4.7</td>
</tr>
<tr>
<td>1st-line PFS, mo</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;6</td>
<td>61/69 (88)</td>
<td>54/68 (79)</td>
<td>3.9</td>
</tr>
<tr>
<td>≥6</td>
<td>142/178 (80)</td>
<td>150/179 (84)</td>
<td>4.6</td>
</tr>
<tr>
<td>LDH</td>
<td></td>
<td></td>
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<tr>
<td>≤1.5×ULN</td>
<td>167/207 (81)</td>
<td>168/210 (80)</td>
<td>4.4</td>
</tr>
<tr>
<td>&gt;1.5×ULN</td>
<td>36/40 (90)</td>
<td>36/37 (97)</td>
<td>2.1</td>
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<tr>
<td>CT choice</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Taxane</td>
<td>25/32 (78)</td>
<td>26/32 (81)</td>
<td>3.2</td>
</tr>
<tr>
<td>Non-taxane non-vinorelbine</td>
<td>156/191 (82)</td>
<td>151/188 (80)</td>
<td>4.4</td>
</tr>
<tr>
<td>Vinorelbine</td>
<td>22/24 (92)</td>
<td>27/27 (100)</td>
<td>2.4</td>
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</tbody>
</table>
CONCLUSIONS: PFS was statistically significantly improved with the addition of BEV to 2nd-line CT in BEV-pretreated pts, meeting the primary objective of TANIA. This effect was seen consistently within subgroups based on stratification factors. Within the limitations of exploratory subgroup analyses with small sample sizes, further subgroup analyses may suggest some potential inconsistencies in treatment effect. These hypothesis-generating observations require further exploration of potential differences in disease and pt characteristics in TANIA, which may have influenced physicians' CT choice.
Title: Epidemiology of stage IV breast cancer patients: A review of the National Cancer Database 2000-2011

Jessica Gries¹, Van Do TH² and Peter Silberstein¹,². ¹Creighton University School of Medicine, Omaha, NE and ²VA Omaha Medical Center, Omaha, NE.

Body: Background: Breast cancer patients tend to have distinctive characteristics that change as the cancer progresses to stage IV, especially in race and socioeconomic status. A previous SEER-based study reported blacks were diagnosed with a more advanced stage and larger tumor size compared to white patients'. This is the largest study to determine multiple factors associated with patients presenting with stage IV breast cancer.

Methods: A population-based study was conducted using the National Cancer Database (2000-2011), which contains 70% of all cancer diagnoses in the United States from 1658 American College of Surgeons Accredited-Hospitals. The initial diagnosis of stage IV disease in women represented 3.56% (81,476) of the total patient population (2,294,058). The demographics of the stage IV patients were compared to the entire database (all stages) of breast cancer patients using the chi square test.

Results: There was an increased incidence of stage IV Breast Cancer in patients with the following characteristics: Black, no insurance, Medicaid, Medicare, less educated, lower household income, and higher comorbidity (Table-1). Stage IV cancer incidence was higher in black patients (17%) compared to all stages (10%). There were 5,137 more black patients with stage IV than expected. Stage IV patients are three 3x more likely to have no insurance and 2x as likely to have Medicaid when compared to all stages. Stage IV patients tend to have less education and were twice as likely to have 2+ comorbidities. There was no increased incidence of stage IV disease in either Hispanics or in different age groups, and only a slight difference in distance traveled (not shown in table).

Conclusion: The following characteristics are more common in patients with stage IV disease: low income, Black, no insurance, Medicaid, less education, and higher comorbidity. Patients who were White, had private insurance, higher education and income status, and lower comorbidity had less representation in stage IV disease. These factors influence the occurrence of a more advanced stage of the disease. Identifying or more promptly treating patients in high-risk populations may reduce their incidence of stage IV disease.


Table-1: Epidemiology of Stage IV Breast Cancer Patients (2000-2011)

<table>
<thead>
<tr>
<th>Race/ Ethnicity*</th>
<th>Stage (%)</th>
<th>All Stages (%)</th>
<th>Observed/ Expected # of Patients**</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>74</td>
<td>80</td>
<td>60,519/ 65,425</td>
</tr>
<tr>
<td>Black</td>
<td>17</td>
<td>10</td>
<td>13,611/ 8,474</td>
</tr>
<tr>
<td>Hispanic</td>
<td>5</td>
<td>5</td>
<td>4,219/ 3,748</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Insurance*</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6</td>
<td>2</td>
<td>4,792/ 1,792</td>
</tr>
<tr>
<td>Medicaid</td>
<td>10</td>
<td>5</td>
<td>8,326/ 3,911</td>
</tr>
<tr>
<td>Medicare</td>
<td>39</td>
<td>35</td>
<td>32,614/ 28,843</td>
</tr>
<tr>
<td>Private</td>
<td>40</td>
<td>54</td>
<td>32,614/ 43,916</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Education* % Without HS Degree*</th>
<th>Stage (%)</th>
<th>All Stages (%)</th>
<th>Observed/ Expected # of Patients**</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Lower value=more education)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;31%</td>
<td>16</td>
<td>11</td>
<td>12,609/ 8,962</td>
</tr>
<tr>
<td>23-30.9%</td>
<td>17</td>
<td>15</td>
<td>14,169/ 11,895</td>
</tr>
<tr>
<td>18-22%</td>
<td>16</td>
<td>15</td>
<td>12,991/ 12,058</td>
</tr>
<tr>
<td>Household Income* (per year)</td>
<td>12-17.9%</td>
<td>&lt;12%</td>
<td>18,854/ 19,717</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------</td>
<td>------</td>
<td>----------------</td>
</tr>
<tr>
<td>&lt;$28,000</td>
<td>12</td>
<td>8</td>
<td>9,330/ 6,274</td>
</tr>
<tr>
<td>$28,000-32,999</td>
<td>13</td>
<td>11</td>
<td>10,422/ 8,718</td>
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<tr>
<td>$33,000-38,999</td>
<td>17</td>
<td>16</td>
<td>14,188/ 13,199</td>
</tr>
<tr>
<td>$39,000-48,999</td>
<td>23</td>
<td>23</td>
<td>18,585/ 18,658</td>
</tr>
<tr>
<td>&gt;$49,000</td>
<td>31</td>
<td>37</td>
<td>25,269/ 30,391</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comorbidity*</th>
<th>0</th>
<th>64</th>
<th>66</th>
<th>51,832/ 54,019</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11</td>
<td>9</td>
<td></td>
<td>8,855 /7,007</td>
</tr>
<tr>
<td>2+</td>
<td>4</td>
<td>2</td>
<td></td>
<td>3,178 /1,548</td>
</tr>
</tbody>
</table>

*p-value < .0001  **Observed # of patients / % of all stages in category x total # of stage IV patients (Expected)
2014 San Antonio Breast Cancer Symposium

**Publication Number:** P5-19-27  
**Average Grade:** 0

**Title:** IMMU-132, a new antibody-drug conjugate (ADC) against Trop-2, as a novel therapeutic for patients with relapsed/refractory, metastatic, triple-negative breast cancer (TNBC): Results from Phase I/II clinical trial (NCT01631552)

Aditya Bardia¹, Alexander Starodub², Rebecca L Moroose³, Ingrid A Mayer⁴, Jennifer R Diamond⁵, Ellen Chuang⁶, Serengulam V Govindan⁷, Robert M Sharkey⁷, Pius Maliakal⁷, William A Wegener⁷, Steven A Hamburger⁷, Allyson J Ocean⁷, David M Goldenberg⁷,⁸ and Linda T Vahdat⁷. ¹Massachusetts General Hospital Cancer Center, Harvard Medical School, Boston, MA; ²Indiana University Health Center for Cancer Care, Goshen, IN; ³UF Health Cancer Center, Orlando, FL; ⁴Breast Cancer Program, Vanderbilt-Ingram Cancer Center, Nashville, TN; ⁵University of Colorado Cancer Center, Aurora, CO; ⁶Weill Cornell Medical College, New York, NY; ⁷Immunomedics, Inc, Morris Plains, NJ and ⁸Garden State Cancer Center, Center for Molecular Medicine and Immunology, Morris Plains, NJ.

**Body:**

**Background:** TNBC, comprising 15-20% of all invasive breast cancers, represents an aggressive phenotype with high risk of recurrence and mortality. Trop-2 is a cell-surface glycoprotein expressed on many human carcinomas, including TNBC. High Trop-2 expression is associated with more aggressive disease and poor prognosis in several cancers, including breast cancer. We report interim results from a Phase I/II trial evaluating a novel ADC, IMMU-132 (isactuzumab govitecan), comprising a humanized anti-Trop-2 antibody conjugated to the topoisomerase I inhibitor, SN-38 (active metabolite of irinotecan). The drug:antibody ratio of 7.6 facilitates the delivery of high-dose chemotherapy preferentially to the tumor cells.

**Methods:** Patients (pts) with relapsed/refractory metastatic epithelial tumors were enrolled at escalating IMMU-132 doses (8 to 18 mg/kg), given on days 1 and 8 of a 21-day cycle. The Phase II dose at this schedule was 10 mg/kg. CT scans were performed every 6-8 weeks to assess response using RECIST 1.1. During the dose-escalation portion, evidence of antitumor activity, including 3 partial responses (TNBC, small-cell lung cancer and colorectal cancer) and many with durable stable disease (SD), was observed, leading to Phase II expansion.

**Results:** As of Sept. 25, 2014, a total of 132 pts have been enrolled, including 30 with advanced/metastatic TNBC. Currently evaluable TNBC pts (N=17) had a median age of 50 (33-77), with a median of 4 prior drug regimens (range 1-8), and 67% having received prior platinum-containing regimens. In this heavily pre-treated population, there were 4 PRs (25%) and 9 SDs (56.3%) per RECIST v1.1, representing a disease control (PR+SD > 4 mos) of 53% among evaluable pts with adequate follow-up. A maximum shrinkage of target lesions of 33%, 44%, 51%, and 60% for pts with PRs, and 14%, 19%, and 27% for 3 pts with SD, was determined. Biomarker CA15.3 directional changes correlated with RECIST. All but one pathology specimen were Trop-2+ by immunohistochemistry. HPLC analysis of serum samples found <5% unbound SN-38. The half-life of IMMU-132 was 23 h, which is similar to the predicted half-life from in vitro serum stability studies. Grade 3/4 toxicities were: neutropenia (G3, 4 pts, 23.5%) with 1 febrile neutropenia (5.9%), and lymphocytopenia (1 Gr 3, 1 pt, 5.9%). Grade 1/2 events were fatigue (35.3%), diarrhea (41.2%), and alopecia (29.4%). No pt discontinued therapy due to toxicity.

**Conclusions:** Based on laboratory and initial clinical results, IMMU-132 is an ADC that selectively delivers a topoisomerase I inhibitor to cancer cells without the need for enrichment by a companion diagnostic. It is safe, well-tolerated, with preliminary evidence of encouraging efficacy in heavily-pretreated pts with relapsed/refractory metastatic TNBC. Randomized Phase III and combination trials are being planned.
**Title:** Evaluation and clinical impact of intra-tumor heterogeneity (ITH) in primary HER2-overexpressing breast cancers (HER2+BC) treated with adjuvant trastuzumab and chemotherapy (CT)

Sherene Loi¹, Peter Savas¹, Ingrid Lonnstedt², Debora Fumagalli³, Franco Caramia¹, Jason Li¹, Roberto Salgado³, Andrew Rowan¹, Fabrice André⁵, Carsten Denkert⁶, Patrick Neven⁷, Sibylle Loibl⁸, Christos Sotiriou³, Charles Swanton⁴ and Terence P Speed². ¹Peter MacCallum Cancer Centre, East Melbourne, Victoria, Australia; ²Walter and Eliza Hall Medical Research Institute, Parkville, Victoria, Australia; ³Institute Jules Bordet, Brussels, Belgium; ⁴Translational Cancer Therapeutics Laboratory, Cancer Research, London, United Kingdom; ⁵Gustave Roussy, Villejuif, France; ⁶Charité Hospital, Berlin, Germany; ⁷UZ Leuven, Gasthuisberg, Leuven, Belgium and ⁸German Breast Group Forschungs GmbH, Neu-Ilsenburg, Hessen, Germany.

**Body:** Background: Despite the success of trastuzumab, when added to CT, for the treatment of women with primary HER2+ BC, diversity in clinical responses and survival are still observed. ITH or the presence of subclonal populations is theorized to be a cause of drug resistance. In this study, we investigated if ITH that could be estimated from a single sample taken at primary surgery from patients with newly-diagnosed HER2+ disease and if ITH was associated with poorer clinical outcomes despite adjuvant trastuzumab-based treatment.

**Methods:** Fifty-two frozen tumor and matched germline samples were taken from patients diagnosed with primary HER2+BC and treated with adjuvant trastuzumab and CT with a median follow-up of 6.3yrs (range 1.5-13). There were 10 (19%) invasive relapses, 7 (13%) distant relapses and 5 (9.6%) deaths. We performed tumor and germline whole exome sequencing (WES), tumor and germline copy number (CN) data profiling (Affymetrix SNP6.0), tumor gene expression (Affymetrix U133 2.0Plus). Variants were called with MuTect, CNs were called using GISTIC. We developed a novel bioinformatics method that integrated WES variant allele fractions, purity estimates and SNP data to delineate the presence of single or multiple genetically distinct subclones. We confirmed our estimated tumor ploidy using flow cytometry on 9 samples. We investigated the association between the presence of ITH and survival using multivariate Cox regression analyses.

**Results:** We observed in HER2+ BCs the presence of substantial chromosomal instability, numerous CN alterations and aneuploidy. With regards to their genomic architecture, 76.9% (40/52) displayed the presence of at least one aneuploid subclone, where the median ploidy was 3.3 (range:1.7-6.3) copies. Multiple distinct subclones or the presence of ITH could be clearly detected in 35/52 (67.3%) samples, 8/52 (15.4%) had evidence of only a single clone, whilst in 9/52 (17.3%) samples their genomic structure could not be definitively determined. The existence of ITH was significantly associated with invasive relapse after adjustment (HR:4.38;95%CI:1.61-7.14;p=0.002) with estrogen receptor and nodal status also remaining significant in the Cox model—in contrast, the absence of ITH was associated with excellent outcomes (no distant relapses). Other surrogates of genomic instability (gene expression signature [CIN70] and SNP signature [Genomic Instability Index]) were not significantly associated with survival in this dataset.

As exploratory analyses, potential genetic drivers of ITH were investigated. *PIK3CA* (n=13,p=0.008), *MED1* (n=3,p=0.03) and *IKZF1* (n=3,p=0.03) mutations, amplifications in 8q22.2 (n=7,p=0.008) as well as deletion in 1q24.2 (n=5, p=0.006) were associated with the presence of a single clone, whereas we did not find any genetic drivers with significant associations with ITH.

**Conclusions:** We show for the first time that the presence of ITH in primary HER2+ tumors is associated with worse outcomes despite adjuvant trastuzumab and CT. These findings should be evaluated in larger cohorts.
Title: Broad exonic DNA diversity is associated with resistance to taxane-FAC chemotherapy in triple negative breast cancer

Tingting Jiang¹, Weiwei Shi¹, William F Symmans², Charles Li¹, James Platt¹, Rosanna Lau², Vikram B Wali¹, Richard Lifton¹, Lajos Pusztai¹ and Christos Hatzis¹. ¹Yale University, New Haven, CT and ²MD Anderson Cancer Center, Houston, TX.

Body: Purpose: Previous efforts to develop transcriptional markers of chemotherapy sensitivity in TNBC had limited success due to the heterogeneity of this disease. The purpose of this study was to identify genomic differences between extremely chemotherapy sensitive and highly chemotherapy resistant TNBC through whole exome sequencing and to assess measures of genomic heterogeneity as predictive markers.

Methods: Twenty nine cases were selected from a prospectively collected cohort of fine needle aspiration specimens obtained before preoperative chemotherapy (MDACC) to represent two extreme response cohorts including pathologic complete response (pCR, N=17) or extensive residual cancer (eRD, N=12). DNA was extracted from specimens stored in RNAlater, exomes were captured using NimbleGen SeqCap EZ Exome Library preparation and paired-end sequencing of 75 base pair fragments was performed on Illumina HiSeq 2000. Alignment and variant calling were performed with BWA and GATK haplotype caller. Variants were filtered against the 1000 Genomes and TCGA normal breast samples to identify candidate somatic variants. Fisher-exact test was used to identify variant genes associated with sensitivity to chemotherapy. We calculated overall mutational load and normal-adjusted clonal entropy of driver mutations as measures of genome heterogeneity. The chi-squared test was used to compare differences in mutational spectra and genome heterogeneity between the two response groups.

Results: The mean coverage was over 150X and 93% of target regions had > 20X coverage. The number of non-silent COSMIC mutations was similar in tumors from the two response groups (pCR: 63, range 49-82; eRD: 59, range 43-78) as well as the number of novel non-silent mutations (eRD: 223, range 113-388; pCR: 192, range 125-293). Gene level aggregation of variants identified 4 genes (MUC21, SLCO5A1, LRBA, STNE1) with response-associated mutational patterns. However, mutations were non recurrent and p-values were modest, ranging from 0.04 to 0.005. We observed greater overall mutational load and subclonal heterogeneity (clonal entropy of cancer related mutations) associated with eRD compared to pCR. Both measures suggest that higher genomic DNA diversity is associated with chemotherapy resistance. However, some genes (BRCA1 and MKI67) had higher mutational load (sum of minor allele frequencies per gene) associated with pCR compared to eRD. In general, a higher proportion of C>T transition (P=0.011) and lower A>G transition (P=0.028) was associated with eRD. The same mutational spectrum shift was previously described in the comparison of trunk and branch driver events suggesting that eRD tumors may undergo heterogeneous branched evolution.

Conclusion: We observed greater genomic diversity and distinctive mutational spectra in original pre-treatment samples of TNBC that were associated with extensive residual disease compared to pCR. Our analysis suggests that broad measures of genomic diversity may serve as markers of resistance to chemotherapy.
2014 San Antonio Breast Cancer Symposium

Publication Number: PD3-3
Average Grade: 3.60

Title: Impact of neoadjuvant chemotherapy on the clonal composition of breast cancer

Matthew P Goetz¹, Michael T Barrett², Krishna R Kalari¹, Vera J Suman¹, Sarah A McLaughlin³, Alvaro Moreno-Aspitia³, Ann M Moyer¹, Donald W Northfelt², Richard J Gray², Jason Sinnwell¹, Douglas Mahoney¹, Poulami Barman¹, Peter Vedell¹, Xiaojia Tang¹, Kevin Thompson¹, Travis Dockter¹, Katie Jones¹, Sara J Felten¹, Amy Conners¹, Jeanette Eckel-Passow¹, Hughes Sicotte¹, Steven N Hart¹, Jia Yu¹, Daniel W Visscher¹, Eric D Wieben¹, Cloann Schultz¹, Minetta C Liu¹, James N Ingle¹, Liewei Wang¹, Richard W Weinshilboum¹ and Judy C Boughey¹. ¹Mayo Clinic, Rochester, MN; ²Mayo Clinic, Scottsdale, AZ and ³Mayo Clinic, Jacksonville, FL.

Body: Background
Cancer genomic investigations have identified recurrent genomic aberrations critical for cancer initiation, progression, and metastases. However, these investigations are typically performed in isolation, and the effects of treatment on the clonal selection of tumor cells are mostly unknown. We hypothesized that molecular profiling of residual tumors after neoadjuvant chemotherapy (NAC) would identify new drug targets/pathways in patients at high risk for disease recurrence. To better identify clonal populations of resistant breast cancer cells, we utilized DNA content-based flow sorting of nuclei to identify and isolate clonal populations for aCGH and next generation sequencing (NGS) both before and after NAC.

Methods
The Breast Cancer Genome Guided Therapy Study (BEAUTY) (NCT 02022202) is a prospective study of patients with high-risk breast cancer treated with neoadjuvant 12 weekly paclitaxel (T) +/- trastuzumab followed by 4 cycles of anthracycline based chemotherapy. Tumor tissue from baseline, residual disease from surgery, distant metastases, and patient derived xenografts (PDX) are obtained for cell sorting by DNA ploidy, aCGH, RNA and exome sequencing.

Results: 140 patients have been enrolled, 104 have completed surgery and 30 unique PDX have been established corresponding to 26 patients prior to chemotherapy and 4 from residual disease at surgery. Baseline exome and RNA sequencing is complete in 140. Currently, genomic analyses of flow sorted matched baseline, surgical, PDX, and distant disease samples are available in 6 patients. Substantial genomic variation was observed in the surgical sample compared to the primary tumor including gain of oncogenic drivers (EGFR) and loss of negative regulators (ATG5) (Table). The PDX recapitulated these events with excellent fidelity compared to the corresponding human tumor. In patients with TNBC, RNA seq obtained from matched samples demonstrated changes in immune related pathways. Evaluation of drug targets/pathways identified in the resistant tumors are ongoing using the PDX and sequencing of the remaining matched baseline/surgical disease will be reported.

Clonal changes occurring over time in patients with residual disease or disease recurrence after NAC

<table>
<thead>
<tr>
<th>Tumor Subtype</th>
<th>Residual Cancer Burden</th>
<th>Disease Status</th>
<th>Clonal Aberration Changes (baseline and post NAC)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNBC (AR subtype)</td>
<td>3</td>
<td>Contralateral lymph node recurrence at 150 days</td>
<td>2p25.2 - p25.1 amplicon lost at recurrence; 6p21.32 -p21.31 amplicon lost at recurrence</td>
</tr>
<tr>
<td>TNBC (BL 1)</td>
<td>3</td>
<td>Bone/liver/lymph node recurrence at 135 days</td>
<td>5q11.2 amplicon gained at surgery; 12p13.33 - p13.2 amplicon gained at surgery</td>
</tr>
<tr>
<td>TNBC (BL 1)</td>
<td>3</td>
<td>Disease-free at day 150</td>
<td>6q21 amplicon lost at sugery</td>
</tr>
<tr>
<td>TNBC (BL 2)</td>
<td>0</td>
<td>Brain recurrence at 390 days</td>
<td>Chr 2 chromothripsis at surgery</td>
</tr>
<tr>
<td>Luminal B</td>
<td>3</td>
<td>Progression during chemotherapy</td>
<td>7p12.1 - p11.2 amplicon gained at surgery</td>
</tr>
<tr>
<td>Luminal HER2</td>
<td>3</td>
<td>Disease-free at day 360</td>
<td>9q33.2 amplicon lost at surgery; 15q13.1 - q13.3 amplicon lost at surgery; 15q22.2 - q24.1 amplicon lost at surgery</td>
</tr>
</tbody>
</table>
Conclusions: We observed substantial evolutionary changes in residual breast tumors remaining after NAC. Our findings suggest that a comprehensive assessment of the mutational landscape that has evolved during NAC can inform drug development in high risk breast cancer patients.
Title: Reliability of whole exome sequencing for assessing intratumor heterogeneity from breast tumor biopsies

Weiwei Shi¹, Anees Chagpar¹, Tingting Jiang¹, Donald R Lannin¹, Brigid Killelea¹, Nina Horowitz¹, Raymond Lim², James Platt¹, Charlotte KY Ng², Vikram B Wali¹, Britta Weigelt², Jorge S Reis-Filho², Christos Hatzis¹ and Lajos Pusztai¹. ¹Yale University, New Haven, CT and ²Memorial Sloan Kettering Cancer Center, New York, NY.

Body: BACKGROUND: False positive findings introduced by analytical noise in sequencing and bioinformatics pipelines constitute a challenge for accurate massively parallel sequencing (MPS). Reports of intratumor genomic heterogeneity based on MPS rarely estimate the impact of false positive mutation calls. The purpose of this study was to measure apparent genomic heterogeneity in different regions of the same tumor and to assess the technical noise in variant calling in replicate sequencing of the same DNA.

METHODS: Three anatomically distinct biopsies were obtained from 3 different regions of 11 breast cancers (33 samples) including 6 low/intermediate grade, estrogen receptor (ER)-positive and 5 high-grade, triple-negative (TNBC) cancers. DNA from 8 different biopsies was split and independently processed on different days to obtain technical replicates. The NimbleGen SeqCap EZ Exome Library preparation method was used for exome capture and paired-end sequencing of 75 base pair fragments was performed on Illumina HiSeq 2000. Read alignment and variant calling were performed with BWA and GATK haplotype caller. Concordance in variant calls and in minor allele frequencies (MAF) was assessed in the 3 biopsies of the same tumor and 8 technical replicates. We adjusted for uneven sequence coverage and analyzed known germline variants from dbSNP, known cancer related variants from COSMIC separately from novel variants (i.e. not previously reported in dbSNP or COSMIC). We estimated intratumor genomic heterogeneity of genes after removing alterations identified in areas where mapping is difficult and variant calls that had low analytical reliability in the technical replicates.

RESULTS: The mean coverage was over 150X and > 90% of target regions had ≥ 20X coverage. We validated the specificity (98.2%) and sensitivity (86.7%) of the variant calling pipeline on the GIAB reference data. The concordance for germ line SNPs and variants in COSMIC in technical replicates was 94.9% and 92.7%, respectively. Novel variants had very low concordance, 55.9%, in technical replicates. The concordance between MAF estimates from the technical replicates was high (0.974, 0.957 and 0.969 for single nucleotide variations, insertions and deletions, respectively). The concordance for germline SNPs and COSMIC variants in pairwise comparisons of biopsies from the same tumor was 93.2% and 91.1%. For known variants, lower concordance was observed in TNBC (88.3%-98.5%) compared to ER-positive tumors (93.6%-98.8%, P<0.05) indicating greater intratumor heterogeneity. We identified variants in a small number of genes (DNAH9, PPM1E, and MAP3K1) that were called inconsistently in most technical replicates, even after excluding low mappability regions. We assessed intratumor heterogeneity in the triplicate biopsies, after excluding the technically unreliable variants. On average, two different biopsies from the same tumor shared 14272 +/− 1379 common variants and differed in 816 +/− 416 variants.

CONCLUSION: We observed heterogeneity to be slightly greater in high-grade, ER-negative compared to low-grade, ER-positive breast cancers. Differences in variants observed in multiple biopsies of the same tumor are only slightly greater than those expected by technical noise alone.
Title: Whole exome sequencing (WES) of HER2+ metastatic breast cancer (MBC) from patients with or without prior trastuzumab (T): A correlative analysis of TBCRC003

Nikhil Wagle¹, Nancy U Lin¹, Andrea L Richardson³, Ignaty Leshchiner², Ingrid A Mayer⁴, Andres Forero-Torres⁵, Timothy J Hobday⁶, Elizabeth C Dees⁷, Rita Nanda⁸, Mothaffar F Rimawi⁹, Hao Guo¹, William T Barry¹, Ron Bose¹¹, Wei Shen¹¹, Antonio C Wolff¹⁰, Stacey B Gabriel², Levi A Garraway¹, Eric P Winer¹ and Ian E Krop¹.

¹Dana-Farber Cancer Institute, Boston, MA; ²Broad Institute, Cambridge, MA; ³Brigham and Women’s Hospital, Boston, MA; ⁴Vanderbilt-Ingram Cancer Center, Nashville, TN; ⁵University of Alabama, Birmingham, AL; ⁶Mayo Clinic, Rochester, MN; ⁷UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC; ⁸University of Chicago, Chicago, IL; ⁹Baylor College of Medicine, Houston, TX; ¹⁰Johns Hopkins Hospital, Baltimore, MD and ¹¹Washington University School of Medicine, St Louis, MO.

Body: Background: Although the spectrum of genomic alterations in primary, treatment-naïve breast tumors has been described, the genomic landscape of HER2+ MBC remains underexplored. Furthermore, tumor genomic alterations that arise after progression on anti-HER2 therapy are largely unknown.

Methods: We prospectively collected metastatic tumor biopsies from patients (pts) enrolled on TBCRC003 (NCT00470704), a phase II study evaluating the combination of lapatinib (L) and T in pts with HER2+ MBC who had varying degrees of prior T exposure. We performed WES on baseline metastatic biopsies and normal DNA from 57 pts. In 36 pts, we also performed WES on pre-treatment primary tumors. Tumors were analyzed for point mutations, insertions/deletions, and copy number alterations.

Results: Total accrual was 116 pts. 87 pts were registered in one of two efficacy cohorts: Cohort 1 included pts w no prior T for MBC. Pts with prior adjuvant T were included if the interval from last T to 1st recurrence > 12 months. Cohort 2 included pts with 1-2 prior lines of T for MBC or recurrence within 12 months of adjuvant T. An additional 29 pts were enrolled in a biomarker cohort (Cohort 3). Per-protocol efficacy analyses for 85 pts deemed evaluable are shown below:

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Objective Response Rate</th>
<th>Clinical Benefit Rate</th>
<th>Median Time to Progression</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort 1</td>
<td>50% (90% CI 33.8-66.2%)</td>
<td>57.5% (95% CI 40.9-73.0)</td>
<td>7.4 months</td>
</tr>
<tr>
<td>Cohort 2</td>
<td>22.2% (90% CI 11.2-37.1%)</td>
<td>42.2% (95% CI 27.7-57.8)</td>
<td>5.3 months</td>
</tr>
</tbody>
</table>

As we previously reported (Wagle et al, ASCO 2014), across 57 metastatic tumors, significant recurrently mutated genes were TP53 (n=30; 53%) and PIK3CA (n=19; 33%). The frequency of mutant TP53 and PIK3CA was not significantly different from 119 primary, treatment-naïve HER2+ tumors sequenced in the TCGA study (50%, p=0.8 and 27%, p=0.5, respectively). Recurrent copy number alterations were also similar to TCGA data. Comparing the 38 pts who received any prior T with the 19 pts who did not, there was no significant difference in the incidence of mutant TP53 (53% vs 53%, p=1.0) and PIK3CA (37% vs 26%, p=0.6).

We identified mutations in the HER2 kinase domain in 4/38 pts who received prior T (11%), as compared to 0/19 T-naïve pts. HER2 kinase domain mutations have been identified in ~2% of HER2-negative cancers but <1% of primary HER2+ cancers. 3 of the mutations were L755S, which has been shown to be resistant to L and sensitive to irreversible HER2 inhibitors. The 4th mutation was D742N, a novel kinase domain mutation. None of the 4 pts with HER2 kinase domain mutations had an objective response, though 1 pt had stable disease for 29 weeks.

An analysis comparing paired archival primary tumors and baseline metastatic biopsies from 36 pts to identify genomic alterations acquired or enriched in the metastatic tumors will be presented.

Conclusions: We present an analysis of the genomic landscape of HER2+ MBC, including comparisons between matched primary tumors and metastatic biopsies. Somatic HER2 kinase mutations in pts with HER2+ MBC treated with prior T suggests...
that these mutations may be involved in resistance to T, and may predict poor response to additional anti-HER2 therapy with combined L and T. Novel therapeutic approaches may be required for these pts.
2014 San Antonio Breast Cancer Symposium

Publication Number: PD3-6
Average Grade: 3.60

Title: ConvertHER: Evolution of genomic alterations from primary to metastatic breast cancer

Ana Maria Gonzalez-Angulo¹, Ana Lluch², Agda K Eterovic¹, Angel Guerrero³, Xiaofeng Zheng¹, Ramon Perez⁴, Shuying Liu¹, José I Chacón⁵, Ken Chen¹, Silvia Antolin⁶, Gordon B Mills¹, Jaime Ferrer², Octavio Burgues², Begona Bermejo⁷, Elia Munoz⁷, Rosalia Caballero⁸, Eva Carrasco⁸, Eduardo Martinez⁹ and Funda Meric-Bernstam¹. ¹University of Texas MD Anderson Cancer Center; ²INCLIVA BioMedical Research Institute, Hospital Clínico de Valencia; ³Instituto Valenciano de Oncología; ⁴Clínica Quirón; ⁵Hospital Virgen de la Salud; ⁶Complejo Hospitalario Universitario A Coruña, A Coruña, Spain; ⁷Hospital de La Plana; ⁸GEICAM, San Sebastián de los Reyes and ⁹Hospital Provincial de Castellón.

Body: Background: Changes in breast cancer receptor status over disease progression and treatment have been described to a point that could alter response to therapy. There is growing interest in delivering biomarker/genomically-based targeted therapies. We aimed to determine the concordance of genomic alterations between primary (P) and metastatic (M) breast cancer in a prospective collection study.

Methods: Targeted capture and next-generation sequencing was performed on formalin-fixed paraffin-embedded (FFPE) samples, profiling 202 cancer relevant genes in 61 pairs (primary and corresponding recurrence/metastasis). Tumors were classified at baseline as [hormone receptor (HR)+/HER2-, HR+/HER2+, HER-/HER2+, and triple negative breast cancer (TNBC)]. We aligned data to human reference assembly hg19 using Burrows-Wheeler Aligner’s (BWA) and removed duplicated reads. We identified somatic mutations variants and called copy number alterations (CNA) using an algorithm which reports gain or loss status of each exon. Alterations potentially targetable with established or investigational therapeutics were considered “actionable.”

Results: Of the 61 cases, 15% changed breast cancer subtype. Of 747 mutations detected in 156 genes, 309 (41%) were discordant. Median number of mutations were 10 (range 6-11) in P and 8 (range 6-10) in M. Most common mutations occurred at NOTCH2, PCLO, MAP3K1, MLL3, NOTCH4, CRIPAK, TP53, PIK3CA, and FLG. Mutations were less common in HR-/HER2+ tumors in both P and M. Mutation discordance was not different in cases of changed breast cancer subtype (P=.31). Of 986 CNA detected in 173 genes, 758 (77%) were discordant. There was an increased frequency of EGFR1, ERBB2, FGFR3, CRIPAK, MEN1 and WT1 amplifications in M. CNA were less common in HR-/HER2+ tumors in both P and M. CNA discordances were more common in cases of changed breast cancer subtype (P<.0001) and driven by HER2- tumors. Fifty-eight (95%) patients had actionable alterations that could inform targeted treatment options.

Conclusion: Deep targeted exome sequencing of cancer-related genes revealed potentially targeted alterations. We found 41% and 77% mutation and CNA discordance between P and M. CNA were more common when breast cancer subtype changed.
Title: Plasma circulating tumor DNA as an alternative to metastatic biopsies for mutational analyses in breast cancer

Michail Ignatiadis¹, Française Rothe¹, Jean-François Laes², Diether Lambrechts³⁴, Dominiek Smeets³⁴, Delphine Vincent¹, Marion Maetens¹, Debora Fumagalli¹, Stefan Michiels⁵, Stylianos Drisis¹, Carine Moerman¹, Jean-Pol Detiffe², Denis Larsimont¹, Ahmad Awada¹, Martine Piccart¹ and Christos Sotiriou¹. ¹Institut Jules Bordet, Université Libre de Bruxelles, Belgium; ²OncoDNA, Gosselies, Belgium; ³Katholieke Universiteit Leuven, Belgium; ⁴Vesalius Research Center, VIB, Belgium and ⁵Gustave Roussy, Univ. Paris-Sud, France.

Body: Background: Molecular screening programs are using next-generation sequencing (NGS) for cancer gene panels in metastatic biopsies. We interrogated whether plasma can be used as an alternative to metastatic biopsies.

Patients and methods: The Ion AmpliSeqTM Cancer Hotspot Panel v2 (Ion Torrent), covering approximately 2,800 COSMIC mutations from 50 cancer genes was used to analyze 70 primary and/or metastases and 29 plasma samples from 17 metastatic breast cancer patients. The targeted coverage for tissue DNA was 1000x and for plasma circulating DNA 25000x. Whole blood normal DNA was used to exclude germline variants. The Illumina technology was used for independent validation.

Results: Twelve patients had estrogen receptor (ER)+/ human epidermal growth factor receptor 2 (HER2)-, 1 ER+/HER2+, 2 ER-/HER2+ and 2 ER-/HER2- tumors. Evaluable NGS results were obtained for 61 primary/metastases and 29 plasma samples from 17 patients. When primary/metastases were analyzed, 12 of 17 patients had at least 1 mutation (median 1 mutation per patient, range 0-2) in either p53, PIK3CA, PTEN, AKT1 or IDH2 gene. When plasma was analyzed, 11 of 17 patients had at least 1 mutation (median 1 mutation per patient, range 0-2) in either p53, PIK3CA, PTEN, AKT1, IDH2 and SMAD4. All primary/metastases/plasma mutations were independently validated using the illumina technology. When we focused on metastases and plasma samples collected at the same time point, we observed that in 4 patients, no mutation was identified in either metastases or plasma, in 9 patients the same mutations were identified in metastases and plasma, in 2 patients a mutation was identified in metastases but not in plasma and in 2 patients a mutation was identified in plasma but not in metastases (Table1). Thus, in 13 of 17 (76%) patients, metastases and plasma analysis provided concordant results whereas in 4 of 17 (24%) demonstrate discordant results providing complementary information (Table1). Conclusion: Plasma can be tested as an alternative tissue source in molecular screening programs.

Table Mutational status of synchronous metastatic biopsies and plasma samples analysed using the Ion AmpliSeqTM Cancer Hotspot Panel v2

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Gene</th>
<th>Mutation</th>
<th>Metastasis (MAF)</th>
<th>Plasma (MAF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>PTEN</td>
<td>p.Q171E</td>
<td>YES (27.5%)</td>
<td>YES (25.9%)</td>
</tr>
<tr>
<td>3</td>
<td>SMAD4</td>
<td>p.E394*</td>
<td>NO</td>
<td>YES (10.9%)</td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
</tr>
<tr>
<td>6</td>
<td>PIK3CA</td>
<td>p.H1047R</td>
<td>YES (20.5-47.7%)</td>
<td>YES (4.6%)</td>
</tr>
<tr>
<td>7</td>
<td>TP53</td>
<td>p.V274A</td>
<td>YES (35.6-56.1%)</td>
<td>YES (20.7%)</td>
</tr>
<tr>
<td>8</td>
<td>IDH2</td>
<td>p.R140R</td>
<td>YES (28.2)</td>
<td>YES (0.5%)</td>
</tr>
<tr>
<td>10</td>
<td>PIK3CA</td>
<td>p.H1047R</td>
<td>YES (19.7%)</td>
<td>NO</td>
</tr>
<tr>
<td>11</td>
<td>PIK3CA</td>
<td>p.E453K</td>
<td>YES (4.6-17.8%)</td>
<td>YES (2.8%)</td>
</tr>
<tr>
<td>11</td>
<td>PIK3CA</td>
<td>p.E453K</td>
<td>YES (13.1-23%)</td>
<td>YES (3.4%)</td>
</tr>
<tr>
<td>14</td>
<td>PIK3CA</td>
<td>p.H1047R</td>
<td>YES (24.8%)</td>
<td>NO</td>
</tr>
<tr>
<td>14</td>
<td>PIK3CA</td>
<td>p.H1047R</td>
<td>YES (0-13.8%)</td>
<td>YES (0.5%)</td>
</tr>
<tr>
<td>16</td>
<td>TP53</td>
<td>p.Y103*</td>
<td>YES (59.8-86.3%)</td>
<td>YES (49%)</td>
</tr>
<tr>
<td>17</td>
<td></td>
<td></td>
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<td>NO</td>
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<tr>
<td></td>
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<td>Mutation</td>
<td>Call</td>
<td>MAF</td>
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<tr>
<td>---</td>
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<td>----------</td>
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<td>-----</td>
</tr>
<tr>
<td>20</td>
<td>PIK3CA</td>
<td>p.E545K</td>
<td>NO</td>
<td>YES (14.3%)</td>
</tr>
<tr>
<td>30</td>
<td>TP53</td>
<td>p.M237K</td>
<td>YES (27.6-50%)</td>
<td>YES (51.8%)</td>
</tr>
<tr>
<td>37</td>
<td>TP53</td>
<td>p.H193L</td>
<td>YES (61.6-82.9%)</td>
<td>YES (55.5%)</td>
</tr>
<tr>
<td>38</td>
<td>AKT1</td>
<td>p.E17K</td>
<td>YES (26-68.2%)</td>
<td>YES (10%)</td>
</tr>
<tr>
<td>38</td>
<td>TP53</td>
<td>p.R248W</td>
<td>YES (23.6-56.8%)</td>
<td>YES (5.9%)</td>
</tr>
<tr>
<td>39</td>
<td>TP53</td>
<td>p.R136H</td>
<td>YES (7.5%)</td>
<td>NO</td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td>NO</td>
<td>NO</td>
</tr>
</tbody>
</table>

MAF: Mutant allele frequency; For patients with multiple metastases samples at the same time point, the MAF range is provided. Patients 11 & 14 had 2 timepoints with synchronous metastases/plasma samples.
Title: Circulating tumor DNA and circulating tumor cells in metastatic triple negative breast cancer patients

Francois-Clement Bidard, Jordan Madic, Anna Kiialainen, Fabian Birzele, Guillemette Ramey, Quentin Leroy, Thomas Rio Frio, Virginie Raynal, Virginie Bernard, Alban Lermine, Inga Clausen, Nicolas Giroud, Roland Schmucki, Carsten Horn, Olivia Spleiss, Olivier Lantz, Marc-Henri Stern, Martin Weisser, Ronald Lebofsky and Jean-Yves Pierga. 1Institut Curie, Paris, France; 2Roche Pharma Research and Early Development (pRED), Innovation Center, Basel, Switzerland; 3Institut Roche de Recherche et Médecine Translationnelle, Boulogne-Billancourt, France and 4Roche Pharma Research and Early Development (pRED), Innovation Center, Penzberg, Germany.

Body: Background
Preliminary reports suggested that circulating tumor DNA (ctDNA) can be used as a prognostic marker in a way akin to circulating tumor cells (CTC) in metastatic breast cancer patients. However ctDNA detection is often performed on multiple mutations, combining heterogeneous techniques. Here we used the high prevalence of TP53 mutations in triple-negative metastatic breast cancer (TNMBC) to compare CTC and ctDNA detection rates and prognostic value.

Methods
A cohort of 40 patients treated at the Institut Curie (Paris, France) was enrolled before starting a new line of treatment for TNMBC. CTC were detected by the CellSearch system (in 7.5 mL of blood). Using massively parallel sequencing (NGS), TP53 mutations were first characterized in tumor tissue, then in plasma DNA extracted from fresh frozen plasma samples (from 15-20 mL of blood). ctDNA detection was performed using high depth targeted sequencing using two platforms in parallel (Illumina HiSeq 2500 and Roche 454). Libraries for Illumina were prepared following the TAm-Seq procedure (Forshew et al, Sci Transl Med 2012), with preamplification of all coding TP53 exons and flanking untranslated regions followed by both paired-end 150bp Illumina and 454/Roche sequencing. CTC, ctDNA and usual patient characteristics were correlated with time to progression (TTP) and overall survival (OS).

Results
Archived tumor (FFPE or frozen) tissue was available for 36 patients, and 31 were successfully sequenced: TP53 mutations were found in 27 patients. As measured on the Illumina platform, ctDNA was detected in 21/27 patients (81%), ranging from 48 to 648,000 copies/mL of plasma (median 1620). Mutant allele fraction in circulating cell-free DNA ranged from 2 to 70% (median 5%). Comparison between ctDNA levels measured by Illumina and 454/Roche platforms in plasma displayed a good correlation (R² = 0.903), with a single discordance. ≥1 CTC were detected in 19 of these 27 patients (70%). Strikingly, high ctDNA levels had prognostic impact neither on OS, nor on TTP, whatever the dataset used (Illumina or 454) whereas CTC≥5/7.5 mL were correlated with OS (p=0.04) and marginally with TTP (p=0.06). Other known usual factors, such as poor performance status, elevated LDH and number of previous treatment lines had also significant prognostic factors in this cohort. CTC and ctDNA early changes during treatment were available for 12 patients and changes (increase/decrease) of the two biomarkers were globally similar.

Conclusion
Demonstrating a good sensitivity (81%), ctDNA by the TAm-Seq is more frequently detected than CTCs in the 27 TNMBC with TP53 mutations. The observed correlation between the 2 massively parallel sequencing approaches suggested that ctDNA levels data were quantitative. In contrast to other usual prognostic factors, baseline ctDNA level did not demonstrate a prognostic impact, in this proof-of-principle study, suggesting that mechanisms of ctDNA release in TNMBC rely on biological features that do not dramatically impact patient’s outcome.
2014 San Antonio Breast Cancer Symposium

Publication Number: PD4-1
Average Grade: 7.00

Title: Timing, severity and risk factors for arthralgia in the IBIS-II trial: A retrospective and exploratory analysis

Ivana Sestak¹, Anthony Howell², John F Forbes³, Patrick Neven⁴ and Jack Cuzick¹. ¹Centre for Cancer Prevention, Queen Mary University, London, United Kingdom; ²Institute of Cancer Sciences, University of Manchester, Manchester, United Kingdom; ³School of Medicine and Public Health, University of Newcastle, Newcastle, Australia and ⁴UZ Leuven, Leuven, Belgium.

Body: Background: Arthralgia is a well known side effect of aromatase inhibitors and low oestrogen levels and postmenopausal status are associated with this event. Anastrozole reduced the incidence of oestrogen receptor positive, invasive breast cancer by 58% in the IBIS-II trial. However, timing, severity and risk factors for arthralgia have not been assessed in detail in this trial.

Methods: The IBIS-II trial randomised postmenopausal women at high risk to receive 1mg anastrozole or matching placebo for 5 years. Date of occurrence of arthralgia along with severity (mild, moderate, severe) were recorded at each yearly follow-up visit. Age, body mass index (BMI), and previous hormone replacement therapy (HRT) were investigated as potential risk factors for arthralgia. All analyses were done by the use of logistic regression.

Results: 3864 postmenopausal women (anastrozole: 1920, placebo: 1944) were enrolled in the IBIS-II trial. 58.5% of women randomised to anastrozole reported arthralgia at any time during the trial compared with 52.8% on placebo (OR=1.26 (1.11-1.43), P=0.0004). The majority of arthralgias were reported within the first 18 months of randomisation, with a decline thereafter (Table). 17.5% of women who reported arthralgia withdrew from the trial compared to 13.9% without any of these symptoms (OR=1.31 (1.10-1.57)), and the withdrawal was significantly greater for those with severe symptoms compared to mild (OR=5.97 (4.27-8.33)). Women who used HRT before trial entry had a significant higher risk of developing arthralgia than their counterparts irrespective of allocated treatment (OR=1.45 (1.27-1.64), P<0.001). Increasing BMI (lowest vs. highest BMI group: OR=1.30 (1.11-1.53)) and age (lowest vs. highest age group: OR=1.23 (1.01-1.50)) were also significant risk factors for arthralgia. HRT and BMI remained highly significant in a multivariate model.

Conclusion: Arthralgia was common in the IBIS-II trial irrespective of treatment. However it increased in severity in the active treatment arm mainly in the 18 month period after randomisation. Severe arthralgia led to significantly more withdrawals from the trial than mild symptoms irrespective of treatment. Major risk factors for arthralgia in both arms were previous HRT use and obesity.

<table>
<thead>
<tr>
<th></th>
<th>Overall</th>
<th>0-18 months</th>
<th>18-30 months</th>
<th>30-42 months</th>
<th>42-54 months</th>
<th>&gt;54 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anastrozole</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women at risk</td>
<td>1920</td>
<td>1150</td>
<td>842</td>
<td>673</td>
<td>543</td>
<td>401</td>
</tr>
<tr>
<td>Number with arthralgia</td>
<td>1123</td>
<td>770</td>
<td>170</td>
<td>86</td>
<td>53</td>
<td>44</td>
</tr>
<tr>
<td>Percentage (%)</td>
<td>58.5</td>
<td>67.0</td>
<td>20.2</td>
<td>12.8</td>
<td>9.8</td>
<td>11.0</td>
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</table>

<table>
<thead>
<tr>
<th>Placebo</th>
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<th></th>
<th></th>
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<th></th>
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</thead>
<tbody>
<tr>
<td>Women at risk</td>
<td>1944</td>
<td>1294</td>
<td>979</td>
<td>782</td>
<td>618</td>
<td>481</td>
</tr>
<tr>
<td>Number with arthralgia</td>
<td>1026</td>
<td>650</td>
<td>153</td>
<td>109</td>
<td>69</td>
<td>45</td>
</tr>
<tr>
<td>Percentage (%)</td>
<td>52.8</td>
<td>50.3</td>
<td>15.6</td>
<td>13.9</td>
<td>11.2</td>
<td>9.4</td>
</tr>
</tbody>
</table>

OR (95% CI) (A vs. P) 1.26 (1.11-1.43) 1.33 (1.17-1.52) 1.29 (1.01-1.65) 0.92 (0.67-1.25) 0.87 (0.59-1.29) 1.17 (0.74-1.86)
Title: Cardiovascular toxicity following aromatase inhibitor use in 13,273 survivors cared for in a HMO

Reina Haque¹, Joanne E Schottinger¹, Jiaxiao Shi¹, Joanie Chung¹, Chantal Avila¹, Britta Amundsen¹ and Rowan T Chlebowski².
¹Kaiser Permanente Southern California, Pasadena, CA and ²Los Angeles BioMedical Research Institute at the Harbor-UCLA Medical Center, Torrance, CA.

Body: Background
Aromatase inhibitors (AIs) reduce breast cancer incidence in primary prevention trials (MAP3, IBIS2). However, controversy regarding AI's influence on cardiovascular disease (MI, angina, and cardiac failure) (Amir et al JNCI 2011) could limit use in prevention settings.

Methods
We assembled a cohort of 13,273 postmenopausal breast cancer patients initially CVD (cardiovascular disease)-free at diagnosis in a large managed care organization. Women were diagnosed 1991-2010, and followed through 2012. The outcome, CVD risk was compared across endocrine treatments (AI, tamoxifen [TAM], both, or neither). Information on demographics, comorbidity (diabetes, hypertension, etc.), and covariate medications (antihyperlipidemics, antihypertensives, and other CVD drugs) were available from electronic medical records. We conducted Cox models using time-dependent endocrine drug use variables adjusted for age, demographics, comorbidity, and CVD drug use, cancer treatment, tumor characteristics and tumor laterality.

Results
Among the 13,273 cohort, postmenopausal women who used AIs exclusively had a similar risk of ischemic disease (HR=0.97, 95% CI: 0.78-1.22) and stroke (HR=0.97, 95% CI: 0.70-1.33) versus those who used TAM only (HR=1.00, reference). However, women who used AIs only had a higher risk of other CVD disease combined (CHF, cardiomyopathy, dysrhythmia, valvular dysfunction, pericarditis) (HR=1.26, 95% CI: 1.11-1.43) than those exposed to TAM only. The risk of other CVD disease was greater among women exposed to sequential TAM and AI treatment. The results are based on 3,711 CVD events occurring in 72,886 woman-years of follow-up.

<table>
<thead>
<tr>
<th></th>
<th>Tamoxifen Only</th>
<th>AI Only</th>
<th>Both Tam and AI</th>
<th>No Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MUTUALLY EXCLUSIVE CATEGORIES</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ischemic</td>
<td>1.00 (ref)</td>
<td>0.97 (0.78 - 1.22)</td>
<td>1.04 (0.83 - 1.29)</td>
<td>1.02 (0.84 - 1.23)</td>
</tr>
<tr>
<td>Stroke</td>
<td>1.00 (ref)</td>
<td>0.97 (0.70 - 1.33)</td>
<td>1.02 (0.75 - 1.37)</td>
<td>0.93 (0.70 - 1.24)</td>
</tr>
<tr>
<td>Other CVD</td>
<td>1.00 (ref)</td>
<td>1.26 (1.11 - 1.43)</td>
<td>1.28 (1.13 - 1.44)</td>
<td>1.23 (1.09 - 1.38)</td>
</tr>
<tr>
<td>COMPOSITE CVD</td>
<td>1.00 (ref)</td>
<td>1.15 (1.04 - 1.28)</td>
<td>1.19 (1.07 - 1.31)</td>
<td>1.14 (1.04 - 1.25)</td>
</tr>
</tbody>
</table>

Based on a subset of 7,982 patients who underwent breast irradiation, the risk of CVD overall was greater among women who used AIs only and received left-sided irradiation (HR=1.21, 95% CI: 1.02-1.44).

<table>
<thead>
<tr>
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<th>Tamoxifen Only</th>
<th>AI Only</th>
<th>Both Tam and AI</th>
<th>No Hormones</th>
</tr>
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<tbody>
<tr>
<td><strong>Right-sided breast irradiation</strong></td>
<td></td>
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<tr>
<td>COMPOSITE CVD</td>
<td>1.00 (ref)</td>
<td>1.16 (1.00 - 1.34)</td>
<td>1.20 (1.04 - 1.38)</td>
<td>1.08 (0.95 - 1.23)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Tamoxifen Only</th>
<th>AI Only</th>
<th>Both Tam and AI</th>
<th>No Hormones</th>
</tr>
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<tbody>
<tr>
<td><strong>Left-sided breast irradiation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>COMPOSITE CVD</td>
<td>1.00 (ref)</td>
<td>1.16 (1.00 - 1.34)</td>
<td>1.20 (1.04 - 1.38)</td>
<td>1.08 (0.95 - 1.23)</td>
</tr>
</tbody>
</table>
Discussion
These results indicate that variation exists in the type of CVD events that occur in breast cancer patients receiving AIs in comparison to tamoxifen users. For example, the risk of ischemic disease or stroke was not elevated in those who used AIs only versus TAM users. However, overall CVD events were greater in women who used AIs only (or sequentially after TAM), especially if they received left-sided breast irradiation. While these observational study results require cautious interpretation, they provide a basis for comparing the benefits and risks of endocrine treatments.
Title: Prevalence, health care utilization and costs of concomitant depression among breast cancer survivors

Diana D Jeffery1. 1Defense Health Agency, Healthcare Operations Directorate, Clinical Support Division, Falls Church, VA.

Body: Purpose: Concomitant conditions in a cohort of non-elderly breast cancer survivors (BCS) were examined with a focus on the prevalence, healthcare utilization, and costs of diagnosed depression. Little research has been conducted on how concomitant depression among cancer survivors impacts utilization and costs of care.

Methods: Using administrative claims data from the Military Health System Data Repository (MHSDR), a cohort of 2,851 BCS was identified with at least 2 years survival from the time of diagnosis. Concomitant conditions were based on ICD-9 codes; codes 296.2, 296.3, 298.0, and 311 were used to identify depression. Fiscal year 2009 was used as the index year to calculate healthcare utilization and costs. Bivariate analyses and logistic regression analysis were used to examine group differences and predictors of having received a diagnosis of depression.

Findings. The most common concomitant chronic conditions in the BCS cohort were hypertension (50.0%), mood disorders or adjustment disorders (37.5%), heart disease (23.0%), diabetes (19.9%), history of tobacco use (19.7%), asthma or chronic obstructive pulmonary disease (16%), and obesity (16.8%). About 15.9% of the BCS were diagnosed with depression in the year prior to, at the time of, or in the 2-year follow up period after the cancer diagnosis. With bivariate analysis, significant differences were found between BCS with and without depression: those with depression had higher mean number of hospital stays (.33 vs .11), mean number of bed days (1.94 vs .58), mean number of ambulatory visits (34.26 vs 20.42), and mean number of pharmacy prescriptions (45.49 vs 27.60). For follow up care, BCS with a diagnosis of depression cost, on average, $7174 more annually then those without a diagnosis of depression ($15,471 vs $8,297). No demographic characteristics significantly increased the likelihood of having received a diagnosis of depression.

Discussion. The results show much higher annual health care utilization and costs for BCS diagnosed with depression compared to BCS without a diagnosis of depression. These findings may reflect the health care plan provided to military-related beneficiaries, a plan that has few restrictions for cancer follow up care if medically ordered. Claims data contains no information about cancer stage, a correlate of health care utilization and costs. Overall, the findings provide empirical evidence that there is a fiscal incentive to screen and manage mild symptoms prior to patients meeting diagnostic criteria for clinical depression. Assumedly, timely screening and rapid intervention will lead to improved quality of life for the patient, decreased utilization of health care resources, and cost savings for health care plans. In this respect, the findings support the 2014 ASCO recommendations regarding screening and treatment for depression and anxiety. Conversely, the data intimate that adoption of the ASCO recommendations will lead to higher costs as more individuals are positively screened and referred for treatment. Who, what, or how such services will be afforded merits sustained inquiry.

The opinions expressed herein are those of the author and are not necessarily representative of the opinions or policies of the Department of Defense.
Title: Blood draws, injections, blood pressure readings in the at-risk arm, and flying might not be associated with increases in arm volume: A prospective study

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Body: Introduction:
Breast cancer related lymphedema (BCRL) is a swelling caused by compromise of the lymphatic system after breast cancer treatment. Commonly-cited risk factors include treatment related variables such as axillary lymph node dissection (ALND) and regional lymph node radiation (RLNR), and patient characteristics including BMI. Patients are often advised to avoid blood draws, injections, and blood pressure cuffs on their at-risk arm, airplane travel, and extensive exercise to reduce the risk of developing BCRL; however, data demonstrating the efficacy of such avoidance strategies do not exist. We sought to determine the impact of blood draws, injections, and blood pressure readings in the at-risk arm, and flying on increases in arm volume in a large, prospective cohort of patients.

Methods:
522 patients who underwent treatment for unilateral breast cancer between were included. Patients were prospectively screened for BCRL with Perometer arm measurements pre-operatively, post-operatively, and at 3-8 month intervals thereafter. At each measurement patients were asked to report number of blood draws, injections, and blood pressure readings in the at-risk arm, and number of flights since the last measurement, and their responses were assessed for association with relative volume change (RVC). RVC was analyzed as a continuous variable for association with risk factors.

Results:
522 patients with 2033 post operative measurements were included. Patients were followed for a median of 23 months and 4 post-operative measurements, with a minimum of 1 post-operative measurement and a maximum of 14. 5.56%. 76.8% (401/522) underwent lumpectomy, 23.2% (121/522) underwent mastectomy. 70% (366/522) underwent sentinel lymph node biopsy, and 19% (98/522) underwent ALND. 62.4% (352/521) received radiation to the breast/ chest wall only, and 21.5% (112/521) also received regional lymph node radiation. By univariate analysis, there was no significant association between RVC increase and undergoing one or more blood draws (p=0.36), blood pressure (p=0.88), injections (p=0.79), or number of flights (p=0.89). ALND was significantly associated with increases in arm volume (p=0.0017) by univariate analysis and older age at diagnosis was associated with increased RVC with borderline significance (p=0.059).

<table>
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<th># since last measurement</th>
<th>Blood Draw</th>
<th>Blood Pressure</th>
<th>Injection</th>
<th>Number of flights</th>
</tr>
</thead>
<tbody>
<tr>
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<td>91.2% (1796/1969)</td>
<td>83% (1633/1967)</td>
<td>97.6% (1923/1969)</td>
<td>72% (1461/2031)</td>
</tr>
<tr>
<td>1 or more</td>
<td>8.8% (173/1969)</td>
<td>17% (334/1967)</td>
<td>2.4% (46/1969)</td>
<td>28% (570/2031)</td>
</tr>
</tbody>
</table>

Conclusions:
In our patient population, non-treatment related risk factors including blood draws, blood pressures, and injections in the at-risk arm, and flying were not significantly associated with increases in arm volume. This data can be used to help improve and refine patient education regarding the importance of risk-reducing practices after breast cancer treatment.
Title: Characteristics associated with nonadherence to medications for hypertension, diabetes, and dyslipidemias among breast cancer survivors

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Body: Background: Comorbidity among breast cancer (BC) survivors increases risk of overall mortality and recent reports have described some comorbid conditions to possibly influence risk of second BC events and BC-specific mortality. Therefore, clinical management of chronic conditions such as hypertension (HTN), diabetes mellitus (DM) and dyslipidemias in BC survivors may be important to both cancer- and non-cancer-related outcomes. Medication adherence for chronic conditions such as diabetes is poor in general populations and quality of care may be further impacted by BC diagnosis/treatment. The objective of this study was to describe characteristics associated with nonadherence to medications to treat HTN, DM and dyslipidemias among BC survivors enrolled in a large, integrated health plan.

Methods: Retrospective cohort of 4,216 BC survivors, the Commonly Used Medications and Breast Cancer Outcomes (COMBO) study at Group Health Cooperative. Women in our analysis were diagnosed with stages I-II BC between 1990-2008 and alive and without recurrence or second primary BC in the year 365 days post-BC diagnosis (days 366-730). Medication users were identified by ≥1 dispensings of antihypertensives, oral DM medications, and/or statins. Data on incident BC, patient characteristics, and medications was obtained via linkage to the western Washington SEER registry, medical record and automated health plan data including pharmacy dispensing records. Medication adherence was measured using medication possession ratio (MPR) and classified as non-adherent if MPR<0.80. We estimated odds ratios (ORs) and 95% confidence intervals (CIs) for nonadherence vs adherence to antihypertensives, oral DM medications and statins by various characteristics using multivariate logistic regression.

Results: We identified 1,929 prevalent users of antihypertensives (n=1,779), DM medications (n=499) and/or statins (n=1,072). 37% were non-adherent to antihypertensives; 75% were non-adherent to DM medications; 39% were non-adherent to statins. In adjusted models, younger women (ages <50, 50-64) were more likely to be non-adherent to all 3 therapeutic classes compared to older women (ages ≥65). Women who received radiation therapy (OR=1.21 95% CI 1.00-1.47) or endocrine therapy (OR=1.25 95% CI 1.03-1.52) were more likely to be non-adherent to antihypertensives; women treated with chemotherapy (OR=1.67 95% CI 1.03-2.69) were more likely to be non-adherent to DM medications. Greater BMI (P=0.001) and more frequent primary care provider visits (≥2 vs 0-1 only; OR=0.30 95% CI 0.24-0.38) were associated with better adherence to antihypertensives. Likewise, higher Charlson comorbidity scores (≥2 vs <2) were associated with greater adherence to DM medications (OR=0.49 95% CI 0.23-0.83) and statins (OR=0.54 95% CI 0.28-1.02).

Conclusion: In this population-based cohort of BC survivors, nonadherence to medications for HTN, DM, and dyslipidemias was associated with younger age, and impact of specific BC treatments on medication adherence varied by therapeutic indication. Additional research is warranted to target patients in need of medication management as well as to explore patient preferences and provider factors that may influence medication adherence.
A randomized placebo-controlled trial of acupuncture and gabapentin for hot flashes among breast cancer survivors

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Body: Purpose
Hot flashes are a common and debilitating symptom negatively affecting the quality of life of breast cancer survivors. We sought to compare the short and long term effects of electro-acupuncture (EA) vs. gabapentin for hot flashes among breast cancer survivors.

Patients and Methods
We conducted a randomized controlled trial of EA vs. gabapentin vs. placebos (sham acupuncture [SA] or placebo medication) in women with breast cancer who had completed primary cancer treatments and experienced bothersome hot flashes twice daily or greater. Acupuncturists performed ten EA/SA treatments over eight weeks using a manualized protocol with 2 Hz electro-stimulation delivered by a TENS unit. Acupuncturists administered SA using Streitberger (non-penetrating) needles at non-traditional acupuncture points without electro-stimulation. Gabapentin (900 mg daily) or placebo medication were continued for eight weeks and then weaned off. The primary endpoint was the hot flash composite score measured by the daily diary at the end of the intervention (Week 8). A secondary endpoint, durability of response, was evaluated at Week 24 from randomization. Longitudinal mixed effects models were used to evaluate change in outcomes over time and group differences.

Results
Of 120 randomly assigned patients, the mean age was 52.3, 75% were White, 12.5% were peri-menopausal, and 20%/25%/37.5% had natural/surgically/chemically induced menopause, respectively. By Week 8, significant group differences were observed. Mean reduction in hot flash composite scores was greatest in the EA group, followed by SA and gabapentin, with placebo medication having the lowest reduction in hot flashes (−7.4 vs. −5.9 vs. −5.2 vs. −3.4, p=0.0003). By Week 24 and off treatment, reduction in hot flashes in the EA group persisted whereas the hot flashes in the gabapentin group returned to baseline. The reduction in hot flashes was greatest in the EA group, followed by SA, placebo medication, and gabapentin (−8.5 vs. −6.1 vs. −4.6 vs. −2.8, p=0.0024). No serious adverse events were reported in any groups. The gabapentin group had the highest percentage of participants reporting treatment-related adverse events followed by placebo medication, EA, and SA (48.4% vs. 29.0% vs. 19.3% vs. 3.2%, p=0.004).

Conclusion: Electro-acupuncture was more effective than gabapentin, sham acupuncture, or placebo medication in reducing hot flashes for breast cancer survivors both short term and long term with few side effects. Gabapentin produced significant short term reduction in hot flashes that did not persist off medication and was associated with more side effects.

Clinical Trial Registration: NCT01005108.
**Title:** Results from the phase 2 trial of ridaforolimus, dalotuzumab, and exemestane compared to ridaforolimus and exemestane in advanced breast cancer

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**Body:** Introduction: The combination of a mammalian target of rapamycin (mTOR) inhibitor and an aromatase inhibitor has been shown to significantly increase progression-free survival (PFS) in patients with estrogen receptor-positive (ER+) advanced or metastatic breast cancer. Ridaforolimus is an alternative mTOR inhibitor with high potency and specificity. We hypothesized that triplet therapy with ridaforolimus, dalotuzumab (a humanized monoclonal antibody targeting the IGF-1 receptor [IGFR]), and exemestane (R/D/E) would be more effective than doublet therapy with ridaforolimus and exemestane (R/E).

**Methods:** This phase 2, randomized, open-label trial enrolled 80 postmenopausal patients who had high-proliferation (Ki67 staining) ER+ breast cancer that had progressed following treatment with a nonsteroidal aromatase inhibitor. Patients received either triplet therapy, at the previously determined maximum tolerated dose of oral ridaforolimus 10 mg QD×5, dalotuzumab 10 mg/kg/week IV, and oral exemestane 25 mg/day (R/D/E, n=40), or doublet therapy with R 30 mg QD×5 and E 25 mg/day (R/E, n=40). Dose increases of R to 20 or 40 mg QD×5 were permitted in the R/D/E or R/E arms, respectively, in the absence of grade ≥2 stomatitis after cycle 1. The R dose could be reduced in either arm for toxicity. The primary endpoint was PFS in the ITT population by central review. Adverse events (AE) of clinical interest (Tier 1) included stomatitis, pneumonitis, hearing loss, and hyperglycemia.

**Results:** Baseline characteristics were balanced between treatment groups. The median PFS was 23.3 (95% CI, 8.71, 38.43) weeks for R/D/E versus 31.9 (95% CI, 16.00, 39.29) weeks in the R/E arm (hazard ratio, 1.18; 80% CI, 0.81-1.72; P=0.565). All patients experienced at least one AE. 5 (12.8%) and 3 (7.5%) patients in the R/D/E and R/E arms, respectively, discontinued the study because of AE. Serious drug-related AE occurred in 2.6% of the R/D/E arm and 15% of the R/E arm. Dose modifications due to AE occurred in 10.3% and 50% in the R/D/E and R/E arms, respectively (difference -39.7%; 95% CI, -56.7, -20.4). Tier 1 AE were primarily grade 1-2 in severity. Stomatitis occurred in 76.9% (30/39 patients) in the R/D/E arm vs 95.0% (38/40 patients) in the R/E arm (P=0.021), and grade 3-4 stomatitis was similar between arms (23.1% vs 25%). Pneumonitis occurred in 5.1% vs 22.5% (P=0.027) and hearing loss occurred in 1 patient in each treatment arm (2.6% vs 2.5%), all grade 1-2. Hyperglycemia occurred at a similar rate in both treatment arms (28.2% vs 27.5%), with grade 3-4 events in 4 (10.3%) and 3 (7.5%) patients in the R/D/E and R/E arms, respectively.

**Conclusions:** The combination of R 10 mg QD×5, D, and E did not improve PFS when compared to R 30 mg QD×5 plus E. The incidence rates of AE were lower in the R/D/E arm than the R/E arm for most categories of adverse events, likely because of the higher dose of R in the R/E arm. The efficacy reported for R/E in this study is similar to that reported in previous studies evaluating mTOR inhibitors in combination with exemestane in ABC. Overlapping toxicities and lower doses likely contributed to the lack of improved PFS with the addition of the IGFR inhibitor to this combination.
Ph1b study of the PI3K inhibitor taselisib (GDC-0032) in combination with letrozole in patients with hormone receptor-positive advanced breast cancer

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Body: Background: Taselisib (GDC-0032) is a next-generation PI3K inhibitor with increased anti-tumor activity against PIK3CA mutant (MT) cancers. Taselisib is an orally bioavailable, potent, and selective inhibitor of Class I PI3K alpha, delta, and gamma isoforms, with 30-fold less inhibition of the PI3K beta isoform relative to the PI3K alpha isoform. Preclinical data show that taselisib has enhanced activity against PI3K alpha isoform (PIK3CA) MT breast cancer cell lines and enhanced antitumor activity when combined with letrozole. Clinical data with single-agent taselisib also showed increased tumor shrinkage in patients with PIK3CA MT breast cancer as compared to patients with PIK3CA wildtype (WT) breast cancer.

Material and Methods: A Phase 1b dose escalation study was conducted with evaluation of taselisib doses ranging from 6-9 mg QD in combination with letrozole 2.5mg QD in a modified 3+3 design. A dose expansion cohort was conducted with taselisib 6 mg QD. Safety and tolerability of GDC-0032 was assessed, as well as pharmacokinetics (PK), pharmacodynamic (PD) assessment by FDG-PET, and anti-tumor activity by RECIST.

Results: As of 31 January 2014, 28 patients were enrolled onto this study with the completion of dose escalation and the dose expansion cohort. No dose limiting toxicities (DLTs) were observed at either the 6 mg (n = 20) or 9 mg (n = 8) dose levels. Adverse events (AEs) assessed by the investigator as related to taselisib in ≥10% of patients (any grade) included diarrhea, nausea, stomatitis, fatigue, rash, decreased appetite, hyperglycemia, dysgeusia, mucosal inflammation, vomiting, muscle spasms, asthenia, dry mouth, dry skin, pruritus, and aspartate aminotransferase increased. Grade 3 and 4 adverse events assessed by the investigator as drug-related and occurring in greater than one patient included diarrhea (14%), hyperglycemia (7%), and mucosal inflammation (7%). No apparent PK interactions were observed between taselisib and letrozole. The median number of prior systemic therapies was six, and promising efficacy data has been observed in these heavily pretreated patients. Metabolic partial responses via FDG-PET (≥20% decrease in mean SUVmax) were observed in 11 out of 18 patients assessed (61%). Confirmed partial responses by RECIST have been observed at both the 6mg and 9mg taselisib dose levels. For patients with measurable disease at baseline, the overall response rate of 38% was observed in patients with PIK3CA MT breast cancer and 9% in patients with PIK3CA WT breast cancer. Updated data on safety, PD, efficacy, and biomarker correlates will be presented.

Conclusions: The combination of taselisib and letrozole is a well-tolerated regimen with promising preliminary efficacy in PIK3CA MT breast cancer patients. This preliminary Ph1b clinical data is consistent with taselisib preclinical and single-agent clinical data showing increased anti-tumor activity for taselisib in PIK3CA MT breast cancer as compared to PIK3CA WT breast cancer. Taselisib is being further investigated in the neoadjuvant setting in combination with letrozole in the LORELEI study in patients with untreated hormone receptor-positive breast cancer.
Title: Phase I trial: PI3Kα inhibitor BYL719 plus aromatase inhibitor (AI) for patients with hormone receptor-positive (HR+) metastatic breast cancer (MBC)

Payal D Shah1, Mary Ellen Moynahan1, Shanu Modi1, Betty Ann Caravella1, Farrah M Datko2, Stephen Zamora1, Elizabeth Comen1, Theresa Gilewski1, Steven M Sugarman1, Gabriella D’Andrea1, Diana E Lake1, Shari B Goldfarb1, Sujata Patil1, Anne Covey1, Michael F Berger1, Mario E Lacouture1, Larry Norton1, Clifford A Hudis1, Jose Baselga1, Sarat Chandarlapaty1 and Maura N Dickler1. 1Memorial Sloan Kettering Cancer Center, New York, NY and 2Front Range Cancer Specialists, Fort Collins, CO.

Body: Background: Phosphatidylinositol 3-kinase (PI3K) hyperactivation plays a role in endocrine therapy resistance. Adding an α-selective PI3K inhibitor (BYL719) to hormonal therapy may therefore overcome resistance in HR+ MBC. We report results from a phase I study to evaluate the safety and preliminary efficacy of BYL719 plus an AI in patients (pts) with HR+ MBC. Methods: This 3+3 dose-escalation trial studied daily oral BYL719 added to standard dose letrozole (L, Arm A) or exemestane (E, Arm B), and later examined intermittent dosing (Arm C, L + BYL719 every other week; Arm D, E + BYL719 on 5 of 7 days weekly). Pts with HR+ MBC, any/no PIK3CA mutation, and on L/E were eligible. A cycle (C) was 28 days (d). Endpoints were dose-limiting toxicity (DLT), tolerability (CTCAE 4.0), and efficacy (RECIST v1.1). Paired tumor biopsies were performed for genomic and proteomic correlatives. Serial plasma was collected to quantify cell-free (cf) DNA and mutant allele fraction. Results: 14 pts (median (M) age: 55 (30-69) yrs), 7 each on Arms A and B, received a M of 76d (6-312+) of BYL719 + L or E. All were evaluable for toxicity, 10 for response. PIK3CA status was mutant(MT)/wild-type(WT)/unknown in 8/5/1 pts. M number of prior MBC therapies was 2 (1-12) in Arm A, 6 (2-14) in Arm B. Arms had similar toxicities. On Arm A, BYL719 was given at 300mg daily (DL0) to 3 pts who completed the 28d DLT period. 2 pts had 3 distinct DLTs: maculopapular rash (N=1), hyperglycemia (N=1), abdominal pain (N=1). Dose was de-escalated (DL-1=250mg) with no DLT in 3 enrolled pts. On Arm B, DL0, 1 pt experienced DLT (maculopapular rash) of 3 initially enrolled pts. Arm B expansion at DL0 had 1 additional pt with DLT (rash). Clinically significant, treatment-related toxicities included grade (G): none; G3: maculopapular rash (N=8, including 1 pt treated at DL-1), hyperglycemia (N=1) and G1/2: fatigue (N=7), nausea (N=7), and hyperglycemia (N=6). Toxicity required 6 dose reductions in 4pts and discontinuation in 2 pts. M duration on study for PIK3CA MT vs. WT was 169.5d vs. 69.5d, respectively. In pts with PIK3CA-WT MBC evaluable for response (n=7), 6 had clinical benefit: 1 PR (pt heavily pre-treated, including prior L, MBC to liver, Arm A, now C10+ after DLT); SD (n=5, included -29.9%, -19%, -12%), and POD (n=1). In pts with PIK3CA-WT MBC evaluable for response (n=3), 2 had SD (no changes ≥/+5%) and 1 had POD. Serial cfDNA analysis in 4 pts with SD or PR demonstrated a decrease of >90% in the PIK3CA mutant allele fraction on treatment. Due to toxicity seen with continuous BYL719, the study was amended to explore intermittent dosing schedules (Arm C, L; Arm D, E; DL0=250mg), with 5 pts enrolled, 3 of whom have completed the DLT period with no DLTs, and 1 pt with G3 rash. Correlative studies including serial of DNA collection from these pts is ongoing. Conclusions: Continuously dosed BYL719 with L or E shows promising antitumor activity. Skin toxicity warranted evaluation of alternative schedules. Mutant allele fraction may be an early predictor of response and may serve as a pharmacodynamic marker during intermittent treatment. Safety, efficacy, and correlative data from study Arm C and Arm D will also be presented.
**Title:** Phase I study of the PI3Kα inhibitor BYL719 plus fulvestrant in patients with PIK3CA-altered and wild type ER+/HER2- locally advanced or metastatic breast cancer

**Body:**

**Background:**

BYL719 selectively inhibits the α-isoform of Class I PI3K. PI3Kα is encoded by PIK3CA, a frequently altered gene in human cancers. Preclinical data indicate BYL719 may be more effective in patients (pts) with PIK3CA-altered tumors; however there are data to suggest that PIK3CA-wild-type (wt) tumors may also be sensitive to BYL719. Here, we present updated data from the Phase I study of BYL719 + fulvestrant in pts with PIK3CA-altered or -wt ER+/HER2– locally advanced/metastatic breast cancer (BC) (NCT01219699).

**Methods:**

Adult women with PIK3CA-altered (mutation or amplification) ER+/HER2– BC received continuous oral BYL719 (300–400 mg/day; 28-day cycles) + fixed-dose fulvestrant (500 mg every 4 weeks, plus an additional dose 2 weeks after first dose) during dose escalation and expansion. Pts with PIK3CA-wt ER+/HER2– BC were enrolled into the dose expansion to receive BYL719 400 mg/day + fulvestrant. A Bayesian logistic regression model with overdose control guided dose escalation. Primary objective: to determine the maximum tolerated dose (MTD) and/or recommended phase II dose (RP2D) of BYL719 in combination with fulvestrant, which was declared previously as 400 mg/day. An expansion cohort at the MTD assessed safety (CTCAE v4.0), tolerability, pharmacokinetics (PK), and preliminary efficacy (RECIST v1.0).

**Results:**

As of May 2, 2014, 64 pts (PIK3CA-altered n=41; PIK3CA-wt n=16; PIK3CA status unknown/pending n=7) received BYL719 300–400 mg/day + fulvestrant. Median number of prior antineoplastic therapies: 5 (range: 1–12) for pts with PIK3CA-altered tumors and 5 (range: 4–16) for pts with PIK3CA-wt tumors. Prior fulvestrant treatment: 19 (46%) and 7 (44%) pts with PIK3CA-altered and -wt tumors, respectively. Overall, the most common (>25%) adverse events (AEs; all grades/all doses) suspected to be study drug-related were hyperglycemia (41%), diarrhea (34%), nausea (30%), and vomiting (25%). The most common (>10%) study drug-related Grade 3/4 AEs (all doses) were maculopapular rash (14%) and hyperglycemia (13%). Preliminary antitumor activity was observed in this trial. At data cut-off, partial responses (PRs) were observed in 2 patients with PIK3CA-altered tumors evaluable for response (2/33, 6%), but no PRs were observed in the 15 evaluable patients with PIK3CA-wt tumors. Duration of exposure was >16 weeks in 24 (59%) patients with PIK3CA-altered tumors and in 5 (31%) patients with PIK3CA-wt tumors. PK and exposure of BYL719 + fulvestrant was similar to that observed with single-agent BYL719 at the same dose levels. At data cut-off, treatment was ongoing in 20 (49%) and 2 (13%) pts with PIK3CA-altered and -wt tumors, respectively.

**Conclusions:**

BYL719 + fulvestrant demonstrated a favorable safety profile in pts with PIK3CA-altered and -wt ER+/HER2– BC, with mostly on-target effects (i.e. hyperglycemia, rash). Preliminary clinical activity was seen in pts with PIK3CA-altered and -wt tumors, but confirmed PRs were only observed in pts with PIK3CA-altered tumors. The low number of pts with PIK3CA-wt tumors limits further conclusion.
Title: A phase I study of BKM120 and fulvestrant in postmenopausal women with estrogen receptor positive metastatic breast cancer

Cynthia X Ma¹, Jingqin Luo¹, Michael Naughton¹, Foluso Ademuyiwa¹, Rama Suresh¹, Timothy Pluard¹, Gayathri Nagaraj¹, Kaitlin Arnold¹, Craig Lockhart¹ and Matthew J Ellis¹. ¹Washington University School of Medicine, St Louis, MO.

Body: Background
BKM120, an oral pan-Phosphatidylinositol-3-kinase (PI3K) inhibitor, plus fulvestrant (F) induced synergistic anti-tumor effect in preclinical studies of estrogen receptor positive (ER+) breast cancer. We therefore conducted a phase I trial of BKM120 and F in postmenopausal women with ER+ metastatic breast cancer (MBC) to determine the maximum tolerated dose (MTD), tolerability and preliminary efficacy.

Methods
A 3+3 phase I design was chosen for phase IA with BKM120 administered orally to define MTD [Table 1]. Cycle (C) length was 28 days. F 500mg was administered intramuscularly on C1 day (D)1 and D15 then on D1 of each subsequent cycles. Two expansion cohorts, phase IB (intermittent dosing: 5 days on and 2 days off) and Cohort C (continuous dosing: daily) of BKM120 at MTD, was initiated to further assess the tolerability and efficacy. Patients (pts) with ER+ MBC with measurable disease were eligible. No more than 3 lines of systemic therapy in the metastatic setting were allowed in phase 1B or Cohort C. Tumor measurement occurred every 3 cycles. Adverse events (AEs) were assessed by CTCAE 4.0 and response by RECIST 1.1.

Results
Thirty one pts, with median age of 58 (range: 34-71) years, prior exposure to a median of 1 (range: 0-9) endocrine and 0 (range: 0-2) chemo regimens, were enrolled. Majority of pts (83%) had visceral metastasis. Thirty, 25, and 22 pts were evaluable for AE, response and clinical benefit (CB), respectively. Most C1 AEs were grade (G)1 or 2, except 1 G3 diarrhea. No DLT occurred in C1. However, G2/3 AEs required BKM120 interruption and/or reduction in C2 or beyond occurred in 16 (53%) pts, including 9 ALT/AST elevation (G2 10%, G3 17%, G4 3%), 7 rash (G3 23%), and 1 pt each with G2 confusion, G3 hyperglycemia, G2 pneumonitis, and G3 tremor. As of June 3, 2014, 1 pt withdrew consent, 19 pts discontinued therapy due to progressive disease (PD) (n=15) or AE (n=4), 11 pts continue to receive BKM120/F. Fourteen (63.6%, 95% CI: 43.0 - 80.3%) of the 22 evaluable pts derived CB, including 7 partial response (PR) and 7 prolonged stable disease (SD) > 6 months. Archival tumor and circulating tumor DNA are being analyzed for presentation. Data will be updated.

Table 1 Study enrollment and results

<table>
<thead>
<tr>
<th>Cohort</th>
<th>N</th>
<th>BKM120</th>
<th>N pt BKM120 interrupted/reduced (AE)*</th>
<th>PD</th>
<th>PR (Cycle completed)</th>
<th>SD (Cycle completed)</th>
<th>NE</th>
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<tbody>
<tr>
<td>1A DL1</td>
<td>3</td>
<td>80 mg daily</td>
<td>2 (confusion, ALT)</td>
<td>1</td>
<td>0</td>
<td>1 (22)</td>
<td>1 (a)</td>
</tr>
<tr>
<td>1A DL2</td>
<td>6</td>
<td>100 mg daily</td>
<td>3 (ALT, rash)</td>
<td>2</td>
<td>2 (13, 3 (a))</td>
<td>2 (9, 7)</td>
<td>0</td>
</tr>
<tr>
<td>1A DL2b</td>
<td>2</td>
<td>100 mg intermittent</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2 (6, 6)</td>
<td>0</td>
</tr>
<tr>
<td>1B</td>
<td>10</td>
<td>100 mg intermittent</td>
<td>6 (AST, ALT, rash Hyperglycemia)</td>
<td>0</td>
<td>2 (15+, 12)</td>
<td>5 (16+, 9+, 9, 6, 6)</td>
<td>3 (a, a, b)</td>
</tr>
<tr>
<td>C</td>
<td>10</td>
<td>100 mg daily</td>
<td>5 (rash, ALT, diarrhea, tremor)</td>
<td>2</td>
<td>3 (9+, 8+, 4+)</td>
<td>3 (3+, 3+, 3+)#</td>
<td>2 (1+, 2+)</td>
</tr>
<tr>
<td>Total (N)</td>
<td>31</td>
<td></td>
<td></td>
<td>16</td>
<td>5</td>
<td>13</td>
<td>6</td>
</tr>
</tbody>
</table>

*All occurred in C2+ except the diarrhea, CBR: 63%, NE: Not evaluable, a: off due to AE, b: withdrew consent, # NE for CBR

Conclusion
BKM120 100mg, administered daily or intermittently, plus F was tolerable without DLT during C1. Grade 2/3 AST/ALT and rash were common in both dosing schedules in subsequent cycles resulting in BKM120 interruption/reduction. Promising activity observed in this trial warrants further development of this combination in ER+ BC. Phase III studies are ongoing in pts with ER+ MBC progressed on aromoatase inhibitor.
Title: PTEN and PIK3CA but not p4EBP1 are associated with low rates of pathological complete response (pCR) to trastuzumab based chemotherapy in primary HER2-overexpressing breast cancer

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Body: Background: Phosphatidylinositol 3-kinase (PI3K)/AKT pathway and phosphatase and tensin homolog deleted on chromosome 10 (PTEN) aberrations are common in breast cancer (BC). PIK3CA mutation is associated with lower pCR rates especially in patients treated with double anti-HER2 therapy (Loibl et al. JCO accepted). Another mechanism of resistance for trastuzumab-based treatment could be loss of PTEN, resulting also in downstream activation of the pathway. We investigated the correlation between PIK3CA, PTEN, p4EBP1 (phosphorylated E4 binding protein 1) and pCR in pts receiving neoadjuvant therapy.

Methods: In addition to PIK3CA we retrospectively evaluated PTEN and p4EBP1 by immunohistochemistry in HER2+ patients who received EC followed by docetaxel within the G4 study (Untch et al. 2011). The G4 study demonstrated a higher pCR rate by adding trastuzumab to chemotherapy. HER2 and hormone receptors (HR) were centrally assessed. PTEN was assessed using the automated quantitative immunofluorescence analysis (Aqua) using an antibody (Cell Signalling Technology). p4EBP1 assessed by immunohistochemistry with an immunoreactive score ranging from 0-12. PTEN was categorized by an optimized cut-off determined by the cut-off finder software (http://molpath.charite.de/cutoff/) and p4EBP1 was measured as a continuous variable and correlated with PIK3CA genotype and pCR (ypT0, ypN0). Central HER2+ve cases with a tumor cell content of ≥20% were selected (n=181).

Results: Median age was 48 years (22-77); HR+ve 51%; Grade 3, 47.4%; pCR rate 32%. p4EBP1 analysis was available from 137 and PTEN from 108 patients. PIK3CA genotype was available in 83 of these patients. 58 pts had PIK3CA and PTEN assessable, 14/58 had a PIK3CA mutation (mut) (25%). Overall, pCR rate in PTEN low tumors was 27.6% vs 57.1% in PTEN high (p=0.010). Within the PIK3CA mut cohort 13/14 (92.9%) tumors were PTEN low. Within the PIK3CA wild-type (wt) cohort 30/44 (68.2%) were PTEN low (p=0.066). The tumours were grouped into 4 subsets using PIK3CA (mut vs wt) and PTEN (low vs. high). pCR rate was 57.1% (8/14) in PTEN high/PIK3CA wt cohort and decreased to 15.4% in the PTEN low/PIK3CA mut cohort. The group with either PTEN low and PIK3CA wt or PTEN high and PIK3CA mut had a pCR rate of 32.2% (p=0.015). In multivariable analysis after adjustment for baseline parameters PTEN was an independent predictor for pCR in the complete cohort (OR 12.3 [95% CI 1.82-82.9] p=0.010) and in PIK3CA wt cohort (OR 10.3 [95% CI 1.31-81.62] p=0.027). Within the HR+ve group PTEN low tumors had a pCR rate of 22.2% vs 61.5% in the PTEN low/PIK3CA mut cohort (p=0.007). In multivariable analysis PTEN was independently predictive for pCR in the HR+ve group (OR 49.5 [95% CI 3.4-707] p=0.004). p4EBP1 correlated weakly with PIK3CA (p=0.049) but not with PTEN. There was no association of p4EBP1 with pCR.

Conclusion: Low PTEN was significantly associated with lower pCR and added independent predictive information not only in the overall HER2+ve cohort but also in the PIK3CA wt and HR+ve cohort. It will be confirmed in a larger sample size if PTEN assessment adds information to PIK3CA genotype to select patients with low pCR rates after trastuzumab therapy.
Title: HER2/PIK3CA\textsuperscript{H1047R} transgenic mammary tumors develop acquired resistance to triple therapy with trastuzumab, pertuzumab and PI3K inhibitors via multiple mechanisms

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Body: HER2 amplification and activating mutations in PIK3CA, the gene encoding the p110\textalpha subunit of PI3K, often co-occur in breast cancer. We generated a transgenic mouse model of HER2-overexpressing (HER2+), PIK3CA\textsuperscript{H1047R}-mutant breast cancer. In these mice, PIK3CA\textsuperscript{H1047R} accelerates HER2-mediated mammary epithelial transformation and metastatic progression, confers stem cell-like properties to HER2-overexpressing cancers and generates resistance to the combination of trastuzumab and pertuzumab (Hanker et al. PNAS 2013). HER2+/PIK3CA tumor growth was inhibited by treatment with the HER2 antibodies trastuzumab and pertuzumab in combination with the pan-PI3K inhibitor BKM120 (TPB). We sought to discover mechanisms of acquired resistance to the triple therapy by long-term treatment of established HER2+/PIK3CA tumors. We used tumor transplants derived from two HER2+/PIK3CA transgenic mice, #564 and #635. Tumor transplants from model 564 were initially growth inhibited by TPB, but did not regress. A subset of 564 transplants (3/11) resumed growth in the presence of continuous TPB therapy. All transplants (n=9) from model 635 regressed to a volume of <100 mm\textsuperscript{3} within 6 weeks of treatment. All tumors recurred and 2 tumors continued growth when re-treated with TPB. Resistance was maintained following passaging in mice and tumors were cross-resistant to trastuzumab/pertuzumab/BYL719, a p110\textalpha-specific inhibitor. TPB-resistant tumor 635-2 expressed p95 HER2, which was not detected in untreated tumors. In contrast, HER2 expression was significantly reduced in TPB-resistant tumor 635-3. P-AKT remained suppressed in some resistant tumors, but was restored in others. Short-term TPB treatment strongly suppressed P-S6 in sensitive tumors, whereas P-S6 was no longer inhibited in all TPB-resistant tumors from both models. We are currently performing whole-exome sequencing and RNA-sequencing on TPB-resistant vs. untreated tumors in order to identify additional mechanisms of resistance. In parallel, we established human HER2+, PIK3CA-mutant cell lines (MDA-MB 453, UACC893, and HCC1954) resistant to TPB by long-term treatment (>5 months) in the presence of the three drugs. Similar to the TPB-resistant tumors, P-S6 was no longer inhibited following TPB treatment in the resistant cell lines. Treatment with the TORC1/2 inhibitor MLN0128 abolished levels of P-S6 in HER2+/PIK3CA\textsuperscript{H1047R} tumors. Combined treatment with MLN0128 and TPB inhibited growth of the drug-resistant tumors. Interestingly, Both TPB-resistant HER2+/PIK3CA\textsuperscript{H1047R} tumor lines displayed resistance to the antibody-drug conjugate trastuzumab-DM1 (T-DM1) \textit{in vitro} and \textit{in vivo}, despite maintenance of HER2 overexpression. In addition, HCC1954 cells selected for resistance to TPB in culture were 66-fold less sensitive to T-DM1 than parental cells, despite maintaining equal levels of HER2 by western blot. These data suggest that multiple mechanisms may contribute to resistance to dual HER2 and PI3K blockade, including re-activation of mTOR signaling. We speculate that a similar heterogeneity of resistance mechanisms may occur in HER2+/PIK3CA-mutant metastases in patients.
Title: The long noncoding RNA M41 promotes aggressiveness and tamoxifen resistance in ER-positive breast cancers

Felix Y Feng¹, Teng Ma¹, Corey Speers¹, Matthew K Iyer¹, Shuang Zhao¹, John R Prensner¹, James M Rae¹, Lori J Pierce¹ and Arul M Chinnaiyan¹. ¹University of Michigan, Ann Arbor, MI.

Background: Long noncoding RNAs (lncRNAs) have recently been associated with the development and progression of a variety of human cancers. To date, the interplay between known oncogenic drivers, such as estrogen receptor (ER), and lncRNAs has not been well described. In this study, we identify M41 as the top outlier lncRNA in ER-positive vs ER-negative breast cancer and investigate its role in preclinical cancer phenotypes and clinical outcomes.

Methods and Materials: RNA sequencing was performed on 89 breast cancer samples and cell lines, including 42 ER+ cases, and a modified cancer outlier analysis was used to identify lncRNAs enriched in ER-positive disease. To assess ER regulation of the top enriched lncRNA (M41), ChIP-Seq and ChIP-PCR was used to detect binding of ER to M41 promoter and qPCR was used to determine changes in M41 expression following 10 nM estradiol treatment in MCF7 and T47D cells. Following knockdown via siRNA, the impact of M41 expression was assessed on cell invasion, migration, proliferation, and anchorage-independent growth. The impact of M41 knockdown on tamoxifen sensitivity was assessed by cell proliferation studies in MCF7 cells with acquired tamoxifen resistance. Lastly, clinical associations between M41 expression and grade/node status, as well as event-free survival (EFS), was determined using ANOVA and Kaplan-Meier analyses of TCGA samples.

Results: M41, an uncharacterized lncRNA located on chr21q22.2, was identified as the top outlier lncRNA in ER-positive vs ER-negative breast cancer. M41 demonstrated outlier expression (RPKM values>50) in 15% of ER-positive cancers, and was not significantly expressed in normal breast tissue. ChIP studies show that ER robustly binds to the M41 promoter. Estradiol stimulation significantly increased M41 expression in a time-dependent manner. Knockdown of M41 significantly inhibited all assessed oncogenic phenotypes in the ER-positive MCF7 and T47D cells, with a 60-80% decrease in both invasion and anchorage-independent growth, but had no effect in the ER-negative MDA-MB-231 cell line (which has minimal M41 expression). M41 expression was greater than 10-fold higher in tamoxifen-resistant MCF7 cells compared to parental controls (p<0.001), and knockdown of M41 restored tamoxifen sensitivity on cell proliferation studies; studies on the mechanism of M41-mediated tamoxifen resistance are ongoing. M41 overexpression was significantly correlated with node positivity, increasing grade, and luminal B subtype in ER-positive breast cancer samples (p<0.001). In TCGA samples, M41 overexpression was significantly associated with decreased EFS (p=0.003).

Conclusion: We have identified M41 as an ER-associated oncogenic lncRNA that contributes to preclinical cancer phenotype, promotes tamoxifen resistance in cell line models, and associates with poor outcomes in clinical samples. We suggest that M41 represents a novel biomarker candidate for the prognosis of ER-positive breast cancers and provides new insight into the biological complexity of breast tumor biology.
**Title:** FoxA1 gene amplification in ER+ breast cancer mediates endocrine resistance by increasing IL-8

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**Body:** Background: ER transcriptional programming is associated with fundamental changes when endocrine resistance develops. The Forkhead transcription factor, FoxA1, is a pioneer factor for ER-DNA binding. We hypothesize that FoxA1 plays a critical role in ER transcriptional reprogramming in endocrine resistance by augmenting itself and the specific downstream effectors. Methods: Next generation sequencing was applied to characterize a panel of endocrine-resistant (Endo-R) cell models. Genomic PCR amplification and FISH assays were developed to measure FoxA1 copy number gain (CNG). Q-RT-PCR, Western blots, IHC, ELISA, and cytokine arrays were used to determine the levels of FoxA1 and IL-8 in cell culture and in vivo xenograft tumors. Effects of gene knockdown (ER, FoxA1, or IL-8) or inducible FoxA1 overexpression on ER and growth factor receptor (GFR) downstream signaling were determined by cell growth and Western blots. ER and FoxA1 binding at the IL-8 gene locus was measured by ChIP-qPCR. ChIP-seq analysis was integrated with RNA-seq data. Kaplan-Meier analysis evaluated the predictive role of FoxA1 in ER+ breast tumors. Results: Exome-seq revealed that FoxA1 is the most highly amplified gene in TamR vs. P cells from two independent MCF7 models. Genomic PCR and FISH also indicate FoxA1 CNG in Endo-R models of ZR75-1 and BT474. Increased FoxA1 expression was found in multiple Endo-R cells and in MCF7L Endo-R xenograft tumors. Cytokines, especially IL-8, are more highly expressed in multiple Endo-R cell models, similar to our previous microarray data from MCF7 Endo-R xenograft tumors. FoxA1 forced overexpression significantly induced IL-8 expression in MCF7L-P cells. It also activated multiple GFR downstream signaling pathways, and conferred endocrine resistance. Conversely, knockdown of either FoxA1 or ER significantly decreased IL-8 levels in TamR cells, and inhibited cell growth in both P and TamR cells. Knockdown of IL-8 in TamR cells substantially inhibited GFR downstream signaling, and was more cytotoxic than in P cells. A novel FoxA1-binding site (10 kb at 5'UTR of IL-8) recruited more FoxA1 and p300 in MCF7L-TamR than -P cells. ChIP-seq shows a general enhancement of FoxA1 binding around the genes (within 20 kb) that are differentially expressed in TamR vs. P cells. We identified a FoxA1 CNG-associated gene signature from TCGA breast tumors that predicts worse relapse-free survival (RFS) in Tam-treated ER+ tumors (from Loi et al). Meta-analysis showed that FoxA1 mRNA levels in the top 25th percentile predict worse RFS in ER+ patients treated with Tam (N=615), but not in systemically untreated patients (N=500). FoxA1 CNG and overexpression in clinical specimens by using our newly developed FISH and IHC assays are currently being investigated. Conclusions: FoxA1 gene amplification was enriched in two independent MCF7 Tam-R cell models. Clonal selection of FoxA1 gene amplification may occur and lead to endocrine resistance. High levels of FoxA1 may mediate endocrine resistance by directly inducing IL-8. The data suggest that IL-8 signaling is a component of a cytokine loop controlled by the FoxA1/ER transcriptional reprogramming, which might be exploited in therapeutics to overcome endocrine resistance.
2014 San Antonio Breast Cancer Symposium

Publication Number: PD6-3
Average Grade: 5.00

Title: Recurrent ESR1 fusion transcripts are associated with endocrine resistance in estrogen receptor positive, HER2 negative breast cancer

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Body: Breast cancer proliferation measured by Ki67 immunohistochemistry after short-term antiestrogen therapy has been shown to correlate with disease-free survival. This suggests the use of biomarkers of the early effects of endocrine therapy on ER+ tumors will identify resistant cancers. Thus, we hypothesized that profiling operable ER+ tumors after short term treatment with an aromatase inhibitor would discover actionable molecular alterations causally associated with resistance to estrogen deprivation.

We performed whole exome sequencing, RNA-Seq and quantitative immunofluorescence (QIF) of ER, PR, HER2, and Ki67 in biopsies from 130 patients with an operable ER+/HER2– breast cancer that had received letrozole for 10-21 days prior to surgery. Tumors were categorized by the natural log of 2-week post-letrozole Ki67 as sensitive, intermediate, or resistant.

We sequenced RNA from 50 frozen tumors and performed fusion transcript analysis using 4 programmatic algorithms (dRanger, TopHat, DeFuse, Chimera Scan), resulting in 304 candidate gene fusions in 44 tumors. Primers with universal sequencing tags were designed against 3' and 5' sites of breakpoints mapping to RefSeq exon coding regions (n=187); fusion sequences were amplified by qRT-PCR from tumor and breast cancer cell line RNA. Single or multiple distinct product bands were visualized by gel electrophoresis in 96 tumor samples and Sanger-sequenced. Results were mapped to the human RNA reference transcriptome using BLAST.

Overall, 9% of putative fusion transcripts (n=27 from 16 unique tumors) were validated by mapping to the open reading frames of predicted 3’ and 5’ genes. Fusion transcripts called by more than one program were more likely to validate (13 of 24 redundant versus 14 of 269 unique; p<0.001). ESR1 fusions in 4 tumors mapped to chromosome 6q25.1, involving the 5’ UTR of ESR1 and 3’ exons of AKAP12, c6orf211, and CCDC170 (c6orf97). The ESR1:CCDC170 fusion was also detected in MDA361 and MCF7 cells as previously published, as well as in BT474 cells. FISH for multiple probes at 6q25.1 demonstrated structural rearrangements but not amplification in primary tumors and breast cancer cell lines.

Using the 2-week Ki67 to stratify for response to treatment, the validated ESR1 fusions were present only in tumors that maintained high (≥7.4%) to intermediate (>2.7%) Ki67 labeling indices upon estrogen deprivation with letrozole (p=0.01). PR expression was lower (p=0.003) and ER expression higher (p=0.05) in ESR1 fusion+ tumors compared to fusion negative tumors. RNA extracted from 14 additional tumors were screened for ESR1 fusions by qRT-PCR and the ESR1:CCDC170 fusion was validated in 1 of 8 resistant/intermediate and 0 of 6 sensitive tumors.

In summary, biomarkers of early response to antiestrogens are needed in order to identify ER+ cancers that are treatment resistant. In a prospective trial of operable ER+/HER2– breast tumors, we discovered recurrent intrachromosomal ESR1 fusion transcripts associated with intrinsic resistance to estrogen deprivation with letrozole. Additional work investigating the genomic basis and function of the fusion transcripts is underway.
Title: ESR1 gene fusions implicated in endocrine therapy resistance of ER+ breast cancer

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Body: Background. We recently identified an in-frame ESR1 translocation in a tumor and PDX pair from a patient with endocrine therapy resistant advanced disease. The fusion gene preserved the N-terminal 365aa of ESR1 containing the intact activation function 1 (AF1), DNA-binding domain (DBD) and hinge, followed by the C-terminal transactivation domain of YAP1. The ESR1-365>YAP1 fusion drove estrogen-independent and anti-estrogen-resistant tumor cell growth (Cell Reports 4:1116, 2013). We therefore sought to further investigate the role of ESR1 fusion genes in breast cancer.

Methods. 711 cases from TCGA, 82 ER+ cases from neoadjuvant endocrine therapy trials and 25 ER+ systemic relapse samples were screened for fusion genes using RNAseq. Fusion genes involving ESR1 were subsequently assayed for estrogen response element (ERE)-mediated transcriptional activity and ability to drive estradiol independent breast cancer cell growth.

Results. Multiple ESR1 fusions were identified in mostly luminal B cancers. ESR1 fusions resulted most frequently from rearrangements involving another gene on Chromosome (Chr) 6 but also fusions with genes on other chromosomes. The fusions involving partners on Chr6 that retained an intact N terminal AF1 and DNA binding domain (DBD) of ESR1 (≥1-253aa) included an Out-of-Frame (OF) fusion event with CCDC170 (a gene immediately centromeric to ESR1), POLH (in-frame or IF), AKAP12 (IF), PCMT1 (OF), SYNE1 (OF) and GPR126 (OF). Also identified were inter-chromosomal translocations involving Chr12p-NOP2 (IF) and ChrX-PCDH11X (IF) and Chr7q-AKR1D1 (OF). The ESR1 fusions with transcriptional potential (because of a retained DNA binding domain) included variable 5’ ESR1 exons that preserved 253aa, 365aa, 412aa or 458aa of ESR1 sequence: in all cases disrupting ligand binding though loss of C terminal sequence. The effect of the IF and OF 3’ sequences on the transcriptional activity of the relevant ESR1 fragment was highly variable. The most transcriptionally active fusion (more active than the relevant ESR1 fragment alone) was the ESR1-365>YAP1 as well as ESR1-365>PCDH11X and ESR1-253>CCDC170. This is remarkable since PCDH11X is not considered a transcription factor and the CCDC170 sequence was OF – in both cases suggesting the activity of the fusion was due to "neomorphic" properties. Two fusions, NOP2 and POLH were inactive in the ERE reporter assay, yet both stimulated estradiol independent growth. In these cases we suggest that the ESR1 locus can function as a "promoter trap" which allows the identification of genes with previously unknown functions in endocrine therapy resistance.

Conclusions: In luminal-type breast cancer the ESR1 gene was fused to multiple 3’ partners with remarkably heterogeneous functions. The two most transcriptionally active, ESR1-365>YAP1 and ESR1-365>PCDH11X, were both identified in endocrine therapy refractory advanced disease – suggesting a role in fatal disease progression.
Title: Profiling of ESR1-mutated metastatic breast cancers by FoundationOne® allows a broad genomic understanding for potential clinical implications

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Body: Background: Estrogen receptor (ER) inhibition is an important treatment option for advanced breast cancer (BC) pts. Recent studies describe recurring somatic mutations in codons 537 and 538 within the ligand-binding domain (LBD) of ~20% of ER+ metastatic disease, but not treatment-naïve ER+ cancer or ER-negative disease, that render the ER constitutively active and confer partial resistance to endocrine agents. Few studies have described the clinicopathologic and genomic covariates that accompany the ESR1 genomic alterations (GAs). Correlating genomic events as captured by FoundationOne in pts with ESR1-mutated BCs with clinical history may provide clinical insight into these alterations.

Methods: Hybridization capture of 3769 exons of 236 cancer-related genes and 47 introns from 19 genes that are frequently rearranged in cancer were fully sequenced to high, uniform coverage using FoundationOne (Foundation Medicine, Cambridge, MA).

Results: 176 of the 2,208 (7.9%) BC pt cases harbored ESR1 alterations. 1127 short variants (SV) were detected in 176 patient samples for an average of 6.4 GA/sample. 16.5% SV (186/1127) were ESR1 GAs with an average of 1.0 ESR1 GAs/sample. ESR1 GAs consisted of base substitutions (77%) and amplifications (20%, median copy number 9X, range 6-28). Base substitutions occurred at codons 538 (71/145) and 537 (70/145) and at two other novel sites, 341 (2/145) and 563 (1/145). A patient-derived xenograft study suggested that tumors refractory to ER inhibitors and harboring ESR1 amplification could be responsive to higher doses of estradiol. (Li et al, Cell Reports, 2013). The most frequently co-occurring GAs were PIK3CA (37.5%), GATA3 (22.7%), TP53 (24.4%), MAP3K1 (10.2%) and CDH1 (7.9). Collection of data on tissue sites sequenced, treatments and outcomes on these pts with ESR1 GAs are ongoing. Of note, one pt had recurrent disease following adjuvant tamoxifen and letrozole. FoundationOne testing of her recurrent bony disease revealed an ESR1 Y537 alteration along with mutations in PIK3CA, APC, and RAD51. She has been on fulvestrant monotherapy with stable metastatic disease 13 + months.

Conclusions: We found through FoundationOne testing that ESR1 GAs are reasonably common in advanced BCs, with base substitutions accounting for 77% of ESR1 GAs and amplifications for 20%. Identification and characterization of ESR1-mutated advanced BC pts by comprehensive genomic profiling capable of detecting both base substitutions and copy number changes may identify clinically relevant GAs. Clinical correlation is pending.
Title: Estrogen receptor (ESR1) mutations confer resistance to hormone therapy using a common mechanism

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Body: Background: The idea that somatic estrogen receptor gene (ESR1) mutations could play an important role in the evolution of hormone-responsive breast cancers was proposed by us with our original identification of two ESR1 mutations at residues K303 and Y537. Technical issues with ESR1 mutation detection and the resulting paucity of reports in tumors led many to assume that ESR1 mutations were not there. However, with recent Next Generation Sequencing of metastatic tumors, mutation of ESR1 is now an accepted certainty. ESR1 mutant allele frequencies vary over a wide dynamic range, and are usually a minority population within tumors. Therefore how does a minor subclonal tumor population drive resistance in metastatic tumors?

Methods: MCF-7 cells expressing endogenous wild-type ER was transduced with ESR1 mutants K303R, Y537N, Y537S, and D538G lentivirus and stable clones selected. ER transcriptional assays and growth in soft agar were performed. Digital drop (dd) PCR and primer extension snp detection were used to ascertain mutant:WT ESR1 allele frequency in cell lines, 200 primary tumors from patients treated with tamoxifen monotherapy, and 20 metastatic breast tumors.

Results: Mutant ER constitutive transcriptional activity was fully antagonized by the antiestrogens tamoxifen or fulvestrant in MCF-7 stable transfectants. In contrast, soft agar growth of all ESR1 mutant-expressing cells was unexpectedly and completely resistant to the growth inhibitory effects of tamoxifen, although mutant-expressing cells were a minority subpopulation in the stable clones. Therefore, in cells with WT ER co-expression, the mutant resistant phenotype dominates. We found that phosphorylation of IGF1R\texttextsuperscript{β}; was constitutively increased in all ESR1-mutant expressing cells. Treatment with a specific IGF1R\texttextsuperscript{β} inhibitor in combination with tamoxifen drastically restored hormone sensitivity in cells expressing the ESR1 mutations. These results suggest a convergence in resistance mechanisms between the K303R and Y537 ESR1 mutation hot spots. We are exploring whether the dominant mutant ESR1 resistant phenotype occurs via activation of paracrine mediators, and have identified altered IGF-1 and interleukin 6 signaling in mutant-expressing cells. Mutation detection in a retrospective cohort of primary and metastatic breast tumors is ongoing and will be presented.

Conclusions: We hypothesize that the selection of dominant-acting ESR1 mutations in tumors is a key event in breast cancer progression, potentially due to the selective pressure of antiestrogens. The dominant-resistant phenotype of ESR1 mutants in a majority WT background supports the subclonal evolution of ESR1 mutations in breast cancer recurrence. A common resistance mechanism (like constitutive IGF1R\texttextsuperscript{β} activation) should enable biologic targeting of ESR1 mutation-positive metastatic patients a feasible clinical goal.

Support: NIH/NCI R01 CA72038 and CPRIT RP1210732 to SAWF.
Title: In-silico discovery of novel estrogen receptor-α inhibitors as potential therapeutics for tamoxifen resistant breast cancer

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Body: Estrogen receptor-α (ER) positive breast cancer (BCa) represents 75% of all invasive BCAs. Conventional ER-directed drug Tamoxifen targets the estrogen binding pocket (EBP) of the receptor. However, over prolonged periods of treatment, the therapeutic efficacy of Tamoxifen declines due to development of resistance. Numerous factors are causative for this phenomenon, including recently reported mutations in the receptor (T537S). Therefore, there is an urgency to develop novel anti-ER therapeutics that exhibit entirely different mode of ER inhibition. A promising alternative strategy is to prevent receptor-coactivator interaction and block further crucial steps in ER activity. ER-coactivator interface should be less prone to adaptive mutations as any mutations at this site would also likely block the coactivator recruitment, we therefore targeted the Activation Function-2 (AF2) site, a coactivator binding pocket on ER, called to overcome the limitations of Tamoxifen. Although AF2 is a shallow surface pocket, the pharmacophore-rich features of this site make it a druggable target. To identify potential ER AF2 inhibitors, virtual screening was performed. Initial hits were subjected to lead optimization and more potent analogues were rationally designed by exploiting critical features of this site. Potential compounds were tested for their ability to inhibit ER transcriptional activity using the T47D-KBluc cell line stably transfected with an ER-specific luciferase reporter. Consequently, the lead compound VPC-16339 (IC50=8.24µM) was identified. The direct binding between VPC-16339 and the receptor was confirmed by Biolayer Interferometry assay. More importantly, VPC-16339 prevents coactivator recruitment at the AF2 pocket in a dose dependent manner as measured by TR-FRET coactivator recruitment assay. Increasing concentrations of estradiol did not affect the IC50 of the lead compound, thereby ruling out the possibility of VPC-16339 binding to EBP. VPC-16339 demonstrated a strong anti-proliferative effect on MCF7 and Tamoxifen resistant cells, with no effect on ER-HeLa cells, suggesting its selective ER-mediated action, as further validated by ER luciferase assay in Tamoxifen resistant cells. VPC-16339 effectively inhibits mRNA and protein expression levels of the estrogen dependent genes such as pS2, CathD and CDC2. Due to AF2-guided mechanism, VPC-16339 successfully overcomes Tamoxifen resistance and inhibits the constitutively active Tamoxifen resistant form of ER (T537S).

In summary, we report VPC-16339 as an ER AF2 specific inhibitor with promising anti-proliferative effect in BCa cell lines including Tamoxifen resistant cell lines. VPC-16339 effectively inhibits the mutant form of the receptor (T537S) which is responsible for acquired endocrine resistance. It can be anticipated that ER AF2 inhibitors will provide an alternative therapeutic strategy that can be applied concurrently or simultaneously with current anti-ER treatments for BCa patients with advanced disease.
Title: TransCONFIRM: The correlative analysis of breast tumors from patients with advanced hormone receptor positive disease identifies a genetic signature associated with decreased benefit from single agent fulvestrant

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¹Dana-Farber Cancer Institute, Boston, MA; ²Hospital of Prato, Istituto Toscano Tumori, Prato, Italy; ³“Sandro Pitigliani” Medical Oncology Unit, Istituto Toscano Tumori, Prato, Italy and ⁴AstraZeneca Pharmaceuticals, Macclesfield, United Kingdom.

Body: Introduction: Several multi-gene expression based assays have been developed to assess the prognosis and predict response to endocrine treatments in early stage hormone receptor positive (HR+) breast cancer. Although a significant number of patients with metastatic ER+ disease will not respond to endocrine treatments, molecular assays to predict response in this setting are limited. In addition, tissue specimens of metastatic lesions for molecular studies are not always available. In this study we sought to identify a molecular profile in the primary tumors of patients who developed disease recurrence that could predict response to endocrine treatment in metastatic disease.

Methods: We used the primary breast tumor samples from a subgroup of patients participating in the randomized phase III CONFIRM trial, which compared 500mg versus 250mg of fulvestrant in post-menopausal women with HR+ advanced breast cancer. Formalin- fixed paraffin embedded tumors were collected from 130 of the participants and were centrally reviewed for ER, PR, HER2 and Ki67. RNA was sufficient for gene expression profiling in 112 of the cases using the NuGEN Ovation FFPE WTA System and Affymetrix HTA 2.0 GeneChip. The majority of the patients in this analysis developed metastatic disease during adjuvant endocrine treatment (N=55) or had de-novo metastatic disease (N=39) versus relapse after adjuvant treatment (N=18). The association between gene expression and progression free survival (PFS) was investigated using a multivariate Cox proportional hazard model adjusting for statistically significant clinicopatholgical factors. In addition we performed pathway-level analysis and evaluated the PAM50 subtype predictor and Risk of Relapse (ROR) score.

Results: The median PFS was 8 months in our cohort. HER2 level by immunohistochemistry above 1+, high PR level, defined as Allred score of above 6, and Ki67 of above 50% were significantly associated with PFS and were included in the multivariate model. Dose of fulvestrant was not associated with PFS in this cohort. We identified a signature of 25 genes that is inversely associated with PFS on fulvestrant treatment (FDR 20%). When compared to other published datasets of breast cancer tumors, these genes are enriched in tumors with poor outcome and triple negative cancers. Pathway analysis revealed an association between activation of the EGFR pathway and decreased PFS (P=0.01). PAM50 subtypes varied with the luminal subtype being the most common (65%) and were generally concordant with the clinical subtype. However, we did not detect a significant trend between PAM50 subtype or ROR score and PFS or overall survival.

Conclusions: In this cohort of patients with early and de-novo metastatic disease we identified a gene signature in the primary tumors that is associated with decreased response to fulvestrant treatment in metastatic disease. This signature warrants further validation to determine it’s predictive value and potential to assist in treatment decision making for patients with HR+ metastatic disease.
2014 San Antonio Breast Cancer Symposium

Publication Number: S1-02
Average Grade: 3.80

Title: Prognostic effects of gene mutation in estrogen receptor positive breast cancer

Obi L Griffith¹, Malachi Griffith¹, Jingqin Luo¹, Jasreet Hundall¹, Christopher A Miller¹, David E Larson¹, Robert Fulton¹, Richard K Wilson¹, Shuzhen Liu², Samuel Leung², Torsten O Nielsen², Elaine R Mardis¹ and Matthew J Ellis¹. ¹Washington University School of Medicine, St Louis, MO and ²University of British Columbia, Vancouver, BC, Canada.

Body: Background: Relationships between recurrent somatic mutations and outcome in estrogen receptor positive (ER+) breast cancer has not been extensively studied as the original discovery efforts were from either heterogeneously treated patients or follow up was too brief. Targeted massively parallel sequencing (MPS) analysis was therefore conducted on DNA extracted from archived formalin-fixed breast primaries from a cohort of over 600 patients from British Columbia treated with five years of adjuvant tamoxifen monotherapy and followed for over 10 years (Nielsen et al CCR 16:5222, 2010).

Methods: Genes were selected for targeted sequencing by meta-analysis of five large-scale breast cancer sequencing studies and manual review of breast cancer literature. In total 83 genes were identified and 3286 probes were designed to tile across all known exons. Minimum starting input DNA was 50ng (mean=189.1ng). Illumina sequencing libraries were constructed, indexed, pooled, and enriched for target sequences by hybrid-capture followed by paired-end 100bp reads. The Genome Modeling System was used to perform single-tumor somatic variant prediction. Variant calls were filtered to include only targeted regions and exclude variants with global mutant allele frequencies greater than 0.1% in 1000 genomes or NHLBI exome datasets. Kaplan-Meier analysis and multivariable analysis (clinical features and intrinsic subtype by qPCR) was performed for breast-cancer-specific and relapse free survival.

Results: A total of 638 samples met minimum quality controls of 80% targeted space covered at 20X or greater. On average each sample had 332M of aligned bases and a mean coverage of 134.3X. In total 7,159 variants were identified including 3,696 missense, 494 nonsense, and 1,047 frameshift insertions or deletions. Preliminary results indicate significant associations between mutation status and improved survival for PIK3CA, ARID1B, ERBB3, MAP3K1 and GATA3 or worse survival for PTEN, DDR1, TP53 and JAK2. Five Y537N/C, two E380Q and 5 potentially novel ligand-binding-domain mutations were identified in ESR1. Such mutations were recently reported to be associated with resistance to hormone therapy but were discovered here in as much as 1.9% of pre-treatment samples. Analysis will be presented regarding the use of relapse events to differentiate passenger from driver mutations.

Conclusion. Multiple recurrently mutated genes have both positive and negative associations with prognosis in tamoxifen monotherapy treated breast cancer populations. Associations with poor outcome suggest that PTEN, DDR1, and JAK2 are high priorities for pharmacological interventions.

Table 1: Mutations and survival in ER+ breast cancer

<table>
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<tr>
<th>Gene</th>
<th>P-Value</th>
<th>Hazard Ratio</th>
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<td>PIK3CA</td>
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<td>GATA3</td>
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</tr>
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<td>JAK2</td>
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Title: Identification of base pair mutations and structural rearrangements acquired in breast cancer metastases including a novel hyperactive ESR1-DAB2 fusion gene specifically in hormone-resistant recurrence

Ryan J Hartmaier¹,², Shannon L Puhalla¹, Steffi Oesterreich¹,², Amir Bahreini¹,³, Nancy E Davidson¹,², Adam M Brufsky¹ and Adrian V Lee¹,²,³. ¹Women's Cancer Research Center, University of Pittsburgh Cancer Institute & Magee Women's Research Center, Pittsburgh, PA; ²University of Pittsburgh, Pittsburgh, PA and ³University of Pittsburgh, Pittsburgh, PA.

Body: DNA structural variations (SVs) are a major source of genetic instability in cancer, but they remain understudied. Large-insert mate-pair sequencing (MPS) is a powerful method designed to detect SVs, even in highly repetitive regions. Using MPS and other methods, we performed a comprehensive analysis of genomic alterations in breast cancer progression. Matched primary/recurrent frozen tumor samples from 6 patients, including two patients from our rapid autopsy program with multiple metastatic tissues (20 total samples; average 5.5 years to recurrence) were examined by multiple large-insert library (3-5, 5-8, 8-12kb) MPS to identify metastatic acquired SVs. This was supplemented with RNAseq (n=15), whole exome sequencing (n=18; ~75x), whole genome sequencing (n=3; 40-65x), and SNP arrays.

A relatively small fraction (~10%) of somatic single nucleotide variants (SNVs) in the primary tumor were identified in matched metastatic samples, and the majority of metastatic SNVs were not found in the matched primary tumor. This indicates that a rare sub-clone colonizes the metastatic site and evolves extensively before becoming clinically evident. For example, in one patient with an ER+ tumor who initially declined anti-estrogen therapy, the recently described ESR1 Y537S mutation was not present in the primary tumor or in metastatic disease 5 years later. However, after extensive anti-estrogen treatment for metastatic disease, the mutation was identified at rapid autopsy, indicating that this mutation can be acquired even after initial metastatic spread. We observed extensive patient-to-patient variability in the number and types of SVs. In general, the overall pattern of SVs was remarkably similar between matched primary and metastatic samples, however, we identified a number of metastatic specific SVs that likely contribute to disease progression. Specifically, in one patient with an ER+ primary tumor treated with adjuvant Tamoxifen, we identified a novel fusion gene between ESR1 (estrogen receptor-α, ERα) and DAB2 (disabled-2) only in a lymph node recurrence. RT-PCR and western blot analysis confirmed that the fusion RNA/protein was expressed/translated only in the recurrent disease. The fusion retains the DNA-binding domain (DBD) and hinge region of ERα while the ligand-binding domain (LBD) is replaced with the majority of DAB2. We hypothesized that this is a functional genetic alteration conferring ligand-independent ERα-mediated signaling and growth. Confirming this, in vitro ERE-Tk-luc reporter assays showed that the ESR1-DAB2 fusion has ligand-independent activity that is 13-290x higher than wild-type ERα. Chromatin immunoprecipitation assays in metastatic tissue from tumors with mutant ERα show strong enrichment for ERα at classical ERα target genes. We are currently assessing the genome-wide binding of ESR1-DAB2 and the functional contribution of DAB2 to the fusion protein.

This study represents the most comprehensive analysis to date of genomic changes in breast cancer progression and indicates extensive changes occur during metastatic spread. A number of acquired changes likely represent therapeutically targetable metastatic dependencies.
Body: Aims
1. To determine the variability of mutational profiles and sub-clonality in core-cut biopsies from ER+ BC and the impact of 2-weeks’ AI therapy on these.
2. To identify mutations or patterns of mutations associated with poor anti-proliferative response to AI treatment.

Background
DNA alterations may lead to de novo and acquired resistance to medical therapies including AIs. Assessing this requires single time-point or sequential sampling usually with core-cuts but there is little information on their ability to represent mutational profiles or sub-clonal structure. We studied this in paired biopsies from ER+ BC primaries in 60 selected postmenopausal patients from the Peri-Operative Endocrine Therapy for Individualising Care (POETIC) trial (CRUK/07/015) before and after 2-weeks’ non-steroidal AI or no AI (randomised 2:1).

Methods
DNA was extracted from RNAlater-preserved diagnostic and surgical 14-gauge core-cut samples and peripheral blood from 20 no AI (Control) and 40 AI-treated patients (15 poor and 25 good Ki67-responders [PR and GR, respectively]). Patients with low ER+ BC or unsuppressed estradiol on treatment were not considered. Exome sequencing (Illumina HiSeq 2000) achieved >60% coverage across the exome at 15x depth. Variants were validated by re-sequencing (median >100x) together with 79 genes of interest curated from COSMIC and selected publications. Statistically significant genes (SMGs) were determined using MuSiC. Sub-clonality was analysed by SciClone.

Results
Good quality exomes were obtained on 102 samples including 44 pairs (control n=14; PR n=10; GR n=20). There were 5684 mutations (including 3616 missense and 1322 silent) affecting 3261 genes. SMGs in this series were PIK3CA (35.3%), TP53 (27.5%), CDH1 (13.8%), HEATR7B2 (8.8%), GATA3 (5.9%), CENPF (5.9%), MAP3K1 (5.9%), MAP2K4 (4.9%), HTR1A (2.9%) and C22orf23 (1%). PR had more mutations than GR (median 65 vs 36, p=0.04). More PR than GR were HER2+ (5/14 vs 1/24, p=0.019) and/or TP53-mutated (5/10 vs 3/20, p=0.08) but similar proportions were PIK3CA-mutated. The correlation of diagnostic vs surgical variant allele frequencies was strong for the control (r=0.75) and treated (r=0.89) groups (for treated GR r=0.83; for treated PR r=0.65). In the treated group there were fewer mutations at surgery vs diagnosis (p<0.026). PIK3CA and TP53 mutation status was identical between the paired samples in 41/44 and 40/44 cases; less frequently mutated genes showed lower concordance. SciClone plots to infer sub-clonality were possible in 37 pairs; for 8 pairs (22%) there was clear evidence of at least one sub-clone being present in only one core-cut sample.

Conclusion
This is the largest reported study of exome reproducibility in ER+ BC for mutation profiles based on core-cut biopsy. Multiple sub-clones are identifiable in ER+ primary BC. In c.20% tumours, a single core-cut does not allow inference of all sub-clonal populations, probably due to spatial heterogeneity. TP53 mutations but not PIK3CA mutations are associated with PR. Large numbers of BC will be needed to identify any associations of lower frequency mutations with resistance. A trend to fewer mutations after just 2 weeks AI needs confirmation.
Title: In-depth genomic analysis of ER+ breast cancers during development of endocrine resistance

J Michael Dixon¹, Arran K Turnbull¹, Chris Fan², Joel S Parker², Xiaping He², Laura Arthur¹, Carlos Martinez-Perez¹, Lorna Renshaw¹ and Charles Perou². ¹University of Edinburgh, Edinburgh, United Kingdom and ²Comprehensive Cancer Centre, Chapel Hill, NC.

Body: Background: Aromatase inhibitors (AIs) have an established role in the treatment of estrogen receptor alpha positive (ER+) post-menopausal breast cancer. Response rates are only 50-70% in the neoadjuvant setting and up to 40-50% of all adjuvantly treated patients will eventually relapse. Mutations in certain genes have been previously shown to confer resistance to therapy, and molecular subtype has associations with poor outcome (i.e. LumB and HER2E). In order to improve the outcomes of non-responders or patients who become resistant to endocrine treatment, the identification of key mutations, and their interaction with subtype, is crucial. Dynamic profiling of the same tumour demonstrating de novo or developing resistance after responding to one or more lines of endocrine treatment in the neoadjuvant setting provides a unique opportunity to identify such genomic changes.

Methods: This series is unique in that it includes 17 post-menopausal women with ER+ breast cancer treated with neoadjuvant letrozole. 13 of these patients progressed on treatment or initially responded to treatment and then developed acquired resistance and 4 responded well. Dynamic clinical response was assessed for each patient using periodic 3D ultrasound measurements performed during treatment. Fresh tissue was taken before treatment and when the tumor was resistant to treatment (4 patients had 2 biopsies, 9 patients had 3 and 4 patients had 4 available biopsies taken). RNA and DNA were extracted from tumour and normal DNA obtained from either matched blood or normal lymphatic tissue. In total, 51 tumour samples were available and have completed RNA-Seq, with exome sequencing shortly to be completed.

Results: From the RNA-seq data, the intrinsic subtype distribution was 9 LumA, 7 LumB, and 1 HER2-enriched; when stratified according to response, the "progressors" were 7 LumB, 5 LumA and 1 HER2-enriched, while "responders" were 3 LumA and 1 LumB. When examined in an unsupervised hierarchical clustering analysis along with >800 TCGA Breast tumor samples, 13/17 patients had all of their samples grouped immediately together, suggesting that the overall tumor phenotype was maintained. Interestingly, the most dominant change in gene expression was the observation that there were 5 "progressor" patients where the pre-treatment sample was LumB and all subsequent samples were LumA; we only observed one instance of a patient starting as LumA and changing to a LumB, who was also labelled as a "progressor". Full exome sequencing is underway and these results will be presented.

Conclusion: Genomic analysis of progression suggests that an apparent "subtype shift" appears in a number of patients where a shift to LumA is seen; this apparent change may be reflective of decreased proliferation rates caused by therapy, or the acquisition of a true LumA phenotype. We cannot differentiate between these two hypotheses at this time, but expect that the exome sequencing will help to differentiate between these two hypotheses given the large number of mutations and copy number alterations that can differentiate between LumA vs. LumB.
Title: Stromal tumor-infiltrating lymphocytes (S-TILs): In the alliance N9831 trial S-TILs are associated with chemotherapy benefit but not associated with trastuzumab benefit

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Body: Background: Tumor-infiltrating lymphocytes (TILs) at diagnosis are reported to be prognostic in triple-negative breast cancer (BC). Analysis of a small subset of 209 HER2+ patients (pts) with 49 events concluded that higher levels of S-TILs are associated with increased trastuzumab benefit (Loi, 2014). Here we report the largest study to date evaluating S-TILs and their prognostic and predictive association with clinical outcome in N9831 pts treated with either chemotherapy or chemotherapy plus trastuzumab.

Methods: Samples assessed were from primary tumors of pts on N9831 arm A (standard AC→T chemotherapy) and arm C (concurrent chemotherapy with trastuzumab) (Perez, 2011). S-TILs were evaluated on H&E whole tumor slides by a single pathologist with ∼10% of cases read by two pathologists in tandem. The percent of stromal lymphocytic infiltrates (S-TILs) was quantitated in deciles; ≥60% S-TILs was used for the categorical cutoff (Denkert, 2010). The association between S-TILs, treatment (tx) and recurrence-free survival (RFS) was studied and the interaction between S-TILs, trastuzumab benefit and RFS was calculated.

Results: 489 pts from arm A (chemo) and 456 pts from arm C (chemo with trastuzumab) were assessed and were similar to pts in the overall trial; all had RFS information and a median follow-up of 4.4yr. Tumors from 54% of pts in arms A and C were HR+; 14% were node-negative. Tumors with high S-TILs were more likely to be hormone receptor-negative (p< 0.0001). In multivariable analyses including nodal status, hormone receptor status, tx arm, tumor size, tumor grade, and age, ≥60% S-TILs was significantly associated with RFS (HR 0.20; 95%CI 0.06–0.65, p=0.007) in arm A but not in arm C (HR 1.1; 95%CI 0.42–2.8, p=0.87); the interaction term of arm and ≥60% S-TILs was significant (p=0.042). Semi-continuous deciles were associated with RFS in arm A (p<0.0002) but not in C (p=0.37). Hormone receptor status was an independent prognostic factor in arm A (HR 0.61; 95%CI 0.41–0.93, p=0.02) but not in C (HR 0.79; 95%CI 0.44–1.41, p=0.42). In arm A the 10yr Kaplan-Meier estimates for RFS were 90.9% and 64.5% for high S-TILs and low S-TILs pts, respectively (HR 0.23; 95%CI 0.07–0.73, p=0.013). In arm C the 10yr Kaplan-Meier estimates for RFS were 80.0% and 80.1% for high S-TILs and low S-TILs pts, respectively (HR 1.26; 95%CI 0.5–3.2, p=0.63).

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<th>Arm C (N=456)</th>
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<td>1 (0.98, 1.03)</td>
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Conclusions: In exploratory analyses from this subset HER2+ population from N9831, S-TILs were associated with RFS in patients treated with chemotherapy alone, and were not shown to be associated with RFS in patients treated with chemotherapy plus trastuzumab.
Title: HER2 T cell dependent bispecific antibody (HER2-TDB) for treatment of HER2 positive breast cancer

Teemu T Junttila¹, Ji Li¹, Jennifer Johnston¹, Maria Hristopoulos¹, Robyn Clark¹, Diego Ellerman¹, Bu-Er Wang¹, Yijin Li¹, Mary Mathieu¹, Guangmin Li¹, Judy Young¹, Elizabeth Luis¹, Gail Lewis Phillips¹, Eric Stefanich¹, Cristoph Spiess¹, Andrew Polson¹, Bryan Irving¹, Justin M Scheer¹, Melissa R Junttila¹, Mark S Dennis¹, Robert Kelley¹, Klara Totpal¹ and Allen Ebens¹. ¹Genentech, San Francisco, CA.

Body: Based on recent clinical success of tumor immunotherapies that block immune suppressive mechanisms to restore T cell function, there is a profound interest in the clinical development of T cell targeted therapies. We have produced a trastuzumab-based HER2 T cell dependent bispecific antibody (HER2-TDB) that conditionally activates T cells resulting in lysis of HER2 expressing cancer cells at low picomolar concentrations. Due to its unique mechanism of action, which is unrelated to HER2 signaling or sensitivity to chemotherapeutic agents, HER2-TDB can eliminate cells refractory to currently approved HER2 therapies. The potent anti-tumor activity of HER2-TDB was demonstrated using four model systems including MMTV-huHER2 and huCD3 transgenic mice. We demonstrate inhibitory effect of PD-L1 expression on the activity of bispecific T cell recruiting antibodies. This resistance mechanism is reversed by anti-PD-L1 treatment and combination of HER2-TDB with anti-PD-L1 immune therapy resulted in enhanced inhibition of tumor growth, increased response rates and durable responses.

Significance:
This report presents a new immunotherapy for HER2+ breast cancer with an alternative, extremely potent mechanism of action that is effective in cells resistant to current HER2 targeted therapies. Several significant advances are provided to bispecific T cell recruiting antibodies: we characterize a critical resistance mechanism, a potential diagnostic marker, a novel transgenic efficacy model and significantly improve the drug-like properties by using technology based on full length antibodies with natural architecture. Finally we demonstrate the benefit of combining two immune therapies: direct polyclonal recruitment of T cell activity together with inhibiting the T cell suppressive PD-1/PD-L1 signaling results in enhanced and durable long term responses.
Reduced tumor lymphocytic infiltration in the residual disease (RD) of post-neoadjuvant chemotherapy (NAC) triple-negative breast cancers (TNBC) is associated with Ras/MAPK activation and poorer survival

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Body: Background: Tumor-infiltrating lymphocytes (TILs) are associated with improved prognosis in TNBCs, with several retrospective analyses demonstrating that TNBCs with high baseline TILs have higher rates of pathologic complete response (pCR) to NAC. Moreover, the TIL burden in the RD of patients who do not achieve pCR to NAC is also correlated with prognosis. However, insight into the molecular pathways in TNBC which modulate heterogeneity in host anti-tumor immune responses is lacking. To address this gap in knowledge, we analyzed TILs retrospectively in a cohort of clinically and molecularly characterized TNBCs with RD after NAC.

Methods: TILs were scored in H&E stained slides by expert pathologists in the post-treatment tumors of 92 NAC-treated TNBC patients with RD at the time of resection and in 44 matched baseline diagnostic biopsies. Genomic alterations in the RD were assayed using targeted next-generation sequencing (tNGS) while selected transcriptional signatures were evaluated by NanoString as previously published (Balko et al, Cancer Discovery 2014). Differences in pre- and post-NAC TILs were compared between tumors harboring alterations in cell cycle, PI3K/mTOR, growth factor receptors, Ras/MAPK and DNA repair pathways. Associations of TILs with transcriptional signatures were also tested.

Results: A strong positive association of TILs in NAC-treated specimens was observed with RFS (coxPH p=0.0001, relative risk reduction of 3.4% for each % of TILs) and OS (p=0.0016; relative risk reduction of 2.8% for each % of TILs). In multivariate analysis with stage, age, node status and RD tumor cellularity, TILs in the post-NAC disease remained a significant predictor of RFS and OS (p=0.0008 and p=0.007, respectively). TILs tended to decrease with NAC in paired samples, although this decrease was not statistically significant (p=0.07).

Genetic alterations in the Ras/MAPK (amplifications in KRAS, BRAF, RAF1 and truncations in NF1) and cell cycle pathway (alterations in CCND1-3, CDK4, CDK6, CCNE1, RB, AURKA and CDKN2A) were associated with lower TILs in RD (p=0.005 and p=0.05, respectively). A significant inverse linear correlation was detected between a transcriptional signature of Ras/MAPK activation (Pratilas et al, PNAS 2009) and TILs in the RD (Spearman’s r=-0.42; p=0.00028). Total number of alterations of likely functional significance detected by tNGS showed no association with TILs, suggesting that the association of Ras/MAPK deregulation and cell cycle alterations with TILs may be a pathway-specific effect.

In TNBC cell lines, chemical inhibition of MEK transcriptionally up-regulated MHC-I and MHC-II molecules, while simultaneously down-regulating mRNA expression of the immune checkpoint inhibitor PD-L1 (MDA-231 p=0.00002, BT549 p=0.0003, and SUM159PT p=0.009). In vivo experiments confirming these associations are underway.

Conclusions: Our data suggest a strong correlation of Ras/MAPK pathway activation with immune-evasion and outcome in TNBC. With additional mechanistic understanding, rational design of clinical trials combining MEK inhibitors with PD-L1 antibodies in TNBC may be warranted.
**Title:** A phase Ib study of pembrolizumab (MK-3475) in patients with advanced triple-negative breast cancer

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**Body:**

Introduction: The PD-1 receptor-ligand pathway can be used by tumors to evade immune surveillance, thereby allowing neoplastic growth. Pembrolizumab is a highly selective, humanized IgG4/kappa isotype mAb designed to block PD-1 interaction with its ligands PD-L1 and PD-L2, thereby reactivating the immune system to eradicate tumors.

Methods: This is a multi-center, non-randomized trial of single agent MK-3475 treatment given intravenously at 10 mg/kg every 2 weeks, in patients with recurrent/metastatic triple-negative (ER, PR, and HER2 negative) breast cancer (TNBC). PD-L1 expression in tumor or stroma was required for study entry. PD-L1 status was determined by immunohistochemical analysis of patient’s tumor tissues using the Merck proprietary 22C3 antibody. Primary objectives of this study were to determine the safety, tolerability, and anti-tumor activity of MK-3475 in patients with PD-L1 positive, advanced TNBC. Secondary objectives included assessments of progression-free survival, overall survival, and response duration. Adverse events (AEs) reported in any patient receiving at least 1 dose of study treatment were monitored and graded using NCI CTCAE v. 4.0. Radiographic imaging was obtained every 8 weeks and evaluated by both investigator and an independent radiologist to assess clinical responses as defined by RECIST 1.1. This study (Clinicaltrials.gov: NCT01848834) is being conducted in conformance with Good Clinical Practices.

Results: A total of 32 female patients with a median age of 50.5 years (range 29 – 72 years) with PD-L1 positive, recurrent/metastatic TNBC were enrolled in the study. Most of these patients had received and progressed on multiple lines of therapy for advanced disease. A preliminary analysis of data collected as of 23May2014 indicates that 5 patients (15.6%) experienced at least one drug-related serious adverse event (SAE); each of 4 patients experienced one of the following: Grade 3 anemia, headache, aseptic meningitis or pyrexia, and a fifth patient experienced disseminated intravascular coagulation (DIC) with thrombocytopenia and decreased blood fibrinogen. The patient who experienced DIC died. Preliminary analysis of data collected from investigators as of 23May2014 indicates that no patient had a complete response, 16.1% of patients had a partial response, 9.7% had stable disease, and 64.5% had progressive disease. As of 23May2014, all but one of the responders, in addition to three patients with stable disease, remain on treatment.

Conclusion: This is the first report of clinical activity of an immune checkpoint inhibitor in TNBC. The preliminary results from this study suggest that single agent MK-3475 is a well-tolerated and effective treatment with significant therapeutic activity in a subset of heavily pre-treated patients with recurrent/metastatic triple-negative breast cancer.
Title: Theranostic multiparametric tests improve residual risk assessment in early luminal breast cancer

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Body: Background: Despite rapidly expanding availability of multiparametric tests which inform residual risk following adjuvant therapy for early breast cancer current approaches provide minimal information on the appropriate targeted therapy to be selected for patients at high risk of recurrence. We hypothesised that inclusion of key signalling nodes from driver molecular pathways in early breast cancer in residual risk signatures would both improve risk stratification and identify candidate theranostic targets for the next generation of clinical trials.

Methods: RNA was extracted from FFPE luminal breast cancers from the TEAM pathology study (Exemestane versus Tamoxifen-Exemestane). Gene expression analyses were performed for 29 genes mapped across key signalling nodes within the PIK3CA pathway. mRNA assessment for IHC4 (ER, PgR, HER2 and Ki67) was included in the model. Quantitative gene expression was performed using the Nanostring platform. Novel signatures were trained in a randomly selected sub-set of the TEAM pathology cohort (n=˜1700) and validated using the remaining 50% of patients (n=˜1700). Results presented represent those from the validation cohort.

Results: The IHC4-protein and IHC4-mRNA risk scores were highly correlated (Rho=0.72, p=4.12x10-265), suggesting the mRNA abundance-based classifier is able to serve as a good substitute for the protein-based model.

A gene signature including IHC4 markers assessed by mRNA performed significantly better (AUC 0.70 vs 0.66) than conventional IHC4. A gene signature including 4 signalling modules from the PIK3CA pathway significantly outperformed both IHC4 and the 4 gene (ER, PgR, HER2, Ki67) classifier (AUC 0.75; p = 3.23x10-7 vs IHC4 and 1.39x10-3 vs "IHC4mRNA").

Conclusions: Inclusion of PIK3CA signalling modules identified key genes/nodes which are linked to early relapse in luminal breast cancer and provided a significantly improvement in risk classification when compared to a currently validated multiparameter test.
**Title:** The FERGI phase II study of the PI3K inhibitor pictilisib (GDC-0941) plus fulvestrant vs fulvestrant plus placebo in patients with ER+, aromatase inhibitor (AI)-resistant advanced or metastatic breast cancer – Part I results

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**Body: Background:** Preclinical and clinical data indicate a key role for the PI3-kinase (PI3K) pathway in the pathogenesis of resistance to endocrine therapies in hormone receptor-positive (HR) breast cancer (BC) and suggest that combining PI3K inhibitors with endocrine therapy may partially overcome this resistance. FERGI is the first randomized Phase II study testing pictilisib (GDC-0941), a PI3K inhibitor, in combination with fulvestrant to evaluate this hypothesis in MBC patients with and without PIK3CA-mutant tumors.

**Methods:** 168 post-menopausal pts with ER-positive, HER2-negative MBC were randomized (1:1) to receive fulvestrant with either pictilisib 340 mg QD (n=89, “combination” arm) or matching placebo (n=79, “control” arm). To be eligible, pts had to have relapsed during or within 6 mos of completing adjuvant AI treatment or have progressed on an AI for MBC. Pts were stratified based on tumor PIK3CA mutation status, resistance to prior AI therapy and presence of measurable disease. The primary endpoint was PFS by investigator assessment in the intent-to-treat (ITT) group and in pts with centrally confirmed PIK3CA-mutant tumors. The primary analysis was based on a 6 mo median duration follow up.

**Results:** Baseline disease and prior treatment characteristics were similar between study arms. Observed treatment-emergent AEs were consistent with those previously described for single agent pictilisib and fulvestrant (primary toxicities were rash and GI disorders). In the ITT population (84 events) the median PFS (mPFS) was 6.2 mo in the combination arm vs 3.8 months for the control arm (HR, 0.77; 95% CI, 0.50-1.19). For pts with PIK3CA-mutant tumors (37 events), mPFS was 6.2 mo in the combination arm vs 5.1 mo in the control arm (HR, 0.92; 95% CI, 0.48-1.76). For pts without a detectable PIK3CA mutant tumor (43 events), mPFS was 5.8 months in the combination arm vs 3.6 months in the control arm (HR, 0.64; 95% CI, 0.35-1.17). Exploratory post-hoc subgroup analysis suggested improvement in PFS in pts with ER+ and PR+ tumors (centrally confirmed) treated with pictilisib plus fulvestrant. In the ER+/PR+ subgroup (57 events) mPFS was 7.2 mo in the combination arm vs 3.7 mo in the control arm (HR, 0.46; 95% CI, 0.27 to 0.78). This improvement was independent of tumor PIK3CA mutation status. Multivariate analysis suggests that this treatment effect in pts with ER+/PR+ tumors is maintained after adjusting for possible baseline imbalances. A similar analysis on pts with luminal A tumors (per PAM50 analysis) was also consistent with the findings in pts ER+/PR+ disease.

**Conclusions:** This is the first report of a blinded, randomized clinical study evaluating a PI3K inhibitor in pts with MBC. In the ITT population, the addition of pictilisib to fulvestrant was associated with a mPFS improvement of 3.8 mo to 6.2 mo. Exploratory subgroup analyses suggested in pts with ER+/PR+ tumors are more likely to derive benefit from the addition of pictilisib to fulvestrant irrespective of PIK3CA mutation status, though the subgroup analyses are limited by the sample size. Additional biomarker analyses will be reported.
**Title:** Preoperative window of opportunity study of the PI3K inhibitor pictilisib (GDC-0941) plus anastrozole vs anastrozole alone in patients with ER+, HER2-negative operable breast cancer (OPPORTUNE study)

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**Body: Background:** Preclinical and clinical data support a key role for the PI3K pathway in resistance to endocrine therapy in patients with ER+ breast cancer and suggest that combining PI3K inhibitors with endocrine therapy may overcome resistance. Short-term preoperative window of opportunity (WOO) studies are a validated strategy for novel treatments to provide proof-of-concept and define the most appropriate patient population by directly assessing treatment effects in tumour tissue before and after treatment. This is the first WOO study with the PI3K inhibitor pictilisib (GDC-0941) in combination with anastrozole (ANA).

**Methods:** 73 postmenopausal patients (pts) have been randomized (2:1 in favour of the combination) to receive 2-week preoperative treatment with ANA plus pictilisib (n=50, "ANA+PIC" arm) or ANA alone (n=23, "ANA" arm). Pts had newly diagnosed, operable, ER+, HER2-negative breast cancer of ≥ 1 cm size. Pts receiving HRT were excluded. Treatment effects and correlative studies were assessed using FFPE and frozen tumour biopsies taken before and after 14 days of study treatment. The primary endpoint was inhibition of tumour-cell proliferation, as measured by change in Ki67 expression, determined centrally by 2 investigators. Secondary endpoints include induction of apoptosis (Caspase3) and safety. Comprehensive biomarkers analyses include targeted NGS of a comprehensive cancer panel of >400 genes, copy number analyses, and pre- and post-treatment reverse-phase protein arrays (RPPA) and RNA profiling.

**Results:** Baseline (BL) disease characteristics were similar between both study arms. PAM50 analysis showed that 53% and 47% of tumors were Luminal (Lum) A and B, respectively. 65% of tumors had >14% Ki67-positive cells. Observed treatment-emergent AEs were consistent with those previously described for single-agent pictilisib and anastrozole. Mean post-treatment percentage reduction of Ki67 was 84% (95% CI, 75%-89%) for ANA+PIC and 72% (54%-87%) for ANA. Ki67-response (≥50% drop in % of Ki67+ cells) was 86% for ANA+PIC and 60% for ANA. By using the definition that pts with a natural logarithm of %Ki67+ cells of ≤ 1 or 1-2 have a day 15 anti-proliferative response, 93% [ln(ki67): <1, 46%; 1-2, 46%] of ANA+PIC were responders compared with 60% [<1, 47%; 1-2, 13%] of ANA-treated pts (p = 0.01). Preplanned subgroup analyses showed a significant interaction of response to ANA+PIC with molecular subtype and Ki67 levels. Patients with LumB tumors or high BL Ki67 (>14%) had a higher Ki67 response with ANA+PIC compared to ANA (LumB, 83% vs 38%; Ki67>14%, 94% vs 55%), whereas Ki67 response was similar for both treatments for LumA tumors (ANA, 75%; ANA+PIC, 73%) or tumors with low BL Ki67. Mean post-treatment % reduction of Ki67 in LumB tumors was 87% (95% CI, 49%-96%) for ANA+PIC and 56% (16%-77%) for ANA (p=0.03).

Additional data on apoptosis and comprehensive pre- and post-treatment biomarkers analyses will be presented.

**Conclusions:** This first report of a preoperative WOO study evaluating a PI3K inhibitor in early breast cancer demonstrated addition of pictilisib to ANA was associated with increased anti-proliferative response over single-agent ANA.
Title: Comprehensive molecular characterization of invasive lobular breast tumors

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Body: Invasive lobular breast cancer (ILC) is the second most common histological subtype of breast cancer accounting for 10-15% of invasive breast tumors. ILC is typically ER+ and beyond the known mutation and/or loss of E-cadherin function, which contributes to a highly discohesive morphology, little is known about the additional mechanisms driving ILC tumorigenesis, or alterations that differentiate ILC from invasive ductal carcinomas (IDC).

Methods
A dataset of 817 breast tumors from the TCGA Project, including 490 IDC, 127 ILC and 88 samples with a mixed IDC-ILC histology, were profiled on six genomic platforms to develop a comprehensive atlas of mutational, epigenetic, transcriptional and proteomic data. Integrative genomic analyses, both supervised and unsupervised, of ILC tumors and across histological subtypes were performed to identify genomic drivers of ILC oncogenesis.

Results
Comprehensive multi-platform analyses identified distinct molecular events associated with ILC tumors. As expected, lack of E-cadherin protein, as determined by Reverse Phase Protein Array (RPPA), and CDH1 mRNA expression was uniformly observed in ILC cases associated with distinct alterations targeting CDH1. In addition to previously reported CDH1 and PIK3CA mutations, we identified a number of novel ILC-enriched recurrent mutations targeting PTEN, RUNX1, TBX3, and FOXA1. An increased incidence of PTEN inactivating events, both mutations and copy number changes, were identified in ILC (13%) compared to IDC ER+ (7%), which corresponded with altered PTEN protein expression. These alterations were largely mutually exclusive with PIK3CA mutations and correlate with increased Akt activation as evident by increased Akt phosphorylation (pS473 and pT308), thus identifying a potential therapeutic opportunity for ILC patients.

GATA3 signaling, which regulates epithelial cell differentiation, is frequently altered in luminal/ER+ breast cancers. Our analyses determined GATA3 mutations are more frequent in IDC luminal tumors as compared to ILC (19 % vs 5%). ILC luminal tumors show significantly lower GATA3 protein expression, but a higher frequency of mutations in FOXA1 (9% vs 2% in Luminal IDC), a transcription factor required to promote ER transcriptional programs. Within ILC tumors, FOXA1 mutations were found to cluster into a specific region of the Forkhead (FK) DNA binding domain. A broader analysis of FOXA1 mutations in breast and prostate cancer confirm two specific hotspots in the FK domain and the C-terminal transactivation domain. Interestingly, these mutational classes are associated with distinct transcriptional changes suggesting different functional effects.

Finally, mRNA-seq analyses identified three robust molecular subclasses that are characterized by distinct genetic, genomic and proteomic patterns, including an increased immune-related group (Class 2), as well as differences in prognosis.

Conclusions
In this study, we developed a comprehensive atlas of genomic alterations that reveals key molecular differences differentiating ILC (FOXA1) from IDC (GATA3) tumorigenesis, a potential therapeutic target for ILC (Akt), and novel ILC subclasses based on underlying biological events. These findings provide further insight into the molecular heterogeneity of ER+ breast cancer.
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Title: Characterization and clinical relevance of the genomic alterations defining lobular breast cancer

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Body: Background:
Invasive lobular breast cancer (ILC) is the second most common histological subtype of breast cancer, is mostly estrogen receptor-positive (ER+) and has distinct clinico-pathological features when compared to ER+ invasive ductal carcinoma (IDC). In this study, we aimed to characterize the genomic alterations defining ILC in a large cohort of ILC patients with long-term follow up (FU).

Material & methods:
In 499 centrally histologically confirmed ILC patients (median FU= 9.8 years) we analyzed mutational data gathered from targeted sequencing of 360 cancer genes at an average coverage of 106X (alignment done with BWA, substitutions and indels –further referred to as mutations- called with Caveman and Pindel). Matched normal DNA was available for 242 patients. Genome-wide copy number (CN) data were available for 178 patients. E-cadherin (CDH1) and beta-catenin (CTNNB1) stains were carried out using the DakoTM antibodies. Invasive disease free survival (IDFS) was considered as primary survival endpoint.

Results:
A median of 6 [range:0-38] non-silent mutations was identified across the primary tumors of all patients. The most frequently mutated genes (>3%) are listed in Table 1. Of those, CDH1, PIK3CA, TBX3, FOXA1 and the chromatin-related genes MLL2, MLL3, ARID1A and ARID1B were more frequently mutated in our ER+/HER2- ILC (n= 451) compared to the ER+/HER2- IDC (n=266) from The Cancer Genome Atlas, whereas GATA3, TP53 and MAP3K1 were less frequently mutated.

Samples with a CDH1 mutation were associated with changes at the protein level: 97.5% displayed a complete loss of the protein, associated with cytoplasmic staining for CTNNB1, compared to only 63% of the CDH1 non-mutated tumors. CDH1 mutated tumors were further characterized by increased mutational frequencies of the ERBB-genes: 15.4% for the CDH1 mutated tumors versus 3% in the CDH1 non-mutated tumors, most of those mutations being described in the literature as activating the pathway. Almost all tumors (97%) with CN data had a heterozygous loss of CDH1.

The special alveolar, solid and trabecular lobular histotypes were associated with specific CN alterations and mutations. Tumors with mutated ARID1A or ATM were associated with worse IDFS at the univariate level and ARID1A remained significant in a multivariate analysis including standard parameters (HR =2.07, p=0.003). At the CN level, ATM and ARID1A losses, as well as HER2 and VEGFA gains/amplifications were associated with decreased IDFS, all but ARID1A holding significance at the multivariate level (HR_HER2=2.41, HR_VEGFA= 1.99, HR_ATM= 1.79, all p<0.05).

Conclusion:
This is the first and largest study to our knowledge to report genomic alterations present in ILC and their association with survival. This work therefore opens new avenues for a better understanding of the disease and its clinical management.

Table 1: List of the most frequently mutated genes

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<tr>
<td>CDH1 62.9</td>
<td>MAP3K1 7</td>
<td>USP9X 4.4</td>
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Title: Survival advantage of anastrozole compared to tamoxifen for lobular breast cancer in the ABCSG-8 study

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Body: Introduction:
Invasive lobular cancer (ILC) is the second most common histological type of breast cancer, appearing to have a biology distinct from ductal cancer (IDC). In the first 5 years, the prognosis of ILC seems to be more favourable compared to IDC. The aim of this study was to investigate the differences in benefit from adjuvant Tamoxifen (Tam) and Anastrozole (Ana) for these histologic subtypes.

Patients and Methods:
The ABCSG-8 (Austrian Breast and Colorectal Cancer Study Group 8) study was a randomized phase III clinical trial (n=3,714) in hormone receptor-positive cancer addressing a sequence strategy with 3 years of Ana after 2 years of Tam in comparison with 5 years of Tam in a low- to intermediate-risk group (Grade 1/2) of postmenopausal patients not receiving adjuvant chemotherapy. Univariate and multivariate comparisons for overall (OS) and disease-free survival (DFS) between endocrine treatments were conducted per subgroup with respect to histology (ductal or lobular). Multivariate Cox analysis included endocrine treatment, histology and their interaction ± additional covariates (age, T-stage, N-stage, grade, ER, PR). For the time-to-event analyses, starting point for all patients was two years after randomization, i.e. when treatment changed from Tamoxifen to Anastrozole on the experimental arm. To avoid crossover effects for post-study treatment, the total time for this analysis is 3 years.

Results:
The 3-year OS hazard ratio (HR) for Anastrozole versus Tamoxifen in ILC (n=694) was 0.24 (0.08-0.70) vs. IDC (n=2,739) HR 1.08 (0.75-1.58). In multivariate analysis, HR for ILC was 0.23 (0.08-0.68) vs. 1.02 (0.70-1.49) for IDC. The test for interaction of treatment and histology was significant (p=0.01). In ILC, no other clinico-pathologic factor was significantly associated with survival differences compared to IDC, where age, T- and N-stage maintained significance. In the models for DFS, our preliminary analysis after 3-year follow-up did not show significant variations in treatment effect according to histology.

Discussion:
The magnitude of survival benefit from adjuvant Anastrozole vs. Tamoxifen varies by histology in this large phase III randomized trial. A significant reduction in risk of death occurs only in patients with lobular cancer compared to ductal cancer after only 3 years of follow-up. An updated analysis with additional follow-up will be available at presentation.
Title: A randomized phase III trial comparing neoadjuvant chemotherapy with weekly nanoparticle-based paclitaxel with solvent-based paclitaxel followed by anthracyline/cyclophosphamide for patients with early breast cancer (GeparSepto); GBG 69

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Body: Background: Anthracycline/taxane based regimen are standard of care for neoadjuvant therapy in breast cancer. A reverse sequence of taxanes followed by anthracyclines was suggested to achieve higher pCR rates (H.Earl, Lancet Oncol 2014). Dual HER2 blockade was shown to be superior to trastuzumab alone increasing the pCR rate by 20%. Nab-paclitaxel (nP) is a solvent-free formulation of paclitaxel (P) encapsulated in albumin which might further improve the pCR rate in breast cancer patients receiving neoadjuvant treatment and cause lower toxicity.

Methods: In the GeparSepto study (NCT01583426) patients were randomized to either nP (125 mg/m\textsuperscript{2}) q1w or P (80mg/m\textsuperscript{2}) q1w for 12 weeks followed by 4 cycles of conventionally dosed EC (E, epirubicin 90mg/m\textsuperscript{2}; C, cyclophosphamide 600 mg/m\textsuperscript{2}) q3w. The primary objective is to compare the pathological complete response rate (pCR, ypT0 ypN0). Further objectives are to compare the pCR rate in predefined subgroups, pCR by other definitions, clinical response rate, rate of breast conserving surgery and toxicity and compliance. Patients with untreated, histologically confirmed uni- or bilateral, cT2- cT4d carcinoma, and no clinically relevant cardiovascular and other co-morbidities were included. HER2\textsuperscript{+} patients received trastuzumab (loading dose 8mg/kg; 6 mg/kg) plus pertuzumab (loading dose 840 mg; 420 mg) q3w concomitantly. HER2, estrogen receptor, progesterone receptor, Ki67 and SPARC status were centrally assessed prior to randomization for stratification. To increase the pCR rate from 33% with P to 41% with nP, corresponding to an odds ratio of 1.41 with an alpha of 0.05 and a power of 80%, 1200 patients would be needed. A window-of opportunity study was integrated to investigate response to anti-HER2 treatment without chemotherapy, HER2\textsuperscript{+} patients were randomized to receive 6 weeks of either trastuzumab, pertuzumab or the combination with biomaterial collection at the start and the end of the window.

Results: A total of 1204/1229 (window study n=71) recruited patients (7/2012 - 12/2013) from 69 German centers were evaluable, 606 receiving nP. Baseline characteristics are well balanced; median age was 49/50 years (P/nP), 33/33% of the patients presented with HER2\textsuperscript{+} tumors, 23/23% triple negative breast cancer TNBC (ER and PR <1 %), 87/85% ductal invasive, 56/52% G3 tumors; Ki67>20% 69/69%; SPARC positive 15.7/16%. 265 patients reported SAEs (119 P/146 nP) and 4 died on study (1 P: cardiac decompensation; 3 nP: accident at home, multiorgan failure, sepsis during EC). The pCR rate (ypT0 ypN0) is 29% with P and 38% with nP, OR 1.5; p<0.01.

Conclusions: GeparSepto showed that the pCR rate is significantly higher with nab-paclitaxel compared to solvent-based paclitaxel given weekly before anthracycline based chemotherapy. Subgroupanalyses and 2ndary endpoints will be presented at the meeting.

The trial was financially supported by Roche and Celgene. The window-substudy was funded within the EU-FP7 project RESPONSIFY No 278659.
Title: The TNT trial: A randomized phase III trial of carboplatin (C) compared with docetaxel (D) for patients with metastatic or recurrent locally advanced triple negative or BRCA1/2 breast cancer (CRUK/07/012)

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Body: Introduction:
Subgroups within sporadic triple negative breast cancers (TNBCs) appear to share impaired DNA damage response mechanisms with BRCA1/2 mutation-associated breast cancers. This has been hypothesised to confer particular sensitivity to DNA-damaging platinum chemotherapy. The TNT trial, a randomized phase III trial in women with metastatic or recurrent locally advanced TNBC or BRCA1/2 mutation-associated breast cancer, aimed to test this hypothesis and examine treatment effect in biological subgroups.

Patients & Methods:
Eligible patients had either ER-, PR-, HER2- breast cancer or were known BRCA1/2 carriers (any ER/PR/HER2). Patients were randomized (1:1) to receive either C (AUC 6 q3wk) or D (100mg/m2 q3wk) for 6-8 cycles or until disease progression if sooner and could cross over to the alternative treatment on confirmed progression. Ineligible patients included those who had ECOG performance status >2, received adjuvant taxane therapy in the last 12 mths, any previous treatment with a platinum chemotherapy, or previous non-anthracycline chemotherapy for metastatic disease.

For consenting patients a blood sample and archived tissue samples were obtained for BRCA1/2 genotyping and central biomarker analysis (primary tumour, lymph nodes and recurrent tumour biopsy if available) of subtypes within TNBC and biomarkers of DNA repair deficiency.

The primary endpoint was RECIST objective tumour response up to cycle 6 of randomised treatment. Secondary endpoints included toxicity, progression free survival (PFS), time to progression and overall survival.

TNT aimed to detect a 15% improvement in ORR with C compared to D, with planned target sample size range of 370-450 depending on assumed ORR in D patients (2-sided \(\alpha=0.05\), power=90%). 376 (188 C, 188 D) were recruited from 74 UK centres between Apr 08 and Mar 14.

Results:
A snapshot of the data was taken on 30/5/14 at which point 336 (89.4%) patients had experienced a PFS event, with overall median PFS time of 4.4 mths. Median age of patients was 55 yrs (IQR 48-63). 366/376 (97%) patients had TNBC of whom 18 were also known BRCA1/2 mutation carriers, with the remaining 10 patients receptor +ve and BRCA1/2 carriers. 338/376 (90%) had metastatic and 38/376 (10%) recurrent locally advanced disease. 53% had liver or lung metastases affecting the parenchyma and 34% had received previous adjuvant taxane therapy. Median time from initial diagnosis to entering TNT was 2.2 yrs (IQR 1.5-3.5). Primary tumour tissue has currently been received for 277 patients, blood from 286 patients and recurrent tumour tissue from 85 patients.

Discussion:
TNT will report evidence on the activity of single agent platinum chemotherapy compared with single agent taxane in patients with TNBC and BRCA1/2 associated breast cancer. Correlative analyses of BRCA1/2 mutation status, subtypes and DNA repair
biomarkers will also be reported. TNT will be the first randomised trial to report the activity of platinum compared with standard chemotherapy within TNBC subtypes and in relation to BRCA1/2 mutation status and DNA repair biomarkers. Safety, tolerability and response to crossover treatment will also be presented.
2014 San Antonio Breast Cancer Symposium

Publication Number: S3-02
Average Grade: 7.80

Title: NSABP B-36: A randomized phase III trial comparing six cycles of 5-fluorouracil (5-FU), epirubicin, and cyclophosphamide (FEC) to four cycles of adriamycin and cyclophosphamide (AC) in patients (pts) with node-negative breast cancer

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Body: Background

NSABP B-36 was originally designed as a 2X2 factorial randomized study to compare 6 cycles of FEC-100 with 4 cycles of standard AC with or without celecoxib in pts with node-negative breast cancer. The rationale for the trial was based on observations from other adjuvant trials suggesting that longer duration of anthracycline-based therapy may result in improved outcomes and also on accumulating evidence that prostaglandins may contribute to the malignant phenotype in breast cancer. The trial opened in May 2004 but random assignment to celecoxib v placebo was terminated in December 2004 (after 327 pts were enrolled) because of concerns for increased risk of cardiovascular disease with the use of COX-2 inhibitors. The trial continued as a two-arm study and completed accrual in July 2008. The primary endpoint of disease-free survival (DFS), and secondary endpoints of overall survival (OS), recurrence-free interval (RFI), distant recurrence-free interval (DRFI), and adverse events comparing FEC-100 and AC are reported here. Analyses of quality of life indicators will be reported separately.

Methods

2,722 pts with T1-3, pN0 breast cancer were randomly assigned to either adriamycin 60 mg/m2 and cyclophosphamide 600mg/m2 every 21 days for 4 cycles (n=1361) or 5-FU 500mg/m2, epirubicin 100 mg/m2 and cyclophosphamide 500 mg/m2 every 21 days for 6 cycles (n=1361). Hormone- receptor positive pts were to receive physician's choice of hormonal therapy for a minimum of 5 yrs. Trastuzumab for HER2-positive pts was at investigator's discretion but was not to begin for >3 weeks after the last dose of chemotherapy. All women treated with lumpectomy were to receive breast radiotherapy; post-mastectomy chest wall radiotherapy was at physician's discretion. The differences in DFS (OS, RFI, and DRFI) between two treatment arms were assessed by log-rank test stratified by hormone receptor status and type of surgery.

Results

Median follow-up is 82.8 months. Pt and tumor characteristics were equally distributed between the two groups (<50 years old: 40%, lumpectomy: 68%, and hormone positivity: 65%). Overall, Grade 3 and 4 expected toxicities were more frequent in the FEC arm. Combined Grade 3/4 toxicities reaching statistical significance with a difference of 3% or more between AC and FEC arms included fatigue 3.55% v 8.45%, febrile neutropenia 3.70% v 9.42%, and thrombocytopenia 0.74% v 4.41%, respectively. While on treatment, Grade 3 left ventricular systolic dysfunction occurred in one pt in each arm. There were 2 toxicity deaths on AC and 5 on the FEC arm. Primary and secondary endpoint analyses at 8 ys did not reveal any significant differences in DFS, OS, RFI, or DRFI.

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<th>FEC (%)</th>
<th>HR</th>
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<td>DFS</td>
<td>83.0</td>
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<td>0.85,1.26</td>
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Conclusions
Six cycles of the FEC-100 regimen did not result in any efficacy advantage over 4 cycles of standard AC in node-negative breast cancer pts. As anticipated, the FEC-100 regimen resulted in greater toxicity.

Support
NCI: U10-CA-12027, -37377, -69974, -69651, -44066-26; Pharmacia & Upjohn Co.
Title: Ten year update of E1199: Phase III study of doxorubicin-cyclophosphamide followed by paclitaxel or docetaxel given every 3 weeks or weekly in patients with axillary node-positive or high-risk node-negative breast cancer

Body: Background: We had previously reported after a median followup of 5.3 years that disease free survival (DFS) was improved with either adjuvant weekly paclitaxel (hazard ratio [HR] 0.73, p=0.0006) or every 3 week docetaxel (HR 0.77, p=0.02) compared with every 3 week paclitaxel (N Eng J Med 2008; 358; 1663), and that obesity and black race were independently associated with inferior outcomes in estrogen receptor (ER)-positive disease after a longer followup (Cancer 2012: 118: 5937 & JNCI 2012; 104: 406). We now report updated results after a median followup of 12.1 years.

Methods: Eligibility included axillary lymph node positive or high-risk (tumor at least 2 cm) node-negative breast cancer. All patients received 4 cycles of AC (doxorubicin 60 mg/m2 and cyclophosphamide 600 mg/m2) every 3 weeks, followed by either: (1) paclitaxel 175 mg/m2 every 3 weeks x 4 (P3), (2) paclitaxel 80 mg/m2 weekly x 12 (P1), (3) docetaxel 100 mg/m2 every 3 weeks x 4 (D3), or (4) docetaxel 35 mg/m2 weekly x 12 (D1). Patients with ER-positive breast cancer also received endocrine therapy for at least 5 years, including either tamoxifen and/or an aromatase inhibitor. The primary comparisons included taxane (P vs. D) and schedule (every 3 weeks vs. weekly), and the primary endpoint was DFS, defined as local, regional, and/or distant relapse, second primary breast cancer, or death without recurrence.

Results: A total of 4954 eligible patients were accrued between October 1999 and January 2002. At the time of this analysis, 90% of surviving patients were followed for at least 10 years, and there were substantially more DFS events (1639 vs. 1048) and deaths (1283 vs. 686) than the original report. For the primary comparison, there remains no significant difference in DFS by taxane (p=0.33) or schedule (p=0.88), although there was a significant interaction between taxane and schedule (p<0.007). When comparing the standard arm (P3) to the other arms (with a hazard ratio [HR] < 1 favoring the experimental arms using a stratified Cox model), the HRs for DFS were 0.84 (p = 0.011) for arm P1 and 0.79 (p=0.001) for arm D3 arm, and for overall survival (OS) were 0.87 (p=0.09) and 0.86 (p=0.054), respectively. When evaluated by subtypes in exploratory analyses, the P1 arm (but not the D3 arm) was associated with improved DFS (HR 0.69, p=0.01) and OS (HR 0.69, p=0.019) in the 1025 patients with triple negative breast cancer (TNBC), and the D3 arm (but not P1 arm) was associated with improved DFS (HR 0.76, p=0.004) but not OS (HR 0.87, p=0.20) in the 2785 patients with ER-positive, HER2-negative/unknown breast cancer (ERBC). In ERBC but not other subtypes, black race (HR 1.60, p=0.002) and obesity (HR 1.23, p=0.009) were associated with inferior OS, and obesity was associated with a higher recurrence risk between 3-8 years after diagnosis.

Conclusions: Improvements in DFS observed at 10 years for the P1 and D3 arms compared with the P3 arm are qualitatively similar but quantitatively less than at 5 years, and the effects on OS are marginal in the overall population. The relative effectiveness of weekly paclitaxel and every 3 week docetaxel may vary by subtype.
Background: Although approximately 50% of newly diagnosed breast cancers arise in women above 65 years old they are underrepresented in clinical trials. The ICE study was designed to investigate if a mono-chemotherapy with capecitabine in addition to the third generation bisphosphonate ibandronate will improve the outcome compared to ibandronate alone in elderly breast cancer patients with medium and high risk primary breast cancer not suitable for standard chemotherapy.

Methods: This is a prospective, multi-center, controlled, open-label, randomized phase III trial. Female patients ≥ 65 years with unilateral or bilateral breast cancer who are either node-positive or high-risk node-negative (tumor size ≥ 2 cm, grade >1, and/or ER-and PR-negative); and a Charlson Comorbidity Index (CMI) ≤ 2 received either ibandronate alone for 2 years, 50 mg p.o. daily alternatively 6 mg i.v. every 4 weeks or the same ibandronate regimen together with capecitabine 2000 mg/m² on day 1 – 14 q day 22 for 6 cycles to be started within 6 months after axillary dissection. Patients with hormone-sensitive disease received an endocrine therapy according to guidelines. The primary objective is disease-free survival. A total of 1,394 patients were needed (5-year DFS improvement from 65% with ibandronate to 71.5% with ibandronate/capecitabine; \( \alpha = 0.05, \beta = 80\% \)) and we expected 497 events during a median 5 year follow up.

Results: Between 06/2004 and 08/2008, 1409 patients were randomized to ibandronate (N=702) or ibandronate/capecitabine (N=668) in 172 German centers, of whom 1380 patients started treatment (689 and 691 respectively). Median age was 71 (range 74-80) years; 58.6% of patients had a CMI≤1, 48.2% had N+, 35.2% grade 3, and 19.2% had ER/PgR negative disease. 83.8% of patients received all 6 capecitabine cycles. Capecitabine grade 3/4 toxicities were <2% except hand-foot-syndrome (6.8%). 3 patients died in relation with capecitabine treatment. Final DFS analysis will be presented at the meeting.

Conclusion: CALGB 49907 showed that AC or CMF was superior to capecitabine in patients aged ≥65 years but was associated with twice as many moderate to severe toxic effects (64% vs 33%) (Muss H et al, NEJM 2012). The ICE study will provide evidence if capecitabine monotherapy is as active as adjuvant treatment and if it might be an option for frail and/or elderly patients where standard chemotherapy is considered to be too toxic.
Title: Intrinsic subtypes, PIK3CA mutation, and the degree of benefit from adjuvant trastuzumab in NSABP trial B-31

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Body: Purpose: Considerable molecular heterogeneity exists among HER2-positive breast cancer regarding gene expression and mutation profiling. Evidence from preclinical, clinical neoadjuvant, and metastatic clinical trials suggested PIK3CA mutational status and PAM50 intrinsic subtype of a tumor were markers of response to anti-HER2 therapies. We evaluated the predictive value of these two biomarkers in the adjuvant setting using archived tumor blocks from NSABP trial B-31.

Methods: Expression data for 49 genes using the nCounter platform was used to generate PAM50 intrinsic subtypes for 1,578 archived tumor blocks from B-31. Five PIK3CA hotspot mutations were examined by mass spectrometry of the primer extension products in a randomly selected subset (N=671). We examined the heterogeneity of trastuzumab treatment effect across different subsets defined by each marker using Cox regression and disease free survival as the endpoint.

Results: 741/1578 (47.0%) tumors were classified as HER2E subtype; and 166/671 (24.7%) had PIK3CA mutations. Hazard ratios (HR) for trastuzumab in HER2E and other subtypes were 0.44 (95%CI: 0.34-0.58, p<0.001) and 0.47 (95% CI: 0.35-0.62, p<0.001), respectively (interaction p=0.67). HRs for trastuzumab in PIK3CA wild type and mutated were 0.51 (95%CI: 0.37-0.71, p<0.001) and 0.44 (95%CI: 0.24-0.82, p=0.009), respectively (interaction p=0.64).

Conclusion: Unlike results seen in the metastatic and neoadjuvant clinical trials, PIK3CA and PAM50 intrinsic subtypes were not biomarkers for differential response to trastuzumab in NSABP B-31. These data suggest that results from the metastatic and neoadjuvant setting may not be always applicable to the adjuvant setting.

Support: National Cancer Institute, Department of Health and Human Services, Public Health Service, Grants U10-CA-12027, U10-CA-69651, U10-CA-37377, and U10-CA-69974, and by a grant from the Pennsylvania State Department of Health. The latter Department specifically disclaims responsibility for any analysis, interpretations or conclusions.
Title: Mutational analysis of CALGB 40601 (Alliance), a neoadjuvant phase III trial of weekly paclitaxel (T) and trastuzumab (H) with or without lapatinib (L) for HER2-positive breast cancer

Katherine A Hoadley¹, William T Barry², Brandelyn N Pitcher³, Joel S Parker¹, Matthew D Wilkerson¹, William Irvin, Jr¹, Norah Lynn Henry³, Sara M Tolaney⁴, Chau Dang⁵, Ian E Krop⁶, Donald A Berry⁶, Elaine R Mardis⁹, Charles M Perou¹, Eric P Winer⁶, Clifford A Hudis⁷ and Lisa A Carey¹. ¹University of North Carolina, Chapel Hill, NC; ²Alliance Statistics and Data Center, Dana-Farber Cancer Institute, Boston, MA; ³Alliance Statistics and Data Center, Duke University, Durham, NC; ⁴Bon Secours Cancer Institute, Midlothian, VA; ⁵University of Michigan, Ann Arbor, MI; ⁶Dana-Farber Cancer Institute, Boston, MA; ⁷Memorial Sloan Kettering Cancer Center, New York, NY; ⁸Alliance Statistics and Data Center, MD Anderson Cancer Center, Houston, TX and ⁹Genome Institute, Washington University School of Medicine, St Louis, MO.

Body: Background: In CALGB 40601, the HER2-Enriched (HER2-E) molecular subtype had significantly higher pathologic complete response (pCR) rates regardless of treatment arm (single HER2-targeting with T+L or T+H, dual targeting with T+H+L) (Carey et al, ASCO 2014). A TP53 mutation gene expression signature was significant in a multivariable analysis as were treatment, molecular subtype, proliferation, and an immune cell genomic signature. Mutational analysis is now available for this sample set.

Methods: 265 of 305 enrolled patients (pts) had RNA sequencing (RNAseq) of pre-treatment biospecimens; 181/265 had whole exome sequencing (WES) available from tumor and matched normal blood. Somatic mutations were detected by the program UNCeqR, which integrates WES and RNAseq. We examined the association of mutations with in-breast pCR, molecular subtypes, and gene expression signatures.

Results: In this subset, there were 57 HER2-E, 58 Luminal A, 51 Luminal B, 9 Basal-like, 4 Normal-like, and 2 Claudin-low. The pCR rate was 45% (51% THL, 47% TH and 34% TL), consistent with the entire study population. TP53 was the most frequently mutated gene (56%); frequency varied by molecular subtype (Fisher p<0.0001) with the highest in the HER2-E (88%). Type of mutation also varied by molecular subtype: 34% of TP53 mutations in HER2-E pts were nonsense or frame shift mutations compared to 20% in Luminal B and 11% in Luminal A. The presence of a TP53 mutation was significantly associated with achieving pCR (59% compared to 28% in wildtype; odds ratio=3.7, p<0.0001) which did not vary by treatment arm. TP53 mutation status by WES was strongly associated with a gene-expression based predictor (AUC=0.85, p<0.001), suggesting the RNAseq-based signature could be used as a surrogate measure of genotype. PIK3CA mutations were present in 36 pts (20%); 33/36 (92%) were in exons 9 and 20. PIK3CA mutations varied moderately among subtypes and were most prevalent in Luminal B (31%) and HER2-E (25%). Rates of pCR did not vary by PIK3CA mutation status (39% vs 47% in wildtype, p=0.46). GATA3 mutations were identified in 7 pts (4 Luminal A, 3 Luminal B), but only 1 pt achieved pCR. ERBB2 mutations were found in 7 pts: 2 HER2-E, 2 Luminal A, 3 Luminal B. Two were previously identified (the lapatinib-sensitive activating mutation V777L and the lapatinib-resistant mutation L755S), both were trastuzumab resistant in experimental models (Bose et al, Cancer Discovery 2013). Consistent with these results, the V777L pt achieved pCR on the THL arm; the L755S pt did not achieve pCR on the TL arm.

Conclusions: TP53 mutation is a frequent, clinically important event in HER2-positive disease and predicts pCR to chemotherapy plus HER2-targeting. Frequency and type of mutation was dependent on molecular subtype within this clinically HER2-positive cohort. Ongoing analyses are comparing WES data between pre- and post-treatment samples as well as investigating copy number and clonality. This research is supported in part by funds from GlaxoSmithKline and grants from the Breast Cancer Research Foundation.
**Title:** 16 year long-term follow-up of the IBIS-I breast cancer prevention trial

Jack Cuzick¹, Ivana Sestak¹, Simon Cawthorn², Hisham Hamed³, Kaija Holli⁴, Anthony Howell⁵ and John F Forbes⁶. ¹Centre for Cancer Prevention, Queen Mary University, London, United Kingdom; ²North Bristol NHS Trust, Bristol, United Kingdom; ³London Bridge Hospital, London, United Kingdom; ⁴Tampere University, Tampere, Finland; ⁵Institute of Cancer Sciences, University of Manchester, Manchester, United Kingdom and ⁶School of Medicine and Public Health, University of Newcastle, Newcastle, Australia.

**Body:** Background: Several randomised clinical trials have shown the benefit of tamoxifen in healthy women to reduce their risk of breast cancer. Here, we report the blinded median 16 year follow-up of the IBIS-I trial to update the long-term prevention of breast cancer with tamoxifen treatment.

Methods: 7154 pre- and postmenopausal women were randomised to receive daily 20mg tamoxifen (N=3579) or matching placebo (N=3575) for 5 years. The primary endpoint of this analysis was the occurrence of breast cancer (invasive and ductal carcinoma in situ (DCIS)). Secondary endpoints included overall mortality, other cancers, and breast cancer specific mortality. Cox proportional hazard models were used to assess occurrence of breast cancer and survival. All statistical tests were two-sided.

Results: After a median of 16.2 years (IQR 14.4 to 17.7) of follow-up, a total of 589 breast cancers have been reported (tamoxifen: 246 (6.9%) vs. placebo: 343 (9.6%)). Tamoxifen reduced the incidence of all breast cancer overall by 29% (HR=0.71 (0.60-0.83), P<0.0001) (Figure 1). Invasive ER-positive (ER+) breast cancers were reduced by 35% (HR=0.65 (0.53-0.80), P<0.0001) (Figure 1), but no effect was seen for invasive ER-negative (ER-) breast cancers (HR=1.06 (0.71-1.58), P=0.8). A non-significant 30% reduction in DCIS was seen with tamoxifen (36 vs. 51, HR=0.70 (0.46-1.07); P=0.1). The overall risk reduction was similar in years 0-10 (HR=0.71) and years 10-20 (HR=0.70). Similar effects were seen in pre- and postmenopausal women (HR 0.71 vs. 0.71). All-cause mortality was non-significantly increased in women randomised to tamoxifen (173 vs. 158, OR=1.10 (0.88-1.38), P=0.4). The excess in deaths with tamoxifen is smaller than in the 96 month update. No differences in breast cancer mortality was seen (24 tamoxifen vs. 27 placebo; OR=0.89 (0.49-1.60), P=0.7). A non-significant increase in other cancers than breast were reported by women on tamoxifen (350 vs. 315, OR=1.12 (0.95-1.32); P=0.2). Specifically more endometrial cancers (28 vs. 17), non-melanoma skin cancers (108 vs. 85), and lung cancer (32 vs. 20) were found in those randomised to tamoxifen.

Conclusion: This updated analysis of the IBIS-I trial confirms the significant reduction in breast cancer occurrence with tamoxifen in the post-treatment follow-up period. These results indicate tamoxifen has a long-term preventive effect on invasive ER+ breast cancer in both pre- and postmenopausal women.
Title: Randomized comparison of adjuvant tamoxifen (T) plus ovarian function suppression (OFS) versus tamoxifen in premenopausal women with hormone receptor-positive (HR+) early breast cancer (BC): Analysis of the SOFT trial

Prudence A Francis¹, Meredith M Regan¹, Gini F Fleming¹, Istvan Lang¹, Eva M Ciruelos¹, Meritxell Bellet¹, Herve Bonnefoi¹, Miguel A Climent¹, Lorenzo Pavesi¹, Harold J Burstine¹, Silvana Martino¹, Nancy E Davidson¹, Charles E Geyer, Jr¹, Barbara A Walley¹, Robert E Coleman¹, Pierre Kerbrat¹, Manuela Rabaglio-Poretti¹, Alan S Coates¹, Aron Goldhirsch¹ and Richard D Gelber¹. ¹SOFT Investigators, International Breast Cancer Study Group, Breast International Group, and North American Breast Cancer Group, Bern, Switzerland.

Body: Background: The value of adding OFS to tamoxifen in premenopausal women with HR+ early BC is uncertain. SOFT was designed to determine the value of adding OFS to tamoxifen and the role of adjuvant therapy with the aromatase inhibitor exemestane (E) plus OFS in premenopausal women.

Methods: From Nov 2003 - Jan 2011, 3066 premenopausal women with HR+ BC were randomized to 5 years of tamoxifen vs tamoxifen+OFS vs exemestane+OFS. OFS was by choice of GnRH agonist triptorelin, oophorectomy or radiation. SOFT was stratified by the use of prior chemotherapy; 47% received no chemotherapy and 53% remained premenopausal after prior chemotherapy, confirmed by estradiol within 8 months of completion. The comparison of T+OFS vs T alone was the primary objective, to be tested at a 2-sided 0.05 level, when median follow-up was at least 5 yrs. The comparison of E+OFS vs T was a secondary objective. The primary end point was invasive DFS. Secondary end-points included invasive breast cancer-free interval (BCFI), distant recurrence-free interval (DRFI) and overall survival (OS).

Results: As expected, the prior chemotherapy cohort represented a higher risk group (table).

<table>
<thead>
<tr>
<th>T+OFS vs T</th>
<th>No chemo (n=949)</th>
<th>Prior Chemo (n=1084)</th>
<th>Primary Analysis (n=2033)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age yrs</td>
<td>46</td>
<td>40</td>
<td>43</td>
</tr>
<tr>
<td>Node positive</td>
<td>9%</td>
<td>57%</td>
<td>35%</td>
</tr>
<tr>
<td>Tumor &gt; 2 cm</td>
<td>14%</td>
<td>47%</td>
<td>32%</td>
</tr>
<tr>
<td>Grade 1</td>
<td>41%</td>
<td>14%</td>
<td>27%</td>
</tr>
<tr>
<td>Grade 3</td>
<td>7%</td>
<td>35%</td>
<td>22%</td>
</tr>
<tr>
<td>Events @ 67 months</td>
<td>70/47/13/10</td>
<td>229/213/172/96</td>
<td>299/260/185/106</td>
</tr>
</tbody>
</table>

After a median follow-up of 67 months, 5-yr DFS was 86.6% in the T+OFS group and 84.7% in the tamoxifen group (HR=0.83; 95%CI, 0.86-1.04; P=0.10). Multivariable allowance for prognostic factors suggested a greater treatment effect (HR 0.78; 95% CI, 0.62-0.98). Overall survival is not mature with 5-yr OS of 96.7% for T+OFS and 95.1% for tamoxifen (HR 0.74; 95% CI, 0.51-1.09; P=0.13). In the no-chemotherapy cohort, one-third of events were not breast-cancer related and BCFI was > 95% with tamoxifen alone. The cohort who remained premenopausal after chemotherapy had a 4.5% absolute improvement in 5-yr BCFI with T+OFS vs T (table). In the chemotherapy cohort, 5-yr overall survival was 94.5% for T+OFS and 90.9% for T (HR=0.64; 95% CI, 0.42-0.96).
Non-adherence with OFS reached 22% at 4 years. Targeted > grade 3 toxicities were reported for 31.3% of T+OFS group and 23.7% of tamoxifen group. Menopausal symptoms, depression, musculoskeletal complaints, hypertension and diabetes were more frequent with T+OFS. Osteoporosis was reported in 5.8% for T+OFS and 3.5% for T.

**Conclusions:** Adding ovarian suppression to tamoxifen did not show significant benefit in the overall population in SOFT after 67 months median follow-up. However, for the cohort of women who received chemotherapy and who remained premenopausal, the addition of ovarian suppression improved breast cancer outcomes, and further improvement was seen with the use of exemestane plus ovarian suppression.
Title: Patient-reported endocrine symptoms, sexual functioning and quality of life (QoL) in the IBCSG SOFT trial: Adjuvant treatment with tamoxifen (T) alone versus T plus ovarian function suppression (OFS) in premenopausal women with hormone receptor-positive (HR+) early breast cancer (BC)

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Body: Background: SOFT efficacy results reported at this SABCS show that T+OFS provides improved disease control compared with T for the cohort of patients (pts) who received prior chemotherapy (chemo), but relatively little is known about treatment-related endocrine symptoms, sexual function and QoL in premenopausal women receiving adjuvant T+OFS compared with T.

Methods: From Nov 2003 to Apr 2011, 1,722 premenopausal pts with HR+ BC were enrolled and included in the QoL analysis of the randomized phase III trial SOFT, to receive adjuvant treatment with 5 yrs T or T+OFS. A third group received exemestane+OFS and is not included in this report. SOFT enrolled two cohorts: pts who received no chemo, and those who received prior chemo with confirmed premenopausal estradiol levels within 8 mos of completing chemo. Pts completed a questionnaire consisting of global and symptom-specific indicators at baseline, every 6 mos for the first 24 mos, and annually yrs 3 to 6. Differences in change of QoL from baseline between the two treatments were tested at short-, intermediate-, and long-term (6, 24 and 60 mos post-randomization, respectively) for 13 symptoms and 4 global QoL indicators using mixed models with repeated measures, overall and by chemo stratum.

Results: Changes of global QoL indicators (mood, physical wellbeing) from baseline were small and similar between treatments over the whole observation period, but treatment differences were seen with respect to symptom-specific indicators, especially for patients in the no chemo cohort. Overall, pts on T+OFS were substantially more affected by hot flushes than pts on T alone at short- and intermediate-term (each p<.0001). The change of hot flushes from baseline improved for the T+OFS but not for the T alone group over the period of 60 mos. Pts on T+OFS reported more loss of sexual interest (p<.0001) and sleep problems (p<.0001) at short-term, and more vaginal dryness over the whole treatment period (each p<.01). Pts on T alone reported more vaginal discharge up to 60 mos (each p<.05), but only among those pts who did not receive prior chemo. Symptom-specific treatment differences (hot flushes, sleep problems, vaginal discharge) were less pronounced in pts who had received prior chemo. Although pts receiving no prior chemo had less improvement in coping and greater treatment burden with T+OFS vs. T (p<.05), no such treatment differences were seen for pts in the prior chemo cohort.

Conclusion: Global QoL (mood and physical wellbeing) did not differ between groups. Overall, pts receiving T+OFS experienced worse endocrine symptoms and sexual functioning than those receiving T alone during the first two years of treatment; most differences between treatments were no longer apparent thereafter. Differences between T+OFS and T with respect to impaired symptom-specific QoL, being burdened by treatment, and having delayed adaptation during the first two yrs of treatment were less pronounced for pts who received chemo prior to enrolling in SOFT, the cohort that benefited most from OFS in terms of disease control.
Title: Macrophage-specific deletion of STAT5 disrupts normal mammary gland development and accelerates mammary tumorigenesis

Nicholas J Brady¹, Michael A Farrar¹ and Kathryn L Schwertfeger¹. ¹University of Minnesota, Minneapolis, MN.

Body: Fibroblast growth factor receptor 1 (FGFR1) is amplified in 10% of human breast cancers and the ligands for FGFR1 are overexpressed in 60% of triple negative breast cancers. Previous studies using a mouse model of FGFR1-induced mammary tumorigenesis demonstrated that FGFR1 activation in mammary epithelial cells recruits macrophages to hyperplastic regions where they promote angiogenesis and epithelial cell proliferation. Clinically, patients with increased numbers of tumor-associated macrophages have increased risk of relapse and decreased overall survival. While these previous studies demonstrate a role for macrophages during tumor initiation and progression the specific mechanisms that orchestrate the pro-tumor response are poorly understood. Our work uses numerous in vitro and in vivo models to study the dynamic interactions between mammary epithelial cells and macrophages. In an initial screen, we identified the transcription factor signal transducer and activator of transcription 5 (STAT5) as being rapidly activated in macrophages in response to tumor cell-derived factors and modulating pro-tumor functions of macrophages. To assess the role of STAT5 in macrophages during normal mammary gland development, we created a macrophage-specific conditional knockout mouse line with Cre recombinase expression driven by the Csf1r promoter. Mice carrying a macrophage-specific deletion of STAT5 (Stat5 fl/fl ; Csf1r-Cre +) show increased epithelial cell proliferation and impaired ductal elongation at 6 weeks of age compared to littermate controls (Stat5 fl/fl ; Csf1r-Cre -). Based on these data, we hypothesized that the loss of STAT5 in macrophages would accelerate mammary tumorigenesis. To test this, we crossed the macrophage-specific STAT5 knockout mice with a mouse model of FGFR1-induced mammary tumorigenesis. FGFR1 activation was induced in the mice for 2 or 4 weeks beginning at 6 weeks of age. Consistent with previous reports, activation of FGFR1 in mammary epithelial cells results in increased proliferation and lateral bud formation. Surprisingly, mice with STAT5-deficient macrophages show signs of accelerated tumorigenesis, with increased proliferation and numerous instances of hyperplasia in the first 2 weeks of FGFR1 activation. Future studies will focus on understanding the downstream effects of STAT5 deletion in macrophages and the therapeutic potential of targeting this pathway in vivo. All together, these data demonstrate that STAT5 is a critical factor that allows macrophages to regulate normal mammary gland development. In addition, this work illustrates that the loss of STAT5 in macrophages can cooperate with a common genetic event in mammary epithelial cells during breast cancer initiation.
2014 San Antonio Breast Cancer Symposium

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Average Grade: 5.40

Title: Principles governing A-to-I RNA editing in breast cancer transcriptome

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Body: Background: Messenger RNA (mRNA) is the target of a series of post-transcriptional modifications that can affect its structure and stability, one of the most relevant being RNA editing. The most common form of RNA editing in humans is of the A-to-I type and is catalyzed by the adenosine deaminase acting on RNA (ADAR) family of enzymes. Currently, little is known about how RNA editing operates in cancer. The main objectives of this study were to investigate and characterize the extent of A-to-I RNA editing in breast cancer (BC) and to define the principles governing the editing process in this as well as other cancers.

Material and Methods: The exome and transcriptome of 58 BC samples representing the four main known subtypes, namely TN, HER2+, luminal A and luminal B, and 10 matched normal samples were sequenced using the Illumina platform. For the same series, gene expression and copy number profiles were obtained using the Affymetrix platform. RNA-DNA single nucleotide differences (RDDs) were called using a pipeline in line with the most updated bioinformatics tools and validated in an independent cohort of 15 BC samples and breast cell lines.

Results: Overall, we detected 16,027 RDDs present in one or more samples, with all possible base changes represented. Among these, 560 RDDs were located in Alu regions and were all of the A-to-I type consistent with the notion that A-to-I editing occurs predominantly in forward-facing Alu forming double stranded RNA duplexes processed by ADAR. We found that the same sites were edited in normal and tumor breast tissues. However, the editing frequency was significantly higher in tumors compared to matched normal breast tissues. Moreover, high editing frequencies were observed in samples in which more editing sites were detectable and/or in which ADAR expression was high. We identified two key factors that independently determine ADAR expression and therefore A-to-I RNA editing in breast and the majority of other human cancers: 1) the type-I interferon response in tumors and 2) gains in ADAR copy number. The mean editing frequency was found to be significantly correlated with the expression of STAT1 and other type I interferon target genes, both in our patient series, in a large pool of BC datasets and a panel of normal and breast cancer cell lines treated with interferon (IFN) α, β, or γ for 1, 2 and 5 days in vitro. Moreover, the association between editing and STAT1 expression or ADAR amplification was validated in 19 additional cancer types obtained from the TCGA dataset.

Conclusions: Our work, which represents the largest survey so far on RNA editing in BC, shows that A-to-I editing is a pervasive and well-controlled phenomenon in cancer that can drive aberrant transcriptome expression in breast and potentially the vast majority of cancers. Moreover, it suggests that the immune response can profoundly impact the transcriptome sequence in tumor cells and thereby influence the internal mechanisms governing their behavior.
Title: Identification of a notch-driven breast cancer stem cell gene signature for anti-notch therapy in an ER+ presurgical window model

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Body: Background: Resistance to endocrine therapy (ET; tamoxifen or aromatase inhibitors, AI) for ER+ breast cancer is a major cause of mortality and new treatment paradigms are needed. Cancer stem cells drive breast cancer growth and are resistant to standard therapy. Notch signaling aids survival of these resistant stem cells and is inhibited by gamma-secretase inhibitors (GSI). We showed combining GSI with ET in mice caused shrinkage of breast cancer tumors. A presurgical window biomarker modulation model was used to confirm this discovery in humans.

Methods: The GSI MK-0752 was added to ET in patients before definitive surgery (ClinTrials.gov NCT00756717). There were 3 biopsies: day 0 (prestudy), day 14 (after ET alone), and day 25 at definitive surgery (after continued ET plus MK-0752, 350 mg orally 3d on, 4d off, 3d on). Biopsies were analyzed for genes increased or decreased by GSI, to confirm that Notch and cancer stem cell pathways were inhibited. Real-time PCR was used to validate expression of genes identified in pathway analyses of microarray datasets generated from the biopsies. Mammosphere-forming assays were performed to confirm that ET+GSI impacts breast cancer stem cells. The qRT-PCR data were evaluated using ANOVA with repeated measures and ANOVA was performed on mammosphere results.

Results: The accrual goal was met and therapy well-tolerated in 20 evaluable women (PSABCS 2011, abs# S1-5). Of 33 genes identified by analysis of expression microarrays, 19 genes (FDR<8%) were impacted significantly by GSI+ ET (3 increased, 16 decreased) compared to initial biopsy and/or ET alone. Genes with increased expression were DAXX, NOXA (both pro-apoptotic) and LNFG (tumor suppressor). Six of 16 genes that decreased (NOTCH1, NOTCH4, HEYL, HES1, HES5, and HEY2) are Notch pathway-associated genes. The GSI decreased expression of 3 genes from cell cycle and proliferative pathways (Ki67, CCND1, CCNA2) and inhibited 2 genes expressed in cancer stem cells (RUNX1 and ALDH1A1). Five genes directly/indirectly regulated by Notch were decreased by GSI (RICTOR, RPTOR, MMP7, ADAM19, and PRH). Estrogen deprivation for 3 days, mimicking short exposure to an AI, increased mammosphere-forming ability of ER+ breast cancer cells more than 2 fold. The GSI MRK-003 blocked this mammosphere formation by 95%-98%.

Conclusions: A 7-day course of the GSI MK-0752 added to ET in the presurgical window had significant biomarker responses: decrease in Notch signaling, cancer stem cell genes, proliferation-associated genes, the mTORC1 and 2 complex genes RICTOR and RPTOR, metalloproteinases that promote metastasis, and PRH; as well as increase in 3 key genes that promote apoptosis and tumor suppression. These results suggest that 1) GSI inhibited the intended Notch pathway, 2) putative breast cancer stems cells can be targeted by this strategy, and 3) the biomarkers identified create a gene signature for anti-Notch therapy in ER+ breast cancer. Validation of efficacy of the GSI+ET therapy combination and this gene signature in a clinical trial is planned.

Support: Breast Cancer Research Foundation (research grant), Merck Oncology (drug/arrays), Swim Across America (clinical trial costs), and DOD BC073237 (KRC).
Title: Predicting pre-surgical neoadjuvant chemotherapy response in breast cancer using diffuse optical spectroscopic imaging (DOSI): Results from the ACRIN 6691 study

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1University of California, Irvine, CA; 2Brown University, Providence, RI; 3MD Anderson Cancer Center, Houston, TX; 4Dartmouth College, Hanover, NH; 5Boston University, Boston, MA; 6MD Anderson Cancer Center; 7University of Pennsylvania, Philadelphia, PA; 8University of California, San Francisco, CA and 9Massachusetts General Hospital.

Body: Background: DOSI is an experimental imaging technique that employs risk-free near-infrared light for quantitative measurements of breast tissue perfusion, metabolism, and composition without exogenous contrast. A multi-center ACRIN 6691 study was designed to evaluate whether changes from baseline to mid-therapy in a DOSI-derived Tissue Optical Index (TOI) could predict pathologic complete response (pCR) in breast cancer neoadjuvant chemotherapy (NAC).

Methods: DOSI instruments were constructed at the University of California, Irvine and delivered to 6 participating sites. Bedside measurements were conducted by scanning a handheld probe over a region of interest (up to ∼10 x 10 cm²) on both breasts. Instruments were standardized and validated using a common set of tissue simulating phantoms and protocols. DOSI-derived near-infrared absorption and scattering spectra (650-1000 nm) were used to calculate the tissue concentration of oxy- and deoxyhemoglobin (ctO₂Hb, ctHHb), water (ctH₂O), %lipid and the tissue optical index (TOI=ctHHb x ctH₂O/%lipid) in each probe location. Baseline to mid-therapy changes in the tumor to normal (T/N) TOI ratio were evaluated from DOSI images as the primary imaging endpoint for predicting clinical outcome (pCR). 60 female breast cancer patients (ages 28-69 years, mean 48.9±11), with locally advanced disease (tumors >2cm) were enrolled across the 6 institutions. DOSI measurements were performed at baseline, during the first week of therapy, at midpoint, and at the completion of NAC. Logistic regression was used to assess the association between pathologic complete response (pCR) and % change in T/N TOI from baseline to mid-therapy. In addition, area under the receiver operating characteristic curve (ROC AUC) and its corresponding 95% confidence interval were calculated.

Results: Of the 34 participants (mean age 48.4 ± 10.7) with complete and evaluable data, 10 (29%) achieved pCR as determined by central pathology review. The % change in TOI ratio ranged from -82% to 321%, with a median of -36%. Using -40% as a threshold, we found that subjects in the group with a 40% or more decrease in T/N TOI were more likely to be pCR (p=0.0586, OR=4.667, 95% CI: 0.945 to 23.038). The % change in TOI ratio from baseline to mid-therapy has an AUC of 0.604 (95% CI: 0.394 to 0.814) to distinguish pCR from non-pCR.

Conclusions: DOSI has been successfully implemented in a multi-center setting and changes in T/N TOI are a promising predictor of NAC clinical outcome (pCR). A larger study population is needed to fully assess the utility of TOI and other DOSI imaging endpoints for guiding therapies and predicting NAC response in individual subjects.

ACRIN receives funding from the NCI through U01 CA079778 and U01 CA080098.
Impact of intrinsic subtype by PAM50 and other gene signatures on pathologic complete response (pCR) rates in triple-negative breast cancer (TNBC) after neoadjuvant chemotherapy (NACT) +/- carboplatin (Cb) or bevacizumab (Bev): CALGB 40603/150709 (Alliance)

Background: Adding either Cb or Bev to standard NACT significantly increases pCR rates in TNBC (Sikov et al, SABCS 2013). Genomic analysis may help us to identify determinants of response within this clinical phenotype.

Methods: Patients (pts) with clinical stage II-III TNBC received weekly paclitaxel x 12 followed by ddAC x 4 +/- Cb and/or Bev. Pre-treatment biopsies were collected in formalin, RNAlater and OCT; residual disease at surgery was biopsied when possible. Illumina mRNA sequencing (RNAseq) was performed. Gene expression values were normalized to a TCGA subset of clinically TNBC samples prior to downstream analysis. pCR was defined as the absence of residual invasive cancer in the breast (ypT0/is).

Results: PAM50 subtype analysis was performed on 367 pre-treatment samples (of 443 pts who started NACT); pCR results were available for 360, comprising the analysis subset. 87% of these displayed a basal-like gene expression pattern, 2% claudin-low, 4% HER2-enriched, <1% luminal A and 7% normal-like. In pts with basal-like tumors, pCRs rose from 47% to 61% with the addition of Cb (p=0.014), an increment which did not differ significantly from the overall study population (adding in the small number of non-basal-like tumors) (interaction p=0.93). In contrast, the addition of Bev increased pCRs in basal-like tumors from 45% to 64% (p=0.0009), while reducing pCRs in non-basal-likes from 60% to 43% (interaction p=0.024); thus, a basal-like gene expression pattern was predictive of benefit from Bev. Expression of a variety of immune signatures (B cell, T cell, IgG) was positively associated with pCR, but not predictive of increased benefit from either Cb or Bev. High expression of the HER2 amplicon signature was uncommon and not prognostic for pCR overall but was associated with reduced benefit from Cb (interaction p = 0.025). High proliferation, high p53 mutation and low IE (estrogen signaling) signatures were prognostic for higher pCR rates and predictive of benefit from Bev (interaction p=0.031, 0.0017, 0.0002, respectively). In basal-like pts with residual disease, surgical samples often (52%) displayed a normal-like PAM50 pattern, though this might be due to ‘contamination’ in low volume residual disease.

Conclusions: Selection criteria led to accrual of a high % of pts with basal-like tumors, limiting our ability to assess prognostic or predictive impact of intrinsic subtype on pCR. Given that limitation, the magnitude of pCR benefit with Cb was consistent across subtypes, while a basal-like pattern was predictive of greater pCR increment with Bev. Ongoing studies will test a large number of other gene signatures and biomarkers, including the Lehmann et al subtypes. Recognition of clinically relevant subpopulations within TNBC may distinguish pts likely to achieve a pCR from those for whom an investigational approach might be considered.
Title: The Connecticut experiment: 4 years of screening women with dense breasts with bilateral ultrasound

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Body: OBJECTIVE: To determine if the addition of screening breast ultrasound in women with mammographically normal but dense breasts has increased breast cancer detection over the 4 years since the legislation was first enacted in 2009.

METHODS: The study utilized a retrospective chart review. Data collected included: (1) total number of screening mammograms; (2) total number of dense breast screening ultrasounds; (3) screening ultrasound Breast Imaging Reporting Data System (BI-RADS) code results; (4) biopsy results; and (5) demographic data on women with malignant biopsies. Data was obtained from included sites from October 2009 through December 2013.

FINDINGS: Data was collected from 2 Radiology practices with a total of 5 sites. In year 1, 30670 mammograms and 2706 screening ultrasounds were performed. In year 2, 32050 mammograms and 3351 ultrasounds; in year 3, 32230 mammograms and 4128 ultrasounds and in year 4, 27937 mammograms and 3331 ultrasounds were performed.

In year 1, 151 ultrasounds were BIRADS 4 or 5 with 11 cancers detected. The PPV was 7.1%; the detection rate was 4/1000 and 22% of eligible women had the study (assuming 40% of women have dense breasts). In year 2, 180 ultrasounds were BIRADS 4 and 5 with 11 cancers detected. The PPV was 6.1% and the cancer detection rate was 3.2/1000 with 26% of eligible women having the study. In year 3, 148 ultrasounds were BIRADS 4 and 5 with 13 cancers detected. The PPV was 8.1% and the cancer detection rate was 3.2/1000 with 32% of eligible women having the study. In year 4, 53 ultrasounds were BIRADS 4 and 5 with 11 cancers. The PPV was 17.2% with the cancer detection rate of 3.3/1000 and 28% of eligible women having the study.

Four Years of Screening Breast Ultrasound Data

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<th>Year 1</th>
<th>Year 2</th>
<th>Year 3</th>
<th>Year 4</th>
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<td>32230</td>
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<td>4128</td>
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<td>180</td>
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CONCLUSIONS: Based on the data collected from these sites, screening breast ultrasound in women with dense breast parenchyma detects mammographically occult malignancy. Over the 4 years studied, the PPV improved from 7% to 17.2% indicating that the selection of lesions biopsied was more accurate with fewer false positives. The rate of detection in the first year was 4.0/1000 and then remained stable at 3.2/1000 in the three subsequent years. Of concern, the number of eligible women who elect to have the additional test remains low at about 30% which is due to several factors including education and cost.
Title: Accelerated partial breast irradiation using intensity modulated radiotherapy versus whole breast irradiation: 5-year survival results of a phase 3 randomized trial

Lorenzo Livi1, Icro Meattini1, Livia Marrazzo2, Stefania Pallotta2, Gabriele Simontacchi1, Calogero Saieva3, Vieri Scotti1, Carla De Luca Cardillo1, Paolo Bastiani4, Jacopo Nori5, Lorenzo Orzalesi6 and Simonetta Bianchi7. 1Radiotherapy-Oncology Unit, Florence University Hospital, Florence, Italy; 2Medical Physics Unit, Florence University Hospital, Florence, Italy; 3Cancer Research and Prevention Institute, Florence, Italy; 4S. Maria Annunziata Hospital, Florence, Italy; 5Florence University Hospital, Florence, Italy; 6Breast Surgery Unit, Florence University Hospital, Florence, Italy and 7Florence University Hospital, Florence, Italy.

Body: Background. Accelerated partial breast irradiation (APBI) has been introduced as an alternative treatment method for selected patients with early stage breast cancer (BC). Intensity modulated radiotherapy (IMRT) had theoretical advantage of further increase in dose conformity compared to 3D technique, with more normal tissue sparing. We present the results of a randomized equivalence trial comparing local recurrence and survival of APBI using IMRT technique to conventional WBI in early stage BC.

Methods. This study was performed at the University of Florence (Florence, Italy). Women aged more than 40 years affected by early breast cancer, with a maximum tumor diameter of 25 mm, suitable for breast-conserving surgery, were randomly assigned in a 1:1 ratio to receive either external whole-breast irradiation (WBI) or APBI using IMRT technique. Patients in the APBI arm received a total dose of 30 Gy in 5 fractions to the tumor bed. Those in the WBI arm received 50 Gy in 25 fractions, followed by a boost of 10 Gy in 5 fractions. For this equivalence trial the prespecified equivalence margin was local recurrence of 5% in the APBI arm. The primary endpoint was occurrence of ipsilateral breast tumor recurrences (IBTR); overall survival (OS) and treatment toxicity were secondary endpoints. Treatment tolerance was assessed using the acute and late radiation morbidity scoring scheme from Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC). Cosmetic outcome was scored on the Harvard Breast Cosmesis scale. This trial is registered with ClinicalTrials.gov, number NCT02104895.

Results. 520 patients were randomized (260 to external WBI and 260 to APBI with IMRT) between 2005 and 2013. At a median follow-up of 5 years (range 0.6-9.0), we recorded 6 IBTR: 3 sited in the quadrant index (true recurrence) and 6 in other quadrants. The mean time to IBRT was 2.9 years (range 1-4). The 5-year IBTR rate was 1.5% (3 cases) in the APBI group (95% CI 0.1-3.0), within the prespecified equivalence threshold of 5%, in comparison to that of WBI group (1.4%; 95% CI 0-2.8). No statistically significant difference emerged between two groups (log rank test p=0.86).

OS at 5 years did not significantly differ between the groups (p=0.057). We identified 8 deaths (5-year event rate: 2.1%; 95% CI 0.9-3.3), 7 in the WBI group and only 1 in the APBI group. The 5-year overall survival was 96.6% for the WBI group and 99.4% for APBI group.

Concerning acute adverse events, APBI group showed a statistically significant better safety considering any grade of skin toxicity (p=0.0001). No grade 3 toxicity was observed for APBI group. Concerning late side effects, only two cases (0.8%) experienced grade 2 toxicity in WBI group (skin fibrosis). In both treatment groups the cosmetic result was rated as excellent/good for more than 90% of patients. Overall, APBI group showed better outcome to WBI group (p=0.045).

Conclusions. To our knowledge this is the first randomized study using IMRT technique for APBI delivery. No statistical difference in terms of IBTR was shown between the two arms. APBI showed a significant better acute and late toxicity profile compared to WBI.
Title: A large prospectively-designed study of the DCIS score: Predicting recurrence risk after local excision for ductal carcinoma in situ patients with and without irradiation

Eileen Rakovitch 1,2,3, Sharon Nofech-Mozes 1,3, Wedad Hanna 1,3, Frederick L Baehner 4,5, Refik Saskin 2, Steven M Butler 4, Alan Tuck 4, Sandip Sengupta 4, Leela Elavathil 6, Prashant A Jani 7,10, Michel Bonin 11, Martin C Chang 1,2,3, Elzbieta Slodkowska 1, Joseph M Anderson 4, Farid Jamshidian 4, Diana B Cherhavaz 4, Steven Shak 4 and Lawrence Paszat 1,2,3.

Body: Background: DCIS patients need better tools to align the aggressiveness of treatment with the aggressiveness of their disease. The DCIS Score (DS) was validated as a predictor of ipsilateral breast recurrence (IBR; DCIS or invasive) in 327 E5194 patients treated by breast-conserving surgery (BCS) without radiation (RT) (Solin, 2013). This Ontario population based DCIS study of 3335 women with DCIS from 1994 to 2003 (Rakovitch, 2013) was conducted to test the DCIS Score as a predictor of recurrence risk in patients treated with BCS alone and in patients treated with BCS+RT.

Methods: REMARK guidelines were followed. Breast pathologists centrally reviewed all H&E slides. The Onco type DX DCIS Score was obtained by standardized quantitative RT-PCR using fixed paraffin embedded tumor. The pre-specified primary objective was to determine the relationship (hazard ratio (HR)/50 units) between the risk of an IBR and the continuous DS (using Cox models) in patients treated with BCS alone with ER+ tumors and clear margins (CM, no ink on tumor).

Results: Tumor blocks were collected for 1569 patients (47% of parent cohort); 718 received BCS without RT (N=571 with CM) and 846 received BCS+RT (N=689 with CM). Median follow-up was 9.4 years. Among 1260 pts with CM, 100 pts treated with BCS alone had an IBR (DCIS, N=44; invasive, N=57); 86 pts treated with BCS+RT had an IBR (DCIS, N=32; invasive, N=55). In the primary analysis, among 571 patients treated by BCS alone with CM the continuous DS was significantly associated with IBR in ER+ patients (HR 2.26; 95%CI 1.41, 3.59; P=0.001) and in all patients (HR 2.15; 95%CI 1.43, 3.22; P=0.001). The DS was also associated with invasive IBR (HR 1.78; 95%CI 1.03, 3.05; P=0.04); similar but non-significant results were noted in the ER+ subgroup (P=0.08). Among 689 pts with CM treated by BCS+RT, the DS was associated with IBR (HR 2.78; 95%CI 1.77, 4.41; P=0.001). There was no interaction between the DS and RT (P=0.40). In multivariable analysis for IBR in CM cases, the HR/50 units for the DCIS Score among patients treated with BCS alone with ER+ tumors and clear margins (CM, no ink on tumor).

Conclusions: DCIS Score quantifies recurrence risk for DCIS patients treated by BCS with or without RT. Integrating the DCIS Score with established risk factors, such as multifocality, age, and tumor size, can help identify DCIS patients treated with BCS alone with low 10 year risk (<10%) of recurrence and those who still have high 10 year risk of recurrence despite RT who may be candidates for more aggressive treatment.
<table>
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<th>High (≥55)</th>
<th>27.8% (20.0%,37.8%) N=121</th>
<th>27.0% (18.2%,38.9%) N=87</th>
<th>20.5% (15.1%,27.5%) N=202</th>
<th>33.3% (21.9%,48.5%) N=49</th>
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<td>Log rank P-value</td>
<td>&lt;0.001</td>
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Title: Defective stalled replication fork repair and predisposition to hereditary breast cancer

Shailja Pathania1,2, Sangeeta Bade1, Morwenna Le Guillou3, Karly Burke2, Ying Su1, David T Ting4, Kornelia Polyak1,2, Andrea L Richardson1,5, Jean Feunteun3, Judy E Garber1,2 and David M Livingston1,2. 1Dana-Farber Cancer Institute, Boston, MA; 2Harvard Medical School, Boston, MA; 3Laboratoire Stabilité Génétique et Oncogène, Université Paris-Sud, Gustave-Roussy, Villejuif, France; 4Massachusetts General Hospital, Charlestown, MA and 5Brigham and Women's Hospital, Boston, MA.

Body: BRCA1 is a tumor suppressor gene, and germ line BRCA1 mutations increase the risk of breast cancer. While all cells with BRCA1 mutations exhibit a heterozygous BRCA1mut/+ genotype, cancer develops primarily in females, often at young ages and affects almost exclusively the breast and ovaries. Why BRCA1 shows such tissue specificity, and how a normal cell in a BRCA1 mutation carrier (BRCA1mut/+) gives rise to invasive tumor cells are largely unknown.
To determine whether BRCA1 heterozygosity in cells confers defect in any of the multiple, known, BRCA1 functions is a potentially valuable step in achieving a better understanding of BRCA1 mutation-driven cancer predisposition. Thus, we have analyzed a collection of primary mammary BRCA1mut/+ epithelial cells and skin fibroblasts obtained from BRCA1 mutation carriers for such functions.
We, and others have recently shown that BRCA1 exhibits a new DNA damage repair function – i.e. repair of stalled replication forks (SFR). Stalled forks, when not resolved, lead to mutations, or collapse into double strand breaks (DSBs). Both outcomes result in what is commonly referred to as replication stress (RS), which, when chronic, is a driving force behind cancer development. To determine if SFR is defective in normal/healthy breast cells in BRCA1 mutation carriers, and whether this haploinsufficiency results in the kind of genomic changes that lead to cancer, we have now generated 18 primary fibroblast strains from skin punch biopsies and 10 primary mammary epithelial cell (MECs) strains from prophylactic mastectomies performed on BRCA1 mutation carrying women. This collection includes N=23 different BRCA1 mutations, which, together, span almost the entire BRCA1 gene. BRCA1+/+ control MECs were derived from tissue collected during reduction mammoplasties and control fibroblasts were derived from skin punch biopsies from women with no BRCA1 mutation.
Our current data shows that BRCA1mut/+ strains exhibited multiple, normal BRCA1 functions, including the support of homologous recombination- type double strand break repair (HR-DSBR), cell cycle- associated checkpoint functions, centrosome number control, spindle pole formation, Slug expression and satellite RNA suppression. By contrast, nearly all strains were defective in the repair of stalled replication forks and in the suppression of fork collapse, i.e. replication stress. These defects were rescued by reconstituting BRCA1 heterozygous cells with wild-type BRCA1 cDNA, indicating that they are a product of BRCA1 haploinsufficiency.
In addition, the development of sufficient replication stalling rendered BRCA1mut/+ cells defective in an otherwise intact BRCA1 function, HR-DSBR. No such ‘conditional’ haploinsufficiency was detected in any of the other non-haploinsufficient functions, noted above. Given the importance of replication stress in cancer development and of an HR defect in breast cancer pathogenesis, these defects, when they develop serially, could contribute to the BRCA1 breast cancer development pathway. Finally, given the important role of BRCA2, another hereditary breast cancer gene, in stalled fork stability, a similar analysis for BRCA2mut/+ cells from BRCA2 mutation carriers is currently underway and will also be reported at the meeting.
Body: PARPs (poly-ADP-ribose polymerases) catalyze a protein posttranslational modification termed Poly-ADP-ribosylation (PARylation). PARylation is composed of linear and/or branched repeats of ADP-ribose, whose lengths can reach up to 200 units. The first gene encoding a poly-ADP-ribose polymerase, PARP1, was cloned in 1987. Numerous efforts have now led to the identification of 16 additional PARP enzymes. PARP1 is a nuclear protein that is activated as a result of sensing DNA strand breaks. PARylation levels in a quiescent cell are usually very low. In response to genotoxic stress, PARP1 is recruited to nicked DNA and is rapidly activated. This triggers the synthesis of a large number of PARylated proteins and the initiation of DNA damage repair mechanisms. Cancer cells with defects in double-strand break (DSB) repair, such as BRCA1/2-mutated cells, are reliant on PARP1 activity for genome integrity. These cells undergo unsustainable genetic damage, and eventually, apoptosis upon PARP1 inhibition. Indeed, recent late-stage clinical studies revealed that PARP1 inhibitor treatment significantly prolonged progression-free survival of BRCA-deficient breast cancer patients. Contrary to the fruitful efforts in characterizing the upstream inputs regulating PARP1, its genuine downstream targets, however, remain poorly defined. We recently developed the first mass spectrometry-based approach to a global mapping of the human aspartic acid- and glutamic acid-ADP-ribosylated proteome (Zhang et al., Nature Methods 2013). This method allowed us to identify 1,048 in vivo PARylation sites on 340 proteins. These modified proteins are involved in a wide array of nuclear functions including DNA damage repair, transcription regulation, epigenetic modulation, and mRNA processing. Using a quantitative mass spectrometry experiment, we also identified hundreds of novel PARP1 downstream effectors.

The central hypothesis of our current study is that ADP-ribosylation levels of PARP1 substrates can reflect the activity of PARP1 in a cell. We predict that a signature composed of multiple PARylated proteins can then be used to identify cells that are "addicted" to PARP1 activity for genome integrity. To this end, we performed a large scale profiling of the Asp- and Glu-ADP-ribosylated proteome of a benign breast epithelial cell line (MCF10A), and compared it to that of a panel of eight breast carcinoma cell lines, including the ER+ (MCF7, T47D and ZR-75-1), HER2+ (SKBR3), and triple negative (MDA-MB-231, MDA-MB-468, SUM159 and HCC1937) subtypes. Among these lines, MDA-MB-468 (PTEN null), SKBR3 (HER2+) and HCC1937 (BRCA1 null) are known to be sensitive to PARP inhibitors. We correlated the pattern of protein ADP-ribosylation to their IC50, and found events of predictive value. In addition, we also observed that the differential PARylation pattern among these cells can be divided into a "public" (proteins that are commonly modified across all cell lines) class, and a "private" one (proteins that specifically modified in certain cell lines). We envision that this dataset will also serve as a valuable resource for the PARP1/DNA damage research community to investigate cell line-specific Asp- and Glu-ADP-ribosylation events.
Title: A randomized, open-label, multicenter, phase 3 study of epoetin alfa (EPO) plus standard supportive care versus standard supportive care in anemic patients with metastatic breast cancer (MBC) receiving standard chemotherapy


Body: Background: Several investigational studies including MBC reported that erythropoiesis-stimulating agent (ESA) treatment beyond correction of anemia decreased survival and locoregional tumor control, and/or increased adverse effects, especially thrombotic vascular events (TVEs). These studies were reviewed at 3 FDA Oncologic Drugs Advisory Committee Meetings (2004, 2007, and 2008). Other studies and well-conducted meta-analyses did not suggest adverse effects on tumor outcomes when ESAs are used according to the prescribing information in subjects receiving chemotherapy, however, no study rigorously evaluated tumor outcomes.

This Phase 3 study is the largest MBC trial in subjects receiving EPO for chemotherapy induced anemia specifically designed and conducted to assess progression-free survival (PFS) as the primary endpoint. Conducted as a post-marketing requirement, the study has been regularly and intensively monitored by an Independent Data Monitoring Committee (IDMC) of internationally recognized experts in the fields of MBC treatment, clinical research, and statistics.

Methods: EPO-ANE-3010 (ClinicalTrials.gov #NCT00338286) is a multinational (19 countries and 223 participating sites) study with 2,098 subjects who are anemic and receiving first- or second-line standard chemotherapy for MBC. Key inclusion criteria: histologically confirmed MBC, stage IV disease and at least 1 measurable metastatic lesion according to Response Evaluation Criteria in Solid Tumors (RECIST); hemoglobin (Hb) ≤11 g/dL, Eastern Cooperative Oncology Group performance score 0 or 1. Key exclusion criteria: metastasis to bone only, receiving anticoagulants or endocrine therapy. Subjects were randomly assigned (1:1) to receive either standard supportive care for treatment of anemia (SOC) plus 40,000 IU EPO given subcutaneously weekly up to 4 weeks after the last dose of cytotoxic chemotherapy, or to SOC alone. Randomization was stratified by line of chemotherapy and HER2/NEU status. EPO dose was held for Hb >12 g/dL. Disease was assessed every 8 weeks for the first year, and then every 12 weeks until disease progression (PD) or death. Tumor response was determined according to modified RECIST 1.0 criteria by Investigators and Blinded Central Review. Overall survival (OS) follow up continued after PD. The primary endpoint is PFS. Secondary endpoints include OS, time to tumor progression, and overall response rate and safety assessments (including incidence and severity of TVEs). For this non-inferiority study, a sample size of 1,650 disease progression or death events was determined to provide over 80% power to rule out a 15% hazard rate increase (i.e., hazard ratio of 1.15, epoetin alfa vs. control) in PFS with a 1-sided Type I error rate 0.025. The study is fully accrued with 2014 projected key dates: clinical cut-off at 1650 PFS events, July; database lock, September; IDMC agreement on presentation of final results, mid-November. Both primary and secondary endpoints will be fully reported.
Title: Final survival analysis from the randomized Women's Intervention Nutrition Study (WINS) evaluating dietary intervention as adjuvant breast cancer therapy

Rowan T Chlebowski\textsuperscript{1} and George L Blackburn\textsuperscript{2}. \textsuperscript{1}Los Angeles BioMedical Research Institute at the Harbor-UCLA Medical Center, Torrance, CA; \textsuperscript{2}Beth Israel Deaconess Hospital, Boston, MA and \textsuperscript{3}on behalf of the Women's Intervention Nutrition Study Investigators, Torrance, CA.

Body: Between 1994 and 2001, 2,437 women between 48-79 years of age with early-stage breast cancer (73% node negative, 79% estrogen receptor positive, 70% = or < 2 cm) receiving standard cancer management (endocrine therapy +/- chemotherapy if hormone receptor positive or chemotherapy if hormone receptor negative plus radiation therapy if clinically indicated) were randomized, within 6 months of diagnosis, to a dietary intervention or control group from 39 US centers participating in the Women's Intervention Nutrition Study (WINS). The dietary intervention, targeting fat intake reduction while maintaining nutritional adequacy, was initiated during 8 biweekly individual counseling sessions by centrally trained registered dieticians implementing a previously developed low-fat eating plan with subsequent every 3 month dietitian contacts. During 5 years (median) intervention, fat intake was significantly reduced (from 29.2% to 20.3% of calories, P< 0.0001) as was body weight (-2.7 kg, P=0.005) in the dietary intervention group but not in the control group. Relapse-free survival, the primary study endpoint, was favorably impacted in the dietary intervention compared to the control group (9.8% vs 12.4% with events, respectively, HR 0.78 95% CI 0.60-0.98, P = 0.03 from adjusted Cox model) (J Natl Cancer Inst 2006:98;1767). Exploratory analyses suggested a greater dietary effect on women with hormone receptor negative cancers. When intervention ended after 5 years median follow-up with a total of 171 deaths, there were somewhat fewer deaths in the intervention group (6.6% vs 7.3%, HR 0.89 95% CI 0.65-1.21). In the last WINS update, based on national death registry information after 8.1 years median follow-up with a total of 251 deaths, while there were fewer deaths in the intervention compared to the control group (9.1% vs 11.1%), the difference was again not statistically significant (HR 0.78 95% CI 0.59-1.03) (J Clin Oncol 2008:26;522). With this as background, the primary study objective is to determine, with updated survival information after now 15 years median follow-up with a total of 430 deaths, whether a lifestyle intervention targeting fat intake reduction associated with significant weight loss will improve overall survival in early stage breast cancer patients receiving standard breast cancer management. Information on survival is being obtained regarding the status of the 2,081 WINS study participants last known to be alive using the National Death Registry identified through DOBsearch.com. It is anticipated that there will now be a total of approximately 430 deaths in the trial. The new information will be incorporated in updated survival analyses, using time-to-event methods based on intention-to-treat principles, for the overall population and for subgroups of interest. Results will be presented as a late breaking abstract.
Title: Phase 3, randomized, double-blind, placebo-controlled multicenter trial of daily everolimus plus weekly trastuzumab and paclitaxel as first-line therapy in women with HER2+ advanced breast cancer: BOLERO-1

Sara A Hurvitz¹, Fabrice Andre², Zefei Jiang³, Zhimin Shao³, Silvia P Neciosup⁵, Max S Mano⁶, Ling-Min Tseng⁷, Qingyuan Zhang⁸, Kunwei Shen⁹, Donggeng Liu¹⁰, Lydia M Drestoi¹¹, Jifeng Feng¹², Howard A Burris¹³, Masakazu Toi¹⁴, Marc E Buyse¹⁵, David Cabaribere¹⁶, Mary-Ann Lindsay¹⁷, Tiffany Kunz¹⁸, Shantha Rao¹⁸, Lida B Pacaud¹⁸, Tetiana Taran¹⁸ and Dennis Slamon¹.
¹University of California, Los Angeles, CA; ²Institut Gustave Roussy, Villejuif, France; ³Beijing 307 Hospital of PLA, Beijing, China; ⁴Cancer Hospital of Fudan University, Shanghai, China; ⁵Instituto Nacional de Enfermedades Neoplasicas, Surquillo, Lima, Peru; ⁶Instituto do Câncer do Estado de São Paulo, São Paulo, Brazil; ⁷Taipei Veterans General Hospital, Taipei, Taiwan; ⁸Tumor Hospital of Harbin Medical University, Harbin, China; ⁹Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China; ¹⁰Sun Yat-sen University Cancer Center, Guangzhou, China; ¹¹University of Pretoria, Gauteng, South Africa; ¹²Jiangsu Provincial Tumor Hospital, Nanjing, Jiangsu, China; ¹³Sarah Cannon Research Institute, Nashville, TN; ¹⁴Graduate School of Medicine, Kyoto University, Kyoto, Japan; ¹⁵International Drug Development Institute, Louvain La Neuve, Belgium; ¹⁶Translational Research in Oncology (TRIO), Paris, France; ¹⁷Translational Research in Oncology (TRIO), Edmonton, Canada and ¹⁸Novartis Pharmaceuticals Corporation, East Hanover, NJ.

Body: Background: Mammalian target of rapamycin (mTOR) is central to multiple signaling pathways regulating cell growth and proliferation. In early studies, an mTOR inhibitor everolimus (EVE) showed antitumor activity in breast cancer and synergy with both trastuzumab (TRAS) and paclitaxel (PAC). The BOLERO-1 study evaluated the addition of EVE to TRAS+PAC as first-line therapy for HER2+ advanced breast cancer (ABC).

Methods: In this phase 3 randomized trial, women with HER2+ ABC without prior TRAS or chemotherapy for advanced disease were randomized 2:1 to receive either EVE (10 mg/d) or placebo (PBO) and weekly PAC plus TRAS. The two primary objectives were to compare the investigator assessed progression-free survival (PFS) between EVE+TRAS+PAC and PBO+TRAS+PAC in the full population and the Hormone Receptor− negative (HR−) subpopulation. Secondary endpoints included overall survival (OS), response rate, and safety.

Results: 719 patients were randomized to receive EVE (n=480) or PBO (n=239). Final PFS analysis was performed after 425 events in the full population. Baseline characteristics/prior therapies were balanced between two treatment arms; median age was 53 years, 70.5% had visceral metastases, 43.3% were HR−; prior therapy included TRAS (10.8%) and taxane (24.9%); baseline characteristics for the HR− subpopulation were generally balanced between two treatment arms and similar to the overall population. Median study follow-up at the time of analysis was 41.3 mo. The study did not meet its primary objective in the full population; median PFS was 15 mo (95%CI: 14.6-17.9) in the EVE arm vs. 14.5 mo (95%CI: 12.3-17.1) in the PBO arm (HR=0.89 [95%CI: 0.73-1.08]; p=0.1166). In the HR− subpopulation (n=311), a clinically relevant 7.2 mo benefit in median PFS was observed in the EVE arm (20.3 mo [95%CI: 15.0-24.1]) vs. PBO arm (13.1 mo [95%CI: 10.1-16.6]; HR=0.66 [95%CI: 0.48-0.91]; p=0.0049), which just fell short of crossing the protocol pre-specified level of statistical significance (p=0.0044). Additional sensitivity analysis of PFS without censoring patients at the start of new antineoplastic therapy yielded hazard ratio consistent with the primary analysis (p=0.0043). PFS based on central assessment corroborated investigator assessed PFS in both the full population and HR− subpopulation. OS data is immature. Safety profile of EVE was consistent with previous observations in ABC; no new signals were identified. Most common adverse events (AEs) in the EVE arm vs. PBO arm were stomatitis (66.5% vs. 32.4%), diarrhea (56.6% vs. 46.6%), and alopecia (46.8% vs. 52.5%); suspected drug-related serious AEs were reported for 21.8% vs. 7.6%, and on-treatment AE related deaths were reported for 3.6% vs. 0.0% of patients, respectively.

Conclusions: First-line therapy with EVE+TRAS+PAC did not show PFS benefit in patients with HER2+ ABC; the HR− subpopulation derived a clinically robust benefit of 7.2 mo in median PFS suggesting that EVE may have a role in this patient subpopulation. EVE+TRAS+PAC toxicity was generally manageable; no new safety signals were identified. (Funded by Novartis; ClinicalTrials.gov number, NCT00876395.)
2014 San Antonio Breast Cancer Symposium

Publication Number: S6-02
Average Grade: 7.33

Title: TBCRC023: A randomized multicenter phase II neoadjuvant trial of lapatinib plus trastuzumab, with endocrine therapy and without chemotherapy, for 12 vs. 24 weeks in patients with HER2 overexpressing breast cancer

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Body: Background: We have previously shown in animal models that combination anti-HER2 therapy leads to complete tumor regression of HER2+ breast cancer (BC). We translated these findings in a neoadjuvant trial of 12 weeks (wks) of L+T (TBCRC006) that demonstrated a meaningful pathologic complete response (pCR) and near pCR (in ER+ tumors) in patients with locally advanced HER2+ BC. In the present trial, we sought to determine whether longer treatment would lead to a higher rate of pCR without the use of chemotherapy by converting near pCR to pCR especially in the ER+ subset.

Methods: TBCRC023 (NCT00999804) is a randomized phase II trial combining a Simon Phase 2 design in the experimental arm with a pick-the-winner design, not powered for direct comparison. Women with HER2+ BC measuring 2 cm or larger were eligible and were randomized in a 1:2 ratio to 12 vs. 24 wks of L+T. Letrozole (along with ovarian suppression if premenopausal) was also administered in patients whose tumors were also ER+. Serial tumor biopsies were obtained at baseline, wk 1, wk 12, and at the time of surgery. All evaluable patients were assessed for pCR, defined as no residual invasive carcinoma in the breast. Patients did not undergo surgery, withdrew consent, or received additional neoadjuvant therapy were counted as non-responders.

Results: Ninety-seven patients were enrolled (33 in 12-wk arm and 64 in 24-wk arm), of whom 95 were evaluable. Seventy-seven percent of patients were white and 18% were black. Twenty percent were of Hispanic ethnicity. Median age was 51 and 55% were postmenopausal. Median tumor size was 5 cm and 65% were ER+. Study treatment was well tolerated with grade 1-2 diarrhea (24% in 12-wk arm, 31% in 24-wk arm) and grade 1-2 acneform rash (12% in 12-wk arm, 19% in 24-wk arm) being the most common toxicities. Grade 3 toxicities were uncommon and were mostly in the 24-wk arm (elevated liver function test: 9%, diarrhea 2%, mucositis 2%), while the 12-wk arm had one grade 3 anemia and the study’s only serious adverse event (acute kidney injury). There were no grade 4 toxicities.

The experimental arm completed stage 2 accrual and pCR rate was numerically superior to control, entirely due to better results in ER+ (Table 1), but lower than the expected pCR rate of 36% needed in the planned analysis to conclude in favor of enhanced efficacy with extended therapy. pCR rates were also lower in the control arm than in the previous study.

Table 1. pCR rates

<table>
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<th>12-week arm % (n)</th>
<th>24-week arm % (n)</th>
</tr>
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<tbody>
<tr>
<td>ER-positive</td>
<td>8.7% (2/23)</td>
<td>33.2% (13/39)</td>
</tr>
<tr>
<td>ER-negative</td>
<td>20% (2/10)</td>
<td>8.7% (2/23)</td>
</tr>
<tr>
<td>Overall pCR</td>
<td>12.2% (4/33)</td>
<td>24.2% (15/62)</td>
</tr>
</tbody>
</table>

Conclusions: Treatment with L+T (with endocrine therapy in ER+ tumors) for 24 weeks leads to doubling of the pCR rate in women with HER2+ breast cancer without using cytotoxic chemotherapy. This approach is effective and well tolerated and warrants study as part of a de-escalation strategy that may spare some patients the cost and toxicity of chemotherapy. Tissue obtained on this trial will provide a valuable resource to validate correlative findings from our prior studies and discover new biomarkers to help guide proper patient selection for treatment.
Purpose: Fulvestrant (F) is a selective estrogen receptor downregulator (SERD) with activity in aromatase-inhibitor (AI) resistant estrogen receptor (ER)-positive metastatic breast cancer (MBC). In preclinical studies, the proteasome inhibitor bortezomib (B) enhances the antineoplastic effects of F, in part by promoting accumulation of large ER-aggregates that lead to cell death (Ishii et al. Clin Cancer Res 2011 17:2292). The objective of this study was to determine if the combination of F+B was more efficacious than F alone in MBC after AI progression.

Patients and Methods: Postmenopausal women with ER-positive MBC who had progressive disease after prior AI therapy were eligible. They were randomized to F alone (500 mg IM days -15, 1, 15 in cycle 1, and day 1 of each subsequent cycle) or in combination with B (1.6 mg/m² IV on days 1, 8, 15). The primary endpoint was progression free survival (PFS), measured from cycle 1, day 1 of starting F. A sample size of 118 was pre-specified in order to provide sufficient power to detect an improvement in median PFS from 5.4 to 9.0 months, and compare PFS rates after 6 and 12 months (1-sided alpha=0.10, beta=0.10). Patients with progression on F could cross over to the F+B combination.

Results: Of 118 patients enrolled, 59 received F alone (arm A), 57 received F+B (arm B), and 2 assigned to arm B never initiated protocol therapy. There were no significant differences in patient characteristics between arms with regard to median age (57 vs. 59 years), ECOG performance status (0 and 1, 64% and 36%, respectively), prior chemotherapy for metastasis (25%), or liver metastases (37%), although patients in arm A had longer median interval between diagnosis and metastasis (49 vs. 28 months) and were more likely to present with metastasis (32% vs. 26%). Patients in arm B had more adverse events (all grades), including nausea (63% vs. 29%), diarrhea (47% vs. 8%), sensory neuropathy (46% vs. 29%), and limb edema (37% vs. 19%), although grade 3-4 events were uncommon, and only 11% discontinued B due to toxicity. At 12 months, the PFS proportion in Arm A and Arm B was 13.6% vs. 28.1%, respectively (P=0.03, 1-sided chi-square test) (95% CI for difference [14.5%] = -0.06%, 29.1%).

Although median PFS was similar in the two arms (2.69 vs. 2.73 months, respectively), the hazard ratio for Arm B vs. Arm A (referred) was 0.73 (95% CI = 0.49, 1.09, P=0.06, 1-sided log rank test). Both results were significant at the pre-specified 1-sided 0.10 alpha level. Of 27 patients on arm A who crossed over to F+B at progression, 4 (15%) were progression-free for at least 24 weeks and had periods of disease control that were longer than when treated with F alone.

Conclusion: Adding bortezomib to fulvestrant in AI-resistant ER-positive MBC enhances its effectiveness by delaying acquired fulvestrant resistance. These results support additional evaluation of proteasome inhibitors in combination with SERDs.

Acknowledgement: Supported by contract N01-CM-62204 to the New York Cancer Consortium (P.I. J. Sparano) and grant P30 CA013330 (P.I. D. Goldman) from the National Institutes of Health, and by a grant from Millennium, Inc.
**Title:** Fulvestrant 500 mg versus anastrozole as first-line treatment for advanced breast cancer: overall survival from the phase II ‘first’ study

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**Body:**

**Background:** Fulvestrant 500 mg showed a clinically significant improvement in median overall survival (OS) vs fulvestrant 250 mg (26.4 vs 22.3 months, respectively; hazard ratio [HR] 0.81; 95% confidence interval (CI) 0.69, 0.96; nominal p=0.02) in the Phase III CONFIRM study, for patients (pts) with hormone receptor positive (HR+) disease following failure on prior endocrine therapy. Further evidence for OS effects of fulvestrant 500 mg was sought in the Fulvestrant fIRst-line Study comparing endocrine Treatments (FIRST), which compared fulvestrant 500 mg with anastrozole as first-line treatment for postmenopausal pts with HR+ locally advanced (LA) or metastatic breast cancer (MBC). In the primary analysis, fulvestrant 500 mg was as effective as anastrozole for clinical benefit rate (primary endpoint) and significantly better for time to progression (TTP; secondary endpoint). In a follow-up analysis, median TTP was 23.4 months for fulvestrant 500 mg vs 13.1 months for anastrozole (HR 0.66; 95% CI 0.47, 0.92; p=0.01). Here we report the only scheduled FIRST OS analysis.

**Methods:** FIRST, a Phase II, randomized, open-label study (NCT00274469), compared fulvestrant 500 mg (im on Days 0, 14 and 28, and every 28 days thereafter) with anastrozole (1 mg/day po). Pts had not received prior endocrine therapy for advanced disease. OS (time from randomization to death) was compared by unadjusted log-rank test after approximately 65% of deaths. Effect of treatment on OS was examined across subgroups (including age, hormone receptor status and visceral disease). Pts alive or not known to have died were right-censored at last known date alive, including 20 pts in centers invited but who did not join the OS follow-up phase. Serious adverse events (SAEs) were recorded.

**Results:** 205 pts (median age 67.0 years) were randomized from 62 centers in 9 countries (fulvestrant 500 mg: n=102; anastrozole: n=103). The first pt enrolled on Feb 6, 2006. As of July 2014, 33/205 pts (16.1%) were known to be alive across both treatment groups and 137/205 (66.8%) pts had died. Median OS was significantly greater for fulvestrant 500 mg (54.1 months) vs anastrozole (48.4 months; HR 0.70; 95% CI 0.50, 0.98; p=0.041). OS analyses in pre-specified subgroups demonstrated a consistent treatment effect for fulvestrant 500 mg vs anastrozole (global interaction test p=0.755). The frequency of SAEs was similar between fulvestrant 500 mg (23.8%) and anastrozole (21.4%).

**Conclusions:** HR+ pts receiving first-line fulvestrant 500 mg lived significantly longer than pts on anastrozole (median OS difference of 5.7 months). A consistent OS treatment effect was observed across predefined subgroups. FIRST is therefore the second randomized trial to show an OS advantage for fulvestrant 500 mg over another endocrine therapy. No new safety signals were identified with longer-term treatment. Improved OS data provide further support for superior efficacy of fulvestrant 500 mg over anastrozole as first-line endocrine therapy for postmenopausal women with HR+ LA or MBC. If confirmation of superiority for fulvestrant 500 mg is seen in the Phase III FALCON study (NCT01602380), fulvestrant 500 mg should be considered for approval as a first-line agent in this setting.
Body: Intro: Male BC is a rare disease (<1% male tumors); knowledge is limited and management extrapolated from female BC. An international consortium, coordinated by EORTC and TBCRC, was created to better characterize and manage this disease, with 3 parts: 1) retrospective joint analysis 2) prospective registry 3) clinical trial(s). We report 1st results of part 1. Methods: Joint analysis of male BC pts with available FU & FFPE samples, treated in 1990-2010, in 23 centers from 9 countries. Clinical data, long term outcomes, local pathology were centrally analyzed at EORTC. FFPE samples were analyzed at 3 central labs (UK, NL, US) for histology, grade, ER, PR, AR, HER-2, Ki67. Cut-off for positivity: Allred score ≥3 for ER, AR, PR; 20% for Ki67; Average Grade: 6.80; 35% use number of non-missing values as denominator. Results: 1822 pts enrolled; 349 (19%) were excluded (no valid central lab assessment); of 1473 eligible pts (1384 from EU, 89 from US) 63% diagnosed in 2001-2010. Median age at diagnosis (Dx) was 68.5 ys. Of pts with known M status at Dx, 56 (5.1%) were M1; of 1046 M0 cases, 60% were N0 and 51% had T1 tumors at Dx; 4% of pts had BCS and 18% had SLNB; half received adjuvant RT. (Neo)adjuvant CT was used in 30% of M0 pts, most (44%) anthracycline-only; 77% of M0 pts received adjuvant ET, most tamoxifen (88.4%). Central pathology: 697 cases with central histology & grade (full series ongoing): 87% ductal, 53% grade 2. In 1473 pts, at least 1 biomarker centrally assessed; 92% ER highly+ (Allred 7-8), PR had wider variation (35% highly+); 87% AR highly+; 25% Ki67 high; 5% HER2pos. Using IHC surrogates: 58% Luminal A-like, 35% LuminalB-like/HER2neg, 6% LuminalB-like/HER2pos; 0.1% HER2pos/non-Luminal; 1% TNBC (16% not classified). Outcome: For 1046 M0 pts, median FU was 5.7 yrs (0-19.2); 63% alive at analysis; in 88% cause of death was reported, mainly 33% non-cancer and 28% progression(PD)/toxicity. Significant OS improvement over time is seen. 75% (42/56) M1 pts died, mainly due PD. In M0 pts, median OS (yrs) was significantly correlated with ER+ p=0.001 [Allred 0-2: 3.9 (0.1, 6.0); 3-6: 7.1 (4.7, 11.7); 7-8: 8.8 (8.3, 10.0)] & with PR+ p=0.022 [Allred 0-2: 7.3 (6.1, 10.3), 3-6: 8.0 (7.0, 9.3), 7-8: 9.5 (8.4, 12.8)]; less correlation for AR; no major differences for HER2 or Ki67. Median OS & grade
(394 cases; full series ongoing): Grade1: 12.8 yrs (6.1-15.6); Grade2: 7.9 (6.5-10.7); Grade3: 9.3 (4.9-21.1). Median OS for IHC surrogates: 8.7 (7.8-9.7) for Luminal A; 8.3 (6.9-9.4) for Luminal B/HER-2neg; 9.3 years (5.9-21.1) for Luminal B/HER-2+. Similar results were seen for RFS. ER/PR/AR using histoscores will be presented. Conclusions: a) 56% pts had T1 tumors at Dx but only 4% had BCS; b) ER was highly + in >90% but adjuvant ET given in only 77% pts; c) Male BC is usually ER+, PR+ & AR+ and of Luminal A-like subtype (5% HER2pos & 1% TNBC); d) Significant improvement in OS over time; e) ER and PR (Allred) are prognostic (high expression/better prognosis), less for AR, not for Ki67 nor IHC surrogates; f) In-depth characterization of samples is ongoing. Funding: BCRF, EBCC Council, Pink Ribbon NL, BRO.
The genomic landscape of male breast cancers

Salvatore Piscuoglio, Melissa Murray, Charlotte KY Ng, Elena Guerini Rocco, Luciano G Martelotto, Francois-Clement Bidard, Carey A Eberle, Nicola Fusco, Rita A Sakr, Leticia De Mattos-Arruda, Raymond Lim, Timour Baslan, James Hicks, Tari A King, Edi Brogi, Larry Norton, Britta Weigelt, Clifford A Hudis and Jorge S Reis-Filho. 1Memorial Sloan Kettering Cancer Center, New York, NY; 2School of Pathology, University of Milan, Milan, Italy; 3Institut Curie, Paris, France; 4Memorial Sloan Kettering Cancer Center, New York, NY; 5Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and 6Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Background: Male breast cancer (MaBC) accounts for <1% of all breast cancers and its genomic landscape has yet to be characterized. The majority of MaBCs are ER-positive invasive ductal carcinomas of no special type and, unlike female breast cancers (FBC), rarely display HER2 gene amplification or a triple-negative phenotype. Given the relative rarity of MaBCs, treatment decisions for MaBC patients are often extrapolated from trials carried out with FBC patients. Here we employed targeted capture massively parallel sequencing to define the repertoire of somatic mutations and gene copy number alterations (CNAs) in MaBCs.

Methods: Subtyping of the 64 MaBCs included in this study was performed by means of immunohistochemistry using the definitions described in the latest St. Gallen’s consensus report. DNA extracted from microdissected tumor and adjacent normal tissue were subjected to massively parallel targeting sequencing of all exons of 273 genes most frequently mutated in FBCs or directly related to DNA repair. Somatic mutations were defined using a combination of MuTect, SomaticSniper, MutationSeq and Haplotype Caller. Selected mutations were validated with Sequenom MassARRAY. CNAs were identified using Varscan2 and GISTIC2.0. Pathway and network analysis of mutations/CNAs was performed using Ingenuity Pathway Analysis and HOTNET. The genomic landscape of MaBCs was compared with that of FBCs of the same subtype analyzed as part of The Cancer Genome Atlas project.

Results: All MaBCs were ER-positive and HER2-negative. Using the St. Gallen’s criteria, 37.5% and 62.5% were classified as luminal A-like or luminal B-like, respectively. The genes most frequently mutated in MaBCs were PIK3CA, GATA3, FLG and PLEC, with PIK3CA being the only significantly mutated gene as defined by MutSigCV (q=0.003). CNA analysis revealed recurrent gains of 1q and 8q and loss of 16q. GISTIC2.0 identified significantly recurrent high-level amplifications in 1q25.3, 8p11 (FGFR1, ZNF703), 8q24.3 (DEPTOR), 17q23 (PPM1D) and 15q26 (IGF1R) and deletions in 11q22 (ATM) and 21q22.12 (RUNX1). HOTNET analysis of the genes mutated and/or targeted by gene amplifications in MaBCs revealed significantly altered subnetworks involving genes related to DNA repair, PI3K and FGF signaling pathways. The most frequently mutated genes in luminal A-like MaBCs were PIK3CA and MLL3, while those of luminal B-like MaBCs were PIK3CA and GATA3. MLL3 and GATA3 mutations were only found in luminal A-like MaBCs (p=0.039) and luminal B-like MaBCs (p=0.048), respectively. Although the mutational landscapes of MaBCs and luminal FBCs were qualitatively similar, PIK3CA and TP53 were less frequently mutated in MaBCs (p<0.001), whereas genes found to be recurrently mutated in FBCs, such as MAP2K4 and NCOR1, were not mutated in MaBCs.

Conclusions: MaBCs are preferentially of luminal subtype and are characterized by recurrent mutations in PIK3CA, GATA3, FLG and PLEC. Genetic alterations in MaBCs often target DNA repair and FGF signaling pathways. The known drivers of luminal FBCs appear to be less frequently altered in MaBCs. Given these important differences between MaBCs and FBCs, caution should be exercised in the extrapolation of biologic and clinical implications from studies in FBCs to the management of MaBCs.
**Title:** LORELEI: A Phase II randomized, double-blind study of neoadjuvant letrozole plus taselisib (GDC-0032) versus letrozole plus placebo in postmenopausal women with ER-positive/HER2-negative, early stage breast cancer

Cristina Saurà, Evandro de Azambuja, Peter Dubsky, Mafalda Oliveira, Kamal S Saini, Christian Fes, Ray S Lin, Timothy R Wilson, Jill Fredickson, Hema Parmar, Jerry Y Hsu, Martine Picard, Michael Gnant and Jose Baselga.

1 Vall d’Hebron University Hospital, Barcelona, Spain; 2 Jules Bordet Institute, Brussel, Belgium; 3 Medical University of Vienna, Vienna, Austria; 4 Breast International Group, Brussel, Belgium; 5 Austrian Breast and Colorectal Cancer Study Group, Vienna, Austria; 6 Genentech, San Francisco, CA and 7 Memorial Sloan Kettering Cancer Center, New York, NY.

**Body:**

Background: Taselisib (GDC-0032) is an orally bioavailable, potent, and selective inhibitor of Class I PI3-kinase (PI3K) alpha, gamma, and delta isoforms, with 30-fold less inhibition of the PI3K beta isoform relative to the PI3K alpha isoform. Preclinical data show that taselisib has enhanced activity against PIK3CA mutant cancer cell lines. Clinical data have also demonstrated confirmed partial responses in patients with PIK3CA mutant breast cancer treated with single-agent taselisib. Preclinical and clinical data also show enhanced antitumor activity when taselisib is combined with either letrozole or fulvestrant.

Study design: LORELEI is a phase II, two-arm, randomized, double-blind, multicenter, neoadjuvant study of letrozole and taselisib versus letrozole and placebo in postmenopausal women with newly diagnosed ER+/HER2-, untreated, stage I-III operable breast cancer. Other relevant eligibility criteria include tumor size ≥ 2 cm, unilateral disease, ECOG PS ≤ 1, and available and evaluable tumor tissue for central review of PIK3CA mutation analysis. Patients will be randomized (1:1) to receive continuous daily letrozole (2.5 mg) with either placebo or taselisib (4mg on a 5 days on/ 2 days off schedule) for 16 weeks. Study treatment is followed by surgery. Adjuvant treatment will be given as per physician’s discretion. Stratification at randomization is based on tumor size and nodal status.

Endpoints: The co-primary endpoints are overall objective response rate (ORR) by centrally assessed breast magnetic resonance imaging (MRI) via modified RECIST criteria and pathologic complete response (pCR) rate in breast and axilla at time of surgery in all enrolled patients and PIK3CA mutant (MT) patients. Secondary endpoints include ORR by centrally assessed MRI and pCR rate in PIK3CA wild-type (WT) patients. Other secondary endpoints performed in all enrolled patients and separately as per PIK3CA mutation status include: assessment of ORR using breast ultrasound, clinical breast exam (i.e. palpation) and mammography; changes in Ki67 levels from baseline to week 3, baseline to surgery and week 3 to surgery; centrally assessed preoperative endocrine prognostic index (PEPI) score; changes in enhancing tumor volume from baseline to surgery as measured by breast MRI via central assessment. Exploratory analyses include expression of biomarkers predictive of response to letrozole plus taselisib from tumor tissue or blood.

Statistical methods:

The sample size was calculated to detect an absolute percentage increase of 24% in ORR via MRI (40% in the letrozole-placebo arm vs. 64% in the letrozole-taselisib arm in the PIK3CA MT cohort) with 80% power at 16% two-sided significance level. The sample size will also detect an absolute percentage increase of 18% in pCR rate (1% in the letrozole-placebo arm vs 19% in the letrozole-taselisib arm in the PIK3CA MT cohort) with 80% power at 4% two-sided significance level.

Target accrual: Approximately 330 pts at 110 global sites across Europe, North and South America, and Asia-Pacific.

Reference Study ID Numbers: GO28888/BIG-3-13/SOLTI 1205/ABCSG 38.
Title: PI3K-Akt-mTOR pathway analysis to obtain further insight in the efficacy of everolimus in combination with exemestane in metastatic, ER-positive breast cancer: A Dutch breast cancer research group (BOOG) study

Dinja T Kruger¹, Karin Beelen², Connie R Jimenez¹, Maurice PHM Jansen³, Stefan Sleijfer³, Sabine C Linn² and Epie Boven¹. ¹VU University Medical Center, Amsterdam, Noord Holland, Netherlands; ²Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands and ³Erasmus University Medical Center, Rotterdam, Zuid Holland, Netherlands.

Body: Background
In patients with hormone receptor-positive breast cancer, activation of the PI3K-Akt-mTOR pathway is associated with resistance against endocrine therapy. Previous research has shown that genetic aberrations in this pathway occur frequently, including mutation and/or amplification in PI3K subunits or PI3K effectors as well as loss of lipid phosphatases (Fu X, et al. The Breast; 2013). Central review of the BOLERO-2 randomized phase III trial in which patients refractory to a non-steroidal aromatase inhibitor were randomized between exemestane combined with the mTOR inhibitor everolimus versus exemestane and placebo has shown a progression-free survival (PFS) of, respectively, 11.0 and 4.1 months [hazard ratio = 0.38 (95% confidence interval 0.31-0.48; log-rank P <0.0001)] (Yardley DA, et al. Adv Ther; 2013). The combination, however, is known to cause more adverse events and is associated with additional costs as compared to exemestane alone. In the present study we will explore whether there are biomarkers that might indicate which patients most likely benefit from co-targeting PI3K and ER pathways.

Trial design/Aims
This is a Dutch prospective, open-label, single-arm, investigator-initiated, multicenter trial in which approximately 30 hospitals will participate. A total of 175 patients will be included for baseline blood sampling and archival tumor tissue collection. From 50 patients, a fresh tumor biopsy is required at baseline and from 30 out of 50, another tumor biopsy will be collected upon progressive disease. Exploratory biomarker assessment includes immunohistochemistry (total and phosphorylated PI3K, AKT, mTOR, p70S6K and 4EBP1), tissue phosphoproteomics and circulating tumor DNA (mutations). The results of the biomarker analysis will be compared with clinicopathological characteristics and PFS.

Eligibility
Postmenopausal patients with hormone receptor-positive, HER2-negative, locally advanced or metastatic breast cancer, refractory to anastrozole or letrozole will be included. No previous treatment with exemestane or mTOR inhibitor for advanced disease is allowed and Informed consent must be signed before enrollment.

Statistical methods
Since the majority of the tests involve the use of new techniques, the study will be mainly explorative in design. The association between potential biomarkers and clinicopathological characteristics will be tested using Fisher exact test or the Mann-Whitney test. PFS curves will be drawn using the Kaplan-Meier method. PFS in association with potential biomarkers will be tested using Cox proportional hazard regression analysis.

Present and target accrual
Recently, the study has been opened for inclusion. A period of 2 years is planned for patient enrollment. Up to May 2014, two patients were included.

ClinicalTrials.gov identifier
NCT02109913

Financial support is received from Novartis, the Netherlands.
Title: Phase Ib dose allocation study of oral administration of lucitanib given in combination with fulvestrant in patients with estrogen receptor-positive and FGFR1-amplified or non-amplified metastatic breast cancer (INES)

Mario Campone1, Thomas Bachelot2, Fabrice André3, Chadi Saba4, Valérie Agrapart4, Marie-Jeanne Pierrat4, Frédéric Dubois4, Thibault Chesnel4 and Camille Poirot4.

1Institut de Cancérologie de l'Ouest – Centre René Gauducheau, Saint-Herblain, France; 2Centre Léon Bérard Centre de Lutte Contre le Cancer (CLCC) de Lyon, Lyon, France; 3Institut Gustave Roussy-Breast Cancer Unit 39 rue C Desmoulins, Villejuif, France and 4Institut de Recherche International Servier 53, rue Carnot, Suresnes, France.

Body: Background: Lucitanib is a potent, oral inhibitor of the tyrosine kinase activity of fibroblast growth factor receptors 1-3 (FGFR1-3), vascular endothelial growth factor receptors 1-3 (VEGFR1-3) and platelet-derived growth factor receptors α/β (PDGFRα/β). FGF aberrancy, as defined as FGFR1- or 11q- amplification, is a hallmark genomic alteration in breast cancer, observed at a frequency of up to 25% of patients and is strongly associated with luminal B type. Breast cancer patients with measurable disease and FGF aberrancy treated in the ongoing Phase 1/2 clinical trial of lucitanib monotherapy experienced an overall response rate of 50% (6 out of 12 patients). FGFR1-knock down was shown to decrease cell proliferation and reverse resistance to endocrine therapy in a FGFR1-amplified breast cancer cell line, hence supporting the idea of combining lucitanib with an endocrine agent such as fulvestrant, at the time of resistance. This has led to this Phase Ib study of lucitanib in combination with fulvestrant in metastatic breast cancer.

Trial design: INES is a multicenter, open-label, 2-part study to assess the tolerability of lucitanib in terms of Maximum Tolerated Dose (MTD) and Dose-Limiting Toxicities (DLTs) when administered with fulvestrant. A Continual Reassessment Method (CRM) will be used for the 1st part. A minimum of 3 patients will be enrolled at the initial dose level of 10 mg daily in combination with fulvestrant. Additional doses of 12.5 mg and 15 mg of lucitanib will be tested with the option of deescalating to 7.5 mg in case of DLTs. A minimum of 9 patients will be included at the MTD. In the 2nd part, 2 cohorts will be opened: fourteen FGF+ patients (FGFR1-or 11q- amplification), and fourteen non-amplified patients. All patients will receive fulvestrant 500 mg monthly and lucitanib at the recommended dose (RD) until unacceptable toxicity according to the investigator, disease progression or patient withdrawal. The main objective is to identify the Phase II RD when lucitanib is combined with fulvestrant. Secondary objectives include determination the Pharmacokinetic (PK) profile of lucitanib and metabolites; Measurement of tumour response; Description of the pharmacodynamic (PD) profile of lucitanib and investigation of any potential exposure dose-response relationships for safety, efficacy and PD.

Eligibility Criteria: Patients with estrogen receptor-positive, HER2 negative, breast cancer after progression or recurrence on prior therapy including fulvestrant. Patients should have ECOG performance status 0 or 1. Patients with uncontrolled hypertension are not eligible. For part 2, the presence of a metastatic site for biopsy to assess the presence of FGFR1- and/or 11q- amplification, which will be analysed centrally using FISH, is required.

Conclusion: INES is a phase Ib trial testing lucitanib in combination with fulvestrant in order to select the RD for phase II and seek preliminary efficacy signal in FGFR1- or 11q- amplified or non-amplified patients. As of June 2014, 3 patients have been enrolled in the 10 mg dose escalation cohort.
Title: OlympiA, Neo-Olympia and OlympiAD: Randomized phase III trials of olaparib in patients (pts) with breast cancer (BC) and a germline BRCA1/2 mutation (gBRCAm)

Mark Robson¹, Andrew Tutt², Judith Balmaña³, ¹², Bella Kaufman⁴, Judy Garber⁵, Charles Geyer⁶, James Ford⁷, Priyanka Sharma⁸, Mary Stuart⁹, Helen Mann⁹ and Peter A Fasching¹⁰,¹¹. ¹Memorial Sloan Kettering Cancer Center, New York, NY; ²King's College London School of Medicine and Institute of Cancer Research, London, United Kingdom; ³University Hospital Vall d'Hebron, Barcelona, Spain; ⁴Sheba Medical Center, Tel Hashomer, Israel; ⁵Dana-Farber Cancer Institute, Boston, MA; ⁶Virginia Commonwealth University, Massey Cancer Center, Richmond, VA; ⁷Stanford University School of Medicine, Stanford, CA; ⁸University of Kansas Medical Center, Westwood, KS; ⁹AstraZeneca, Macclesfield, Cheshire, United Kingdom; ¹⁰University Hospital, Erlangen, Germany; ¹¹German Breast Group, Neu Isenburg, Hessen, Germany and ¹²SOLTI Breast Cancer Research Group.

Body: Background  A Phase II study showed that the PARP inhibitor olaparib (400 mg bid; capsules) exerts antitumor activity in BC pts with a gBRCAm (Tutt et al Lancet 2010). Three Phase III trials of olaparib monotherapy have been initiated in BC pts with a gBRCAm: OlympiA (NCT02032823), Neo-Olympia (D081EC00005), OlympiAD (NCT02000622).

Trial design

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<th>Neo-Olympia</th>
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<tr>
<td>Comparator arm(s)</td>
<td>Placebo</td>
<td>Placebo + weekly paclitaxel 80 mg/m² for 12 wks (Arm B)* Olaparib 100 mg bid (tablet) + weekly paclitaxel 80 mg/m² for 12 wks (Arm C)*</td>
<td>Physician's choice of capcitabine 2500 mg/m² (d1-14 q21d), vinorelbine 30 mg/m² (d1, d8 q21d) or eribulin 1.4 mg/m² (d1, d8 q21d)</td>
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</table>

*Curative-intent surgery to be performed after 12 wks; pts will then receive olaparib 300 mg bid (Arm A), placebo (Arm B), or either weekly paclitaxel 80 mg/m² for 12 wks (then olaparib 300 mg bid) or olaparib 300 mg bid (Arm C). BICR, blinded independent central review; d, days; DDFS, distant disease-free survival; EFS, event-free survival; IDFS, invasive disease-free survival; ORR, objective response rate; pCR, pathological complete response; q, every; PFS2, time to second disease
For each trial, eligible pts will have a BRCAm and will undergo gBRCAm testing (Myriad Integrated BRACAnalysis®) as part of the trial. For OlympiA, pts must be at high risk of recurrence and have completed local treatment and either neoadjuvant (without pCR) or adjuvant chemotherapy. Neo-Olympia pts can have operable, locally advanced or inflammatory BC, must have a tumor >2cm by clinical exam (or >1cm by radiological exam) and have completed four cycles of anthracycline plus carboplatin without progression. OlympiAD pts can have TNBC or HER2– BC, and must have received prior anthracycline and taxane in the adjuvant or metastatic setting, and ≤2 chemotherapy lines for mBC. OlympiA pts will be treated for up to 12 months (m); efficacy will be assessed q3m up to 24m, then q6m up to 60m, then q12m. Neo-Olympia pts will be treated for 12 wks (w) pre-surgery, then for 40w post-surgery. In OlympiAD, PFS will be assessed by RECIST v1.1; radiologic exams will be performed at baseline, q6w up to 6m, then q12w until progression. In OlympiA and OlympiAD, IDFS and PFS will be analyzed using stratified log-rank tests; for Neo-Olympia, pCR rate will be analyzed with an adjusted logistic regression model. Primary analyses will be undertaken after 330 IDFS events (OlympiA), surgery (Neo-Olympia) and 230 PFS events (OlympiAD). Enrollment began in Mar 2014 for OlympiAD, Apr 2014 for OlympiA and is expected to begin in Q3 2014 for Neo-Olympia.
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Body: Background: Endocrine therapy (ET) is the cornerstone treatment for HR–positive, HER2-negative breast cancer (BC) patients. The high response rates with ET in these patients are partially undermined by the resistance developed by most of them over time. On early disease recurrence/progression to AIs, the treatment options include other AI, estrogen-receptor antagonists or chemotherapy (being capecitabine one of the best options). Preclinical data suggest that ER+/HER2- BC are dependent on cyclin-dependent kinases 4/6 (CDK4/6) function; the inhibition of this target may be effective in delaying/reverting endocrine resistance. Palbociclib is an oral novel CDK4/6 inhibitor that seems to be synergistic with ET in preclinical and clinical studies.

Trial Design: This is an international (6 countries) randomized phase III study. Patients are randomized 1:1 to exemestane (25 mg daily) plus palbociclib (125 mg daily x3 weeks every 4 weeks) vs. capecitabine (1,250 mg/m² twice daily x2 weeks every 3 weeks). Postmenopausal patients with HR+/HER2- MBC are eligible if resistant to previous NSAI (letrozole or anastrozole) defined as: recurrence while on or within 12 months after the end of adjuvant treatment or progression while on or within 1 month after the end of treatment for MBC. Previous chemotherapy is permitted either in the (neo)adjuvant setting and/or as first line for MBC. Patients must have measurable disease according to RECIST 1.1 or lytic bone lesions in the absence of measurable disease. The primary objective is Progression-Free Survival (PFS); secondary objectives are overall survival, response rate, clinical benefit rate, response duration, safety, quality of life and biomarker's defined changes. The study will recruit 348 patients to detect a difference of 2.75 months in the median PFS (from 6 to 8.75 months; hazard ratio= 0.686), with a power of 80% and a 5% two sided significance level. The study started recruitment in March 2014 and 14 patients have been included so far (ClinTrials.gov reference NCT02028507).
Title: A phase I study of LDE225 in combination with docetaxel in patients with triple negative (TN) advanced breast cancer (ABC): GEICAM/2012-12 (EDALINE study)

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Body: Background: LDE225 is a potent and selective oral inhibitor of Smo, a key component of the hedgehog (Hh) signaling pathway. Up-regulation of the Hh pathway is implicated in the genesis of a wide range of tumors including triple negative breast cancer. Here we report an ongoing phase I study exploring the combination of LDE225 with docetaxel in TN ABC patients to identify the Maximum Tolerated Dose (MTD) and the Recommended Phase II Dose (RP2D) (ClinicalTrials.gov Identifier: NCT02027376).

Trial Design: Eligibility criteria include patients with TN ABC candidates to receive treatment with docetaxel that have received a maximum of 3 prior chemotherapy regimens. Those patients with CNS involvement are also candidates if treated and clinically stable. Treatment consists of 21-day cycles with docetaxel 75mg/m² on day 1, every 21 day and LDE225 once daily. We use a standard 3+3 design in sequential cohorts (3 dose levels (DL) of LDE225: 400mg once daily (DL1), 600mg once daily (DL2), 800mg once daily (DL3); and a DL-1: LDE225 400mg once daily and docetaxel 60mg/m² every three weeks). The primary endpoint is the MTD and RP2D of the combination; secondary endpoints include evaluation of safety and tolerability, in addition to pharmacodynamic (PD) and pharmacokinetic (PK) studies. Patients are treated until radiologic or symptomatic progression or unacceptable toxicity occurs. PK will be performed to evaluate whether LDE225 influences the pharmacology of docetaxel. PD assessments include Hg gene expression signature associated to pathway activation in tumor samples and changes in Smo related biomarkers in skin and blood correlative samples. Efficacy will be measured in terms of time to progression and objective response rate. A minimum of 9 and a maximum of 18 patients will be included in this phase I. The study is approved by ERBs and Competent Authority and already recruiting patients (two patients included in DL1).
Title: A phase III study of abemaciclib (LY2835219) combined with fulvestrant in women with hormone receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2-) breast cancer (MONARCH 2)

Antonio Llombart, Masakazu Toi, Suzanne R Klise, Martin Frenzel, Edward M Chan and George W Sledge. 1Hospital Arnau de Vilanova, Valencia, Spain; 2Kyoto University, Kyoto, Japan; 3Eli Lilly and Company, Indianapolis, IN and 4Stanford University, Stanford, CA.

Body: Background: Abemaciclib (LY2835219), an oral drug administered twice daily on a continuous schedule, is a cell cycle inhibitor of both CDK4 and CDK6. In Study I3Y-MC-JPBA, abemaciclib demonstrated evidence of single agent activity in a tumor-specific cohort of patients with metastatic breast cancer (MBC) and a median of 7 prior therapies; all responses observed were in women with HR+ disease. Abemaciclib also demonstrated an acceptable safety profile both as a single agent and in combination with fulvestrant. Based on these results, abemaciclib has been entered into a Phase III study (MONARCH 2) in combination with fulvestrant for women with locally advanced or metastatic hormone receptor positive (HR+), human epidermal growth factor receptor 2 negative (HER2-) breast cancer.

Trial design: MONARCH 2 (NCT02107703) is a randomized, double-blind, placebo-controlled Phase III study of fulvestrant with or without abemaciclib for women with HR+, HER2- locally advanced (not amenable to curative treatment by surgery) or metastatic breast cancer. Patients will be randomized 2:1 (Arm A [abemaciclib plus fulvestrant]: Arm B [placebo plus fulvestrant] with stratification based on the nature of disease (visceral metastases versus bone only metastases versus other) and sensitivity to endocrine therapy (no prior endocrine therapy versus primary resistance versus secondary resistance). Abemaciclib (200 mg every 12 hours [Q12H] on Days 1 to 28 of a 28-day cycle) or placebo will be given orally and fulvestrant 500 mg will be administered intramuscularly on Days 1 and 15 of Cycle 1, then on Day 1 of Cycle 2 and subsequent cycles.

Eligibility criteria: Postmenopausal women with HR+, HER2- inoperable locally advanced or metastatic breast cancer who have either relapsed after prior endocrine therapy or have not received prior endocrine therapy are eligible. Patients are required to have either measurable disease or non-measurable bone only disease, adequate organ function and an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of ≤1.

Specific aims: The primary objective of MONARCH 2 is to compare PFS between two treatment arms: abemaciclib plus fulvestrant versus placebo plus fulvestrant for women with HR+, HER2- locally advanced or metastatic breast cancer. Secondary objectives are to compare overall survival, objective response rate, clinical benefit rate, safety, pharmacokinetics and quality of life.

Statistical methods: The study has 90% power to detect an increase in PFS of approximately 42% (hazard ratio = .703). Assuming a median PFS of 6.5 mos. in the control arm, this corresponds to a 2.75 month increase in the median PFS to 9.25 mos. PFS and OS will be hierarchically tested to maintain an overall type I error rate of 2.5%.

Present accrual and target accrual: Target accrual is approximately 550 patients.

Contact information: For further information please contact 1-877-CTLILLY (1-877-285-4559).
Title: Master regulator (MR)-directed therapy in residual breast cancer patient derived xenografts (PDXs)

Kevin Kalinsky¹, Prabhjot Mundi¹, Dawn L Hershman¹, Eileen Connolly¹, Katherine D Crew¹, Hanina Hibshoosh¹, Andrea Calfiano¹ and Matthew Maurer¹. ¹Columbia University Medical Center, New York, NY.

Body: Background: Neoadjuvant chemotherapy (NACT) is standard of care in operable breast cancer (BC). There are no therapies that have demonstrated benefit in patients who failed to achieve a pathologic complete response (pCR). There remains an unmet need to identify appropriate targets in which to direct therapy in this high-risk population to decrease BC mortality. Computational approaches to reverse engineering of cell signaling networks from patient tumor-specific RNA expression data can identify key molecular dependencies that result in the malignant phenotype, i.e. MRs. These approaches out-perform traditional methods, such as identifying mutated or over-expressed genes, in determining true tumor dependencies. Successful targeting of MRs may be a means for rational selection of targeted drugs in the operable BC setting.

Trial Design: A single center pilot study that will accrue 35 subjects with BC who will have received standard NACT and at least 1.5 cm of residual disease. At the time of definitive surgery, resected tissue will be assessed for residual disease and then allocated. After reserving required tissue for pathologic diagnosis, 0.5 cm³ will be procured for xenografting and 0.5 cm³ will be flash frozen for RNA extraction. Tissue for xenografting is immediately sent to a Champions Oncology site for implantation. We will use fresh frozen tissue to perform RNA-seq. Previously validated computational algorithms at our center, including MARINa and VIPER, will be used to interrogate the expression data and identify the top MRs in individual tumors. Upon maturation of a Champions Oncology Tumor Graft model, the second generation expansion group will be divided into four cohorts. We plan to test two drugs that target tumor-specific MRs, one vehicle control, and one negative control (paclitaxel, as these tumors have demonstrated paclitaxel resistance) in each model.

Eligibility Criteria
1. Patients ≥18 years with newly diagnosed BC, deemed candidates for definitive surgery.
2. Subjects must have received standard NACT with taxane (paclitaxel or docetaxel; herceptin +/- pertuzumab if HER2+) and/or adriamycin/cytoxan.
3. Clinical or radiographic evidence of ≥1.5 cm of residual BC.
4. ECOG PS ≤2

Specific Aims:
1) To computationally infer MRs in individual patient tumors resistant to NACT
2) To determine if targeting MRs results in superior tumor growth inhibition in PDX models

Statistical Methods: In the PDX model, pre- and post-treatment tumor volume (TV) is calculated: TV= width² x length x 0.52, and is standardized as a tumor growth inhibition (TGI) percentage compared to the vehicle control. Since we anticipate the MR-directed arms will demonstrate a TGI (80%) twice that of the paclitaxel arm (40%), we have 80% power to detect a difference in 18 patients (one-sided alpha, 0.05). We assume a xenograft take-rate of 50% in the post-NACT setting and thus aim for an N of 35. Final data analysis will be adjusted using ANOVA.

Present and Target Accrual: 35 patients, projected to accrue in 18-24 months. Estimate based on number of locally advanced BC patients seen, competing studies, and likelihood of participation. The study will open in Fall 2014.
Title: Phase I/II trial of ruxolitinib in combination with trastuzumab in metastatic HER2 positive breast cancer

Kevin Kalinsky¹, Dow-Chung Chi¹, Shing Lee¹, Amy Tiersten², Della Makower³, Ellen Chuang⁴, Katherine D Crew¹, Dawn L Hershman, Jose Silva², Andrea Califano¹ and Matthew Maurer⁴. ¹Columbia University Medical Center, New York, NY; ²Icahn School of Medicine at Mount Sinai; ³Albert Einstein College of Medicine, Bronx, NY and ⁴Weill Cornell Medical Center, New York, NY.

Body: Background
Integrated analysis of whole genome RNAi screening with computationally reverse engineered interactome models identified IL6/JAK/STAT as a master regulator pathway essential for growth of ErbB2/HER2 positive breast cancer. Ruxolitinib (R), FDA-approved treatment for myelofibrosis, inhibits JAK1 and JAK2. The combination of R plus Trastuzumab (T) is synergistic in tumor growth inhibition in mouse xenografts of HER2 amplified breast cancer cell lines. These data provide a strong rationale for studying the efficacy of combination R and T in a clinical trial.

Trial Design
A multi-center, open-label, phase I/II (P1/2) trial of R plus T in HER2+ metastatic breast cancer (MBC) who have progressed on T-based therapy. P1 will be an adaptive design with 10 patients, using the time-to-event continual reassessment method. The recommended P2 dose (RP2D) will be used in a non-randomized, open-label P2 trial with 30 evaluable patients (pts). Given the anticipated limited overlapping toxicities, approximately 36 pts (range: 32-40) are expected for the P1/2. The duration of a treatment cycle will be 21 days. R will be taken orally twice a day continuously. The P1 dosing range will be 10-25 mg BID (dose level 0: 20 mg BID). T will be administered on Day 1 of each cycle at standard dosing. Objective Response Rate (ORR) will be assessed by imaging every 9 weeks. Blood samples will be obtained for biomarker analysis, pre-treatment, on-treatment on C2D1, and then at progression. Pre-treatment biopsies from archival tissue or new biopsy, on treatment biopsy on C2D1, and upon progression of disease will be discussed with pts with accessible disease.

Main Eligibility Criteria:
1. HER2 positive MBC
2. Progression on ≥2 HER2-directed therapy in metastatic setting, including Pertuzumab and T-DM1
3. Measurable or non-measurable disease
4. LVEF >50%.
5. No history of prior JAK2 inhibitor
6. No HIV-positive or active infection
7. No concurrent medications that are potent CYP3A4 inhibitor or inducer

Specific Aims
1. Primary: P1: MTD of combined R + T. P2: Progression Free Survival (PFS)
2. Secondary: a) Clinical: ORR, clinical benefit rate (CBR), and tolerability. Pts will be stratified by hormone receptor (HR) status to explore differences in efficacy between HR+ and HR-.
b) Explore potential predictive tumor and blood-based predictive biomarkers at baseline, on treatment, and progression: (tumor: pSTAT3 expression); serum: IL-6, IL-8, C-reactive protein; circulating tumor cell pSTAT3 expression; and tumor gene expression.

Statistical Methods
Assuming a historical PFS of 8 weeks with single-agent agent HER2-targeted therapy in HER2+ MBC after progressing on T-based therapy, we predict that pts receiving the combination of R plus T will have a PFS of at least 13 weeks. With a 2-sided alpha of 0.05, we have 80% power to detect a difference with 30 pts.

Target Accrual
Sample Size: 32-40 pts; projected over 2 years at 4 sites: Columbia, Einstein, Mount Sinai, and Cornell. Trial will start accruing July 2014.
Title: The B-YOND study: A phase II three-arm randomized trial of the combination of tamoxifen plus goserelin acetate with BYL719 or buparlisib (BKM120) in premenopausal patients with hormone receptor-positive/HER2-negative locally advanced or metastatic breast cancer

Yen-Shen Lu¹, Yeon Hee Park², Zhimin Shao³ and Roberta Valenti⁴. ¹National Taiwan University Hospital, Taipei, Taiwan; ²Samsung Medical Center, Seoul, Korea; ³Fudan University Cancer Hospital, Shanghai, China and ⁴Novartis Pharma AG, Basel, Switzerland.

Body: Background:
The incidence of breast cancer (BC) has been rapidly increasing in many countries in the last decades. In contrast to Western countries, approximately 50% of BC patients in Eastern countries are premenopausal, with hormone receptor-positive (HR+) disease, or luminal subtype by molecular classification. Benefit from the most recent advances in endocrine treatments for patients with HR+ BC is currently confined to postmenopausal women. For premenopausal metastatic BC (MBC) tamoxifen and/or ovary ablation/suppression remain the recommended first-line therapies for metastatic disease through category 2 evidence, calling for further clinical investigations.

In the context of growing evidence of the role of PI3K/AKT/mTOR pathway inhibition in enhancing and extending the benefit of endocrine therapies in HR+ MBC pre-clinical and clinical models, the B-YOND study is a phase II, three-arm randomized trial aimed at exploring the PI3K inhibitors buparlisib and BYL719 in combination with tamoxifen in the context of ovarian suppression in premenopausal patients with MBC.

Design & Objectives:
Premenopausal women with HR+/HER2-negative locally advanced or MBC who a) are newly diagnosed or b) recurred during or after adjuvant treatment with tamoxifen monotherapy, and received no endocrine treatment in the metastatic setting, will be randomized to receive tamoxifen plus goserelin (control arm, Arm 3) or the same combination with buparlisib (Arm 2) or BYL719 (Arm 1) until progression. Stratification based on previous treatment with tamoxifen and presence of liver and/or lung metastasis will be applied. Primary objective is the efficacy comparison of Arm 1 vs Arm 3 and Arm 2 vs Arm 3 in terms of 9-month progression free survival (PFS) rate. Secondary objectives include additional efficacy evaluations (median PFS, overall response rate, clinical benefit), safety and tolerability, quality of life, and pharmacokinetics.

Statistical Methods:
Based on previous reports, the estimated proportion of patients who are alive without progression at 9 months is 50% for the control arm (P0=0.50), and we hypothesize that this proportion is 75% for both experimental arms (P1=0.75). Using a two-sided 0.05 level of significance for both the comparisons [Arm 1 vs Arm 3; Arm 2 vs Arm 3], with an overall type-I error not greater than 0.10, with 80% power, the estimated patient numbers needed is 58 patients for each arm. With an assumption that approximately 10% patients will be lost to follow-up, a total of 192 patients will need to be randomized to the three treatment arms in 1:1:1 ratio.

Accrual
This study will be open for recruitment in Taiwan, South Korea, China, Hong Kong and Thailand and potentially extended to additional Eastern countries. Enrollment started in May 2014 and will last for an estimated period of 18 months.
Title: A phase 3, open-label, randomized, parallel, 2-arm multi-center study of the oral PARP inhibitor BMN 673 versus physician’s choice in germline BRCA mutation subjects with locally advanced and/or metastatic breast cancer (EMBRACA study)

Jennifer K Litton¹, Joanne L Blum², Wolfgang Eiermann³, Young-Hyuck Im⁴, Miguel Martin⁵, Lida Mina⁶, Henri Roché⁷, Hope S Rugo⁸, Frances Visco⁹, Charlie Zhang¹⁰, Nathalie A Lokker¹⁰ and Debra L Lounsbury¹⁰. ¹MD Anderson Cancer Center, Houston, TX; ²Baylor Sammons Cancer Center, Texas Oncology, US Oncology, Dallas, TX; ³Interdisziplinares Onkologisches Zentrum Munchen, Munich, Germany; ⁴Samsung Medical Center, Seoul, Korea; ⁵Hospital General Universitario Gregorio Marañón, Madrid, Spain; ⁶Indiana University School of Medicine, Indianapolis, IN; ⁷Institut Universitaire du Cancer Toulouse, Toulouse, France; ⁸UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; ⁹National Breast Cancer Coalition, Washington, DC and ¹⁰BioMarin Pharmaceutical Inc, Novato, CA.

Body: Background: Poly-ADP-ribose polymerase (PARP) represents a family of enzymes of which at least two (PARP1 and PARP2) play important roles in DNA repair. PARP inhibition induces synthetic lethality in tumor cells bearing mutations in the genes encoding breast cancer susceptibility gene 1 (BRCA 1) and breast cancer susceptibility gene 2 (BRCA 2), both of which are key components in the pathway of homologous recombination DNA repair. BMN 673 is the most potent preclinical PARP inhibitor described to date with the highest efficiency at trapping PARP-DNA complexes (Murai et al, 2014). BMN 673 is a novel and highly potent PARP inhibitor and has shown promising single-agent anti-tumor efficacy in several tumor types in an ongoing Phase 1/2 clinical study.

Methods: The purpose of this multi-center, international, open-label, 2:1 randomized Phase 3 trial (EMBRACA) is to compare the safety and efficacy of BMN 673 versus protocol-specific physician’s choice treatment (capecitabine, eribulin, gemcitabine or vinorelbine) in subjects who have locally advanced and/or metastatic breast cancer with germline BRCA mutations. The primary objective of the study is to compare progression free survival (PFS) of subjects treated with BMN 673 as a monotherapy relative to those treated with protocol-specific physician’s choice treatment. Secondary objectives include objective response rate (ORR), overall survival (OS), safety and pharmacokinetics of BMN 673. Exploratory objectives include duration of response (DOR) and health-related quality of life assessment. Patients may be eligible if they are 18 years or older, have histologically or cytologically confirmed carcinoma of the breast, locally advanced and/or metastatic disease appropriate for systemic single cytotoxic chemotherapy, documentation of a deleterious or pathogenic germline BRCA1 or BRCA2 mutation, ≤ 2 prior chemotherapy-inclusive regimens for locally advanced and/or metastatic disease, prior treatment with a taxane and/or anthracycline in the adjuvant or metastatic setting, ECOG performance status ≤ 1, and no prior platinum treatment for metastatic disease. Patients (n=429) will be randomized 2:1 to receive either BMN 673 oral capsules once daily (1.0 mg/day) in 21-day cycles or protocol-specific physician’s choice treatment. All eligible subjects will receive study drug treatment until disease progression or unacceptable toxicity. This trial is enrolling patients from the United States, Europe, Israel, Asia/Pacific, and South America (NCT01945775).
**Title:** A Phase 2, randomized, open-label, multicenter, safety and efficacy study of oral lucitanib in patients with metastatic breast cancer with alterations in the FGF pathway

Maysa Abu-Khalaf¹, Ingrid Mayer², Jason B Litten³, Mitch Raponi³, Andrew R Allen³, Lajos Pusztai¹ and Carlos L Arteaga². ¹Yale Cancer Center, New Haven, CT; ²Vanderbilt-Ingram Cancer Center, Nashville, TN and ³Clovis Oncology, Inc, San Francisco, CA.

**Body:** BACKGROUND: Lucitanib is a potent, oral inhibitor of the tyrosine kinase activity of Fibroblast Growth Factor Receptors 1-3 (FGFR1-3), Vascular Endothelial Growth Factor Receptors 1-3 (VEGFR1-3) and Platelet-Derived Growth Factor Receptors A/B (PDGFRα/B). Aberrant FGF signaling, as defined as \( \text{FGFR1} \) and/or 11q amplification, is a hallmark genomic alteration in breast cancer, observed at a combined frequency of up to 25% of patients. Breast cancer patients with measurable disease and aberrant FGF signaling treated in the ongoing Phase 1/2 clinical trial of lucitanib monotherapy experienced an ORR of 50% and a DCR of 100%. This compelling clinical activity has led to the initiation of a global clinical development program for lucitanib in breast cancer.

TRIAL DESIGN: The current study is comparing PFS for doses of lucitanib (10 or 15 mg daily) in patients with FGF-aberrant metastatic breast cancer after failure of currently available standard therapies. Approximately 160 patients will be randomized 1:1 to the 10 mg and 15 mg daily dosing groups and stratified by FGF pathway alteration (\( \text{FGFR1} \) or 11q amplification) and prior anti-VEGF therapy (yes or no). \( \text{FGFR1} \) and 11q (containing the FGF ligands 3, 4, and 19) amplification is determined locally for patient enrolment and confirmed by central laboratory fluorescent in situ hybridization (FISH) testing. A total of 130 PFS events provides 80% power at a 2-sided significance level of 0.05 to detect a hazard ratio of 0.60 for comparing the 10 mg and 15 mg dose groups. Secondary objectives are ORR, DoR, DCR, OS, PROs, safety and population PK. Exploratory endpoints include tissue and blood-based biomarkers that may be predictive of response or primary resistance to treatment with lucitanib.

ELIGIBILITY CRITERIA: Histologically or cytologically confirmed FGF-aberrant metastatic breast cancer relapsed or refractory to approved standard available treatment. ECOG 0 or 1. Normal organ function. Patients with uncontrolled hypertension are excluded.
Title: A phase 2, 2-stage, 2-cohort study of the oral PARP inhibitor BMN 673 in patients with germline BRCA mutation and locally advanced and/or metastatic breast cancer (ABRAZO study)

Nicholas C Turner¹, Judith Balmana², Susan M Domchek³, Frances Visco⁴, Charlie Zhang⁵, Nathalie A Lokker⁶, Debra L Lounsbury⁵ and Mark E Robson⁶. ¹Royal Marsden Hospital and Institute of Cancer Research, London, United Kingdom; ²Vall Hebron Institute of Oncology, Barcelona, Spain; ³Basser Research Center for BRCA, University of Pennsylvania, Philadelphia, PA; ⁴National Breast Cancer Coalition, Washington, DC; ⁵BioMarin Pharmaceutical Inc, Novato, CA and ⁶Memorial Sloan Kettering Cancer Center, Weill Cornell Medical College, New York, NY.

Body: Background: Poly-ADP-ribose polymerase (PARP) represents a family of enzymes of which at least two (PARP1 and PARP2) play important roles in DNA repair. PARP inhibition induces synthetic lethality in tumor cells bearing mutations in the genes BRCA1 and BRCA2, both of which are key components in the homologous recombination DNA double-strand breaks repair pathway. BMN 673 is the most potent preclinical PARP inhibitor described to date with the highest efficiency at trapping PARP-DNA complexes (Murai et al, 2014). BMN 673 has shown promising single-agent anti-tumor efficacy in several tumor types in an ongoing Phase 1/2 clinical study.

Methods: The purpose of this Phase 2 trial (ABRAZO) is to evaluate the safety and efficacy of BMN 673 in patients with locally advanced or metastatic breast cancer with a deleterious germline BRCA 1 or BRCA 2 mutation. This study is an open-label, 2-stage, 2-cohort Phase 2 study using a Southwest Oncology Group (SWOG) 2-stage design. Eligible subjects will be assigned to either Cohort 1 (n=70) or 2 (n=70) based on prior chemotherapy exposure for metastatic disease: Cohort 1) Subjects who have previously responded to a platinum-containing regimen for metastatic disease with disease progression > 8 weeks following the last dose of platinum; or Cohort 2) Subjects who have received > 2 prior chemotherapy regimens and who have had no prior platinum therapy for metastatic disease. The primary objective is to determine the objective response rate (ORR) of BMN 673 as a single agent for each cohort. The secondary objectives of the study are to determine the following for each cohort: clinical benefit rate (CBR), duration of response (DOR), progression free survival (PFS), and overall survival (OS). Health-related quality of life assessment is an exploratory objective. Patients may be eligible if they are 18 years or older, have histologically or cytologically confirmed carcinoma of the breast, locally advanced and/or metastatic disease, deleterious or pathogenic germline BRCA1 or BRCA2 mutation, prior chemotherapy for metastatic disease based on the above Cohort 1 or Cohort 2 inclusion criteria, ECOG performance status ≤ 1, and no central nervous system (CNS) metastasis except adequately treated brain metastasis documented by baseline CT or MRI scan that has not progressed since previous scans and that does not require corticosteroids for management of CNS symptoms. Eligible patients will receive BMN 673 oral capsules once daily (1.0 mg/day) continuously in 21-day cycles until disease progression or unacceptable toxicity. This trial is enrolling patients from the United States and European countries (NCT02034916).
**Body: Background:**

Inflammatory breast cancer (IBC) is an aggressive form of breast cancer that accounts for 3 to 5% of all invasive breast tumors in the United States. IBC possesses an increase of proangiogenic factors including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and platelet derived growth factor (PDGF) as compared with non-IBC. In particular, FGF family receptors play a critical role in tumorigenesis, morphogenesis, and inducers of angiogenesis. Dovitinib (TKI258) is an oral tyrosine kinase inhibitor with in vitro IC\textsubscript{50} values of approximately 10 nmol/L against FGFR1-3, VEGFR1-3, and PDGFR. These structurally related receptors are important for the growth and survival of endothelial cells during tumor angiogenesis.

**Trial Design:**

This is a single institution, single arm phase II study. Patients receive a single daily oral dose of dovitinib 500 mg for 5 consecutive days, followed by a 2-day rest period (5 days on/2 days off schedule).

**Eligibility:**

Patients have histological confirmation of breast carcinoma with a clinical diagnosis of IBC based on presence of inflammatory changes in the involved breast, including diffuse erythema and edema (peau d’orange). Pathological evidence of dermal lymphatic invasion should be noted but is not required for diagnosis. HER2-negative. ECOG PS 0-2. Baseline MUGA or echocardiogram scans with LVEF of > 50%. Normal hematology, liver and kidney function laboratory studies. Patients must have received at least 2 chemotherapy lines for metastatic disease and have relapsed.

**Research Hypothesis:**

Dovitinib has antitumor activity in patients with HER2-negative advanced IBC.

**Specific Aims:**

Primary objective: to determine the disease control rate (CR, PR, and SD). Secondary objective: to evaluate safety profile. Exploratory biomarkers: circulating tumor cells (CTC), CTC undergoing EMT, and cancer stem cells

**Statistical Methods:**

The primary endpoint is the six-month disease control rate (ORR) as defined by RECIST 1.1. A response is anyone who experiences SD, CR or PR in the first 6 months. We will conduct this study with Simon’s two-stage design using the mini-max criterion and the response rate will be estimated accordingly. It is assumed that dovitinib will have a target ORR of 30%. An ORR of 10% or lower is considered a failure based on the typical ORR with a second line regimen for IBC and the new regimen will be rejected under this circumstance. When the probability of accepting a "bad" regimen (i.e. response rate ≤ 10%) is 0.05 and the probability of rejecting a "good" regimen (i.e. response rate ≥ 30%) is also 0.10, Simon’s design to minimize the maximum sample size requires 22 patients in the first stage. If two or less patients respond to the treatment, the trial will be stopped and the regimen will be declared as ineffective. If at least three of the first 22 patients respond to the treatment, 11 additional patients will be entered in the study to reach a total of 33 patients. By the end of the study, the new regimen will be rejected if response rate is less than or equal to 6 out of 33 patients and will be accepted otherwise.

**Present Accrual and Target Accrual:**

A total of 22 patients were accrued. Target accrual is 33 patients.
Title: A randomized, multicenter, phase II study of ipatasertib (Ipat, GDC-0068), an inhibitor of Akt, in combination with paclitaxel (Pac) as front-line treatment for patients (pts) with metastatic triple-negative breast cancer (TNBC)

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Body: Background: The PI3K/Akt pathway is often activated in TNBC through loss of PTEN expression, low INPP4B expression, and/or increased AKT3 amplification. Activation of Akt may then lead to chemoresistance; thus, inhibition of Akt signaling may result in improved efficacy of chemotherapy in TNBC. Ipat (GDC-0068) is a potent ATP-competitive small molecule inhibitor of all Akt isoforms. In preclinical breast cancer models, the combination of Ipat with taxanes enhanced efficacy, and in a Phase Ib study, the combination of Ipat with Pac was well-tolerated and resulted in clinical responses.

Trial Design: This is a randomized, double-blinded, placebo controlled, international, multicenter, Phase II study designed to estimate the efficacy and safety profile of Ipat combined with Pac versus placebo combined with Pac in pts with metastatic TNBC. Pts will receive Ipat or placebo 400 mg orally once daily on Days 1 to 21 of each 28-day cycle with Pac 80 mg/m² on Days 1, 8, and 15 of each 28-day cycle. Treatment will continue until disease progression, intolerable toxicity, withdrawal, or study completion. Pts will then be followed every 3 months for survival. Archival tumors will be assessed for PTEN expression by immunohistochemistry.

Key Eligibility: Pts ≥ 18 years, ECOG 0 or 1, with histologically documented TNBC that is inoperable locally advanced or metastatic and not amenable to curative resection are eligible. Additional eligibility criteria include availability of a tumor specimen, measurable disease per RECIST v1.1, and adequate hematologic and organ function within 14 days of study. Any previous therapy for TNBC is excluded, except for prior neoadjuvant or adjuvant chemotherapy and/or radiation completed 6 months prior to study. Pts with known brain or spinal cord metastases are also excluded.

Objectives: The primary objective is progression-free survival (PFS) in all TNBC pts and in TNBC pts with PTEN-low tumors. Secondary objectives include estimation of overall survival (OS), objective response rate (ORR), duration of ORR, safety, pharmacokinetics (PK), patient-reported outcomes (PROs), and biomarkers.

Statistical Methods: Approximately 120 pts will be randomized 1:1 and stratified by prior adjuvant/neoadjuvant treatment including chemotherapy and/or radiation (yes vs. no), disease free interval from last dose of chemotherapy (≤ 12 months vs. > 12 months), and tumor PTEN status (low/null vs. moderate vs. high). Primary and secondary efficacy analyses will include all randomized pts, grouped by treatment at randomization. Kaplan–Meier curves will be produced for analyses of PFS, OS, and duration of response, and stratified log rank tests will be used to compare treatments.

Accrual: This study is open for accrual.
Title: Circulating tumor DNA in plasma as a surrogate for tumor biopsy to identify tumor genetic alterations in patients with multi-focal metastatic breast cancer

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Body: Background: While effective targeted therapies exist for patients with ER/PR + (anti-estrogens) and HER2+ (anti-HER2 agents) disease, and some triple-negative cancers respond to DNA-damaging chemotherapy, many patients eventually exhibit disease refractory to all standard breast cancer therapies, particularly in the metastatic setting. Expanding catalogs of tumor-targeted therapies are being developed, and tumor genetics are playing an increasing role in patient selection. However, tumors can exhibit intra- and inter-tumor genetic heterogeneity. If a biopsied tumor is not genetically reflective of all tumors within a patient, then the optimal therapy may be overlooked. Identifying targetable genetic changes for which there are drugs, using non-invasive procedures, will be an increasing challenge for medical oncologists. One way to potentially overcome this issue is through cell-free circulating tumor DNA, which is detectable in the bloodstream. This study represents the first step in this important process: evaluating plasma DNA as a potential route to non-invasive identification of genetic mutations in patients with metastatic breast cancer with several tumors.

Design: Patients with new or progressive metastatic breast cancer with >=3 sites of biopsy-able disease are enrolled. Large bore, large volume blood draw for PT/ INR and plasma DNA will be obtained. Primary tumor will be biopsied if present. Biopsies of >=3 tumors in >=2 different organ sites will be required. Tumor histology and ER/PR/her2 status will be determined. Tumor tissue and plasma will undergo DNA sequencing.

Eligibility: Measureable new or progressive metastatic breast cancer by CT and bone scan or PET scan. >=3 sites of disease with >=2 organ sites appropriate for biopsy. Prior therapy allowed, but all specimens must be obtained prior to change in therapy.

Primary objective: To determine whether the genetic mutations in plasma DNA are reflective of the genetic mutations present in biopsies of all tumors.

Secondary objectives: To determine A) the amount of plasma DNA required to capture all somatic genetic mutations in tumor samples, and B) the extent of genetic heterogeneity between primary and metastatic tumors.

Primary endpoint: Rate of genetic concordance between plasma DNA and >=3 tumor within each patient.

Present accrual: 2 Target accrual: 10.
2014 San Antonio Breast Cancer Symposium

Publication Number: OT1-2-02
Average Grade: 4.33

Title: Trastuzumab in HER2-negative early breast cancer as adjuvant treatment for circulating tumor cells (CTCs) (Treat CTC)

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Body: Background:
The presence of Circulating Tumor Cells (CTCs) in metastatic breast cancer (BC) is associated with worse clinical outcome. Recent data showed an association between CTC(s) detection and reduced disease-free and overall survival in early disease. Patients with persisting CTC(s) after (neo)adjuvant chemotherapy might benefit from additional systemic treatment. Trastuzumab is a part of the standard of care for patients with HER2-positive BC. Recent data have reinforced the hypothesis that the therapeutic effect of trastuzumab depends on immune-related mechanisms. It has been demonstrated that trastuzumab eliminated CTC(s), irrespective of the HER2 status of the primary tumor and of CTC(s) and this was associated with improved relapse-free survival (Bozionellou et al, Clin Cancer Res 2004, Georgoulias et al Ann Oncol 2012).
The Treat CTC trial is designed to explore the effect of trastuzumab in patients with HER2-negative early BC and persisting CTC(s) after (neo)adjuvant chemotherapy and surgery.

Trial Design:
Treat CTC trial is a multicentre (6 countries, 92 centers) European randomized phase II trial, sponsored by the EORTC and run under the BIG umbrella. It will assess the efficacy of trastuzumab in eliminating persisting CTC(s) after the completion of (neo)adjuvant chemotherapy and surgery in patients with HER2-negative early BC. Eligible patients will be randomized in a 1:1 ratio to either 6 cycles of trastuzumab or observation.

Eligibility criteria:
- Adequately excised HER2-negative early BC
- Evidence of CTC(s) detection using the CellSearch technology after completion of (neo)adjuvant chemotherapy
- Completion of adjuvant chemotherapy for node-positive disease or neoadjuvant chemotherapy with residual invasive disease in breast or lymph nodes (no complete pathological response)

Specific aims:
The primary objective is to evaluate whether trastuzumab decreases the detection rate of CTCs in patients with HER2-negative primary BC by comparing the trastuzumab treated arm to the observation arm. Furthermore, clinical outcomes as measured by Recurrence Free Interval (RFI), Invasive Disease Free Survival (IDFS), Disease Free Survival (DFS) and Overall Survival (OS) between the trastuzumab and observation arms will be compared.

Present accrual and target accrual:
It is estimated that 2175 women will be registered to include 174 patients eligible for randomization in a 1:1 ratio. Accrual is expected to be completed in 2 years. Treat CTC started patient screening in May 2013 in Belgium, in March 2014 in Germany and in June 2014 in France. An update of the proportion of patients screened versus patients randomized will be presented during SABCS.

Methods:
The primary test will be a one-sided test to compare the trastuzumab arm to the observation arm for the CTC(s) detection rate at week 18 (superiority test). The comparison for the primary endpoint will be performed on the intention-to-treat population using a one-sided test with overall α of 0.1. The odds ratio and its confidence interval will be estimated using a logistic regression model. The comparison of RFI, IDFS, DFS and OS will be done using a two-sided test in a proportional hazards model for cause specific hazard, adjusted for the stratification factors.
Title: The DETECT-study concept: Treatment based on the phenotype of circulating tumor cells in HER2-negative metastatic breast cancer

Bernadette AS Jaeger¹, Susanne Albrecht¹, Fabienne Schochter¹, Carola A Melcher², Carsten Hagenbeck², Thomas WP Friedl¹, Brigitte Rack³, Volkmar Müller⁴, Peter A Fasching⁵, Wolfgang Janni¹ and Tanja Fehm². ¹University Hospital Ulm, Ulm, Germany; ²University Hospital Duesseldorf, Duesseldorf, Germany; ³University Hospital LMU-Munich, Munich, Germany; ⁴University Hospital Hamburg-Eppendorf, Hamburg, Germany and ⁵University Hospital Erlangen, Friedrich-Alexander University Erlangen-Nuremberg, Comprehensive Cancer Center Erlangen-EMN, Erlangen, Germany.

Body: Background: The prognostic impact of circulating tumor cells (CTC) in metastatic breast cancer (MBC) is well demonstrated. The role of CTCs in predicting specific treatment response and the importance of CTC phenotypes for therapeutic decisions will be investigated within the DETECT-study concept.

Trial Design and eligibility criteria: The DETECT studies are prospective, multicenter, open-label clinical trials designed for patients with HER2-negative MBC and evidence of CTCs in the peripheral blood. DETECT III is a two-arm study for patients with HER2-positive CTCs, randomized to physician’s choice therapy (chemotherapy or endocrine treatment) with or without additional HER2-targeted treatment with lapatinib. DETECT IV combines two single-arm studies aimed at patients with HER2-negative CTCs. Postmenopausal patients with hormone-receptor-positive MBC will be treated with the mTOR-inhibitor everolimus in combination with an endocrine therapy of physician’s choice (everolimus cohort), whereas patients with triple-negative or hormone-receptor-positive MBC and indication to chemotherapy will receive eribulin (eribulin cohort).

Specific aims: The primary objective of the trials is to estimate the clinical efficacy of treatments, assessed by the CTC clearance rate for DETECT III and by progression-free survival (PFS) for DETECT IV.

Methods: Prevalence of CTCs at various time points as well as the HER2 status of CTCs are assessed using the FDA-approved CellSearch System (Veridex, USA). After immunomagnetic enrichment with an anti-EpCam-antibody, cells were labelled with anti-CK8/18/19 and anti-CD45 antibodies to distinguish epithelial cells from leucocytes. A fluorescein conjugate antibody with anti-CK-Fluorescein Isothiocyanate (FITC) was used for HER2 phenotyping. The cut-off for CTC-positivity was 1 CTC and for HER2 1 CTC with strong HER2-staining (+++). Survival endpoints will be estimated using the Kaplan-Meier method.

Present and target accrual: Overall, about 2000 patients with HER2-negative MBC will have to be screened for CTCs to be able to recruit 228 patients with HER2-positive CTCs for DETECT III (which started in February 2012), 400 patients with HER2-negative CTCs for DETECT IV- everolimus cohort (which started in December 2013) and 120 patients for DETECT IV- eribulin cohort (which will start in the second half of 2014). 907 patients have been recruited for CTC screening until June 2014.

Perspectives: One screening for CTCs offers different treatment options for patients with HER2-negative MBC and evidence of CTCs within the DETECT-study concept. DETECT III is the first study to investigate a personalized targeted treatment based on the phenotype of CTCs. The addition of a HER2-targeted therapy in case of HER2-positive CTCs is innovative and in case of success will lead to new treatment strategies in MBC. DETECT IV complements DETECT III with regard to additional therapy indications.
Body: Background:
Circulating tumor DNA (ctDNA) has been proposed as a biological surrogate of the repertoire of molecular aberrations in cancer patients. The genetic information derived from the analysis of ctDNA may be therefore employed as biomarker for diagnosis, prognostication, therapy response monitoring and assessment of genetic mechanisms of resistance. We have initiated a prospective study in a population of BRCA1-mutation carrier at high risk of either relapse and/or new cancer growth. The CirCA01 study is based on the combination of (i) on the clinical side, the systematic inactivation of the TP53 gene by mutations in BRCA1-related cancers, whatever the histological type and the organ of origin, and (ii) on the bench side, an original assay that allows to detect any TP53 mutation in plasma with exquisite sensitivity.

Methods:
CirCA01 is a national prospective study, opened in several cancer centers in France (Institut Curie, Gustave Roussy, Centre Léon Bérard, Centre Eugène Marquis) and funded by the French Ministry of Health (PHRCK1369250N). Inclusion criteria are: patients with no evidence of any invasive tumor mass at inclusion (clinical and, if any, radiological exams), carriers of known germline BRCA1 deleterious mutation (a personal history of cancer being not mandatory), age ≥ 30 years for patient with previous history of cancer or age ≥ 40 years for patient without previous history of cancer, written informed consent. Main exclusion criteria are: patient presenting with invasive tumor masses (e.g. stage IV cancer or localized cancer not yet surgically removed), carriers of germline BRCA1 variant of unknown significance, carriers of germline BRCA2 deleterious mutation or variant, individuals with a low risk of BRCA1-related tumor growth, i.e. women who underwent prophylactic bilateral mastectomy, annexectomy or contralateral prophylactic mastectomy after breast cancer. 200 BRCA1 mutation carriers will be enrolled and followed up regularly, as per national guideline for high-risk patients. Fresh plasma samples will be collected at each follow-up visit to the hospital and will be used to detect any mutation in TP53 exons and flanking non-translated regions by a "digital NGS"-based technique. (NCT registration ongoing).

Results: Correlation with patients outcome will allow to report whether TP53 ctDNA detection may be used as (i) a new screening test that can be repeated easily in BRCA1-carriers with no evidence of tumor growth (ii) a new diagnostic test in BRCA1-carriers who present a clinical or radiological abnormality (typically ACR class 3 mammograms): (A) diagnosis of the malignant vs benign nature of the abnormality, based on TP53 mutation levels, (B) differential diagnosis of a new cancer growth vs relapse of a previously treated cancer based on TP53 mutation characterization (different vs similar TP53 mutations).
Title: Phase II study on radiofrequency ablation in stage 0 and I breast cancer without extensive intraductal components

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Body: Background: Screening mammography makes it possible to identify small size of breast cancer (BC) and minimize surgical management. Previously we reported about a multi-center cohort study on radiofrequency ablation (RFA) in early breast cancer (ASCO2012 #1119). Although various devices and ablation procedure were attempted, 5-year’s recurrence-free survival of ipsilateral breast tumor was 96% in 425 cases of T1BC treated with RFA alone (the mean follow-up, 50 months). To validate complete pathological ablation in BC, we started a non-randomized phase II study in 2013 (UMIN000013836). Eligibility criteria: Unilateral BC patients with stage 0 (TisN0M0) or I (T1N0M0 or T1N1miM0) are eligible. Written informed consent is obtained. Tumor diameter of 2cm or less should be diagnosed by ultrasound and MR mammography. BC with diffuse calcification, extensive intraductal components, or multiple tumors is excluded. Methods: RFA is performed by Cool-tip RF ablation system (Covidien, USA). The needle electrode is inserted into the center of referent tumor under ultrasound guidance. The first ablation is started at an initial electrical power level of 5W for 1 min and the power is increased at 10W for 1 min. After then, it is increased in steps of 10W from every minute until rapid elevation of tissue impedance. The second ablation is allowed by physician’s discretion. One month later, ablated tissue is collected by core needle biopsy or vacuum-assisted breast biopsy. Cell viability is examined by central review of independent pathologists using tumor specimens stained with hematoxylin–eosin and nicotinamide adenine dinucleotide (NADH) diaphorase. In case of complete ablation, breast irradiation and adjuvant therapy will be performed. In case of incomplete ablation, partial mastectomy should be recommended. Aims: The primary endpoint is complete ablation rate. The secondary endpoints are deformity after RFA, relapse-free survival and overall survival for 10 years. Two-step design is used for statistical evaluation. Finally, 32 patients will be needed. Present accrual: As of April 2014, 16 patients were enrolled. One patient was ineligible because of macrometastasis in a sentinel node. Of 13 eligible patients who underwent pathological examination, one patient had viable cancer cells with NADH diaphorase staining. We also investigate optimal histological examination of cell viability and adequate image diagnosis after RFA.
Title: Will chest wall and regional nodal radiotherapy post mastectomy or the addition of regional nodal radiotherapy to breast radiotherapy post lumpectomy reduce the rate of invasive cancer events in patients with positive axillary nodes who convert to ypN0 after neoadjuvant chemotherapy? NSABP B-51/RTOG 1304 a phase III trial

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Body: Background
This phase III randomized post-neoadjuvant chemotherapy trial will evaluate if chest wall and regional nodal XRT (CWRNRT) after mastectomy or whole breast irradiation (WBI) with RNRT after breast-conserving surgery significantly reduces the rate of events for invasive breast cancer recurrence-free interval (ICBR-FI) in patients who present with histologically positive axillary nodes but become histologically negative axillary nodes after neoadjuvant chemotherapy. Secondary aims are OS, LRR-FI, DRFI, DFS-DCIS, and second primary cancer. HYPOTHESIS: Can We Use Tumor and Nodal Response to Neoadjuvant Chemotherapy in Order to Individualize the Use of L-R XRT?
Correlative science will examine the effect of RT by tumor subtype, molecular predictors of outcome for patients with residual disease, and the development of predictors of degree of reduction in loco-regional recurrence.

Methods
Eligible patients with clinical T1-3, N1 breast cancer with pathologic axillary nodal involvement (positive FNA or core needle biopsy) must complete ≥12 weeks of neoadjuvant chemotherapy (anthracycline and/or taxane-based regimen). HER2-positive patients must receive neoadjuvant trastuzumab or other anti-HER2 therapy. After neoadjuvant chemotherapy either breast-conserving surgery or mastectomy will be performed. At the time of surgery, all removed axillary nodes must be histologically free from cancer. 3 or more histologically negative sentinel nodes are acceptable to determine axillary nodal involvement. ER/PR and HER-2 neu status before neoadjuvant chemotherapy is required. All patients will receive additional required systemic therapy.
Site radiation credentialing with a facility questionnaire and case benchmarking is required. Randomization for mastectomy patients will be to no CWRNRT or CWRNRT and for breast-conserving surgery patients to WBI or WBI RNRT.

Statistical Considerations
1636 patients will be enrolled over 5 years with definitive analysis at 7.5 years. The study is powered at 80% to test the main hypothesis that RT reduces the annual hazard rate of events for IBCR-FI by 35% for an absolute risk reduction in the 5-year cumulative rate of 4.6%. Analysis will be on intent-to-treat with 3 formal interim analyses at 43, 86, and 129 events, with a 4th/final analysis at 172 events. Current accrual is 37. (as of 6-10-14)
736 enrolled patients will be evaluated with targeted patient-reported outcome instruments focusing on the effect of RT. Patient assessments will be prior to randomization and then at 3, 6, 12, and 24 months.

Contact Information
Study protocol information can be found under the protocol-specific web page on the CTSU member web site https://www.ctsu.org. For protocol-specific questions contact – NRG Oncology Pittsburgh Clinical Coordinating Department. Phone: 1-800-477-7227. Email: ccd@nsabp.org. All investigators will enroll patients by accessing OPEN at https://open.ctsu.org or from the OPEN tab on the CTSU members’ side of the web site.

Support: NCI PHS U10-CA-12027, -69651, -37377, -69974, and -2166.
Title: HIOB trial - Hypofractionated whole-breast irradiation preceded by intraoperative radiotherapy with electrons as anticipated boost

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Body: ClinicalTrials.gov Identifier: NCT01343459

Brief background discussion:
The commonly accepted standard fractionation schedule for whole breast irradiation (WBI) consists of 25 fractionations with single fractional doses of 2 Gy, resulting in cumulative doses of 50 Gy to the whole breast. Hypofractionation trials from UK and Canada demonstrated a similar outcome in local control and cosmesis compared to conventional fractionation. The combination of IOERT boost and hypofractionation was not yet evaluated.

Trial design:
Prospective one-armed multi-center trial - ISIORT 01

Eligible patients are treated with hypofractionated whole breast irradiation (WBI) of 40.5 Gy in 2.7 Gy per fraction for 15 days, preceded by an intraoperative electron boost (IOERT) to the tumor bed of 10 Gy (90% isodose) during breast conserving surgery.

Eligibility criteria:
Inclusion criteria:
Invasive breast cancer
Age 35 years and older
T1/T2, N1/N2, G1 - G3
Hormone receptor and Her 2 status: no limitations

Exclusion criteria:
Non-invasive breast cancer
Age less than 35 years
T3/T4, N2/N3
Neoadjuvant chemotherapy

Aim of the study:
To assess the effectiveness of IOERT in combination with hypofractionated WBI in terms of in-breast tumor control and cosmetic outcome, by matching or exceeding the best published results for annual LR rates in 3 different age groups in reference to an upper limit (exceeding = inferiority) and a lower limit (undershooting = superiority) which were estimated on the basis of the existing literature as follows:

Age 35 – 40 years:
Upper limit (best published evidence): 1.2 % (EORTC 22881- Boost trial)
Lower limit (commonly expected dimension): 0.72 % (CONSORT trial)

Age 41 – 50 years:
Upper limit (best published evidence): 1.2 % (EORTC 22881- Boost trial)
Lower limit (commonly expected dimension): 0.72 % (CONSORT trial)

Age over 50 years:
Upper limit (best published evidence): 0.7 % (EORTC 22881- Boost trial)
Lower limit (commonly expected dimension): 0.4 % (START B trial)

Primary endpoint:
Proof of superiority or iso-effectiveness.

Secondary endpoints:
Acute toxicity (CTC- toxicity scoring system) and late toxicity (LENT SOMA scoring system), cosmetic results (Van Limbergen - Score), DFS, OS.
Statistical methods:
Sequential Probability Ratio Test (SPRT):
As a consequence of best published analyses for this patient selection, stopping rules for annual local recurrence rates are defined as follows:
Age group 35-40: H0: p1 <= 0.72% vs H1: p1 > 0.72% with p2 = 4.0%
Age group 41-50: H0: p1 <= 0.72% vs H1: p1 > 0.72% with p2 = 2.4%
Age group 51+: H0: p1 <= 0.4% vs H1: p1 > 0.4% with p2 = 1.4%
H0-Hypothesis means no superiority to standard regimen.
H1-Hypothesis means superiority of HIOB against best published evidence.
Present accrual and target accrual:
Start date: January 2011
Estimated enrollment: 1500 patients
542 patients recruited by March 2014
10 international institutions recruiting.
Title: Fulvestrant alone versus fulvestrant and everolimus versus fulvestrant, everolimus and anastrozole: A Phase III randomized, placebo-controlled trial in postmenopausal patients with hormone-receptor-positive stage IV breast cancer: SWOG-Clinical Trials Initiative (CTI)* S1222

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Body: BACKGROUND: The median survival for women with hormone-receptor-positive (HR+) stage IV metastatic breast cancer (MBC) is 36 months. Strategies to improve outcome for postmenopausal women, include combinations of aromatase inhibitors (AIs) with either the estrogen receptor downregulator fulvestrant, or with the PI3kinase/AKT/mTOR signal inhibitor everolimus, as this pathway has been implicated as a mediator of resistance to endocrine therapies. However, fulvestrant 500 mg has not been tested in combination with everolimus alone, or in combination with everolimus and an AI.

SPECIFIC AIMS/TRIAL DESIGN: S1222 (NCT02137837) is a randomized phase III double-blinded, placebo-controlled clinical trial and is currently accruing. Patients receive fulvestrant (500 mg IM every 2 weeks for 3 doses, then monthly) on all arms, everolimus 10 PO daily (Arms 2 and 3, placebo Arm 1), and anastrozole 1 mg daily (Arm 3), or placebo (Arms 2 and 1). The co-primary objectives are to compare progression-free survival (PFS) between fulvestrant and everolimus (Arm 2) vs. fulvestrant (Arm 1), and fulvestrant, everolimus, and anastrozole (Arm 3) vs. fulvestrant (Arm 1). Additional objectives include comparison of PFS between Arms 2 and 3, and overall survival (OS), response and clinical benefit rates, toxicities, feasibility, compliance, and OS among the study arms.

Translational research goals are to test molecular determinants of response in circulating tumor cells (CTCs), assess a previously piloted CTC-Endocrine Therapy Index (CTC ETI), perform CTC-Next Generation Sequencing Analysis (CTC-NGS), and NGS on cancer tissue and germ line DNA.

ELIGIBILITY CRITERIA: Postmenopausal women with histologically confirmed de novo or newly relapsed stage IV MBC, with HER2-negative and HR-positive (≥1% positive nuclear staining) features, with either measurable, or evaluable disease are eligible. Previous neoadjuvant/adjuvant therapy allowed, but adjuvant anti-HR therapy must have been completed ≥ 12 months prior to enrollment.

STATISTICAL METHODS/TARGET ACCRUAL: This is a parallel randomized design with equal allocation to the three arms: (1) fulvestrant + placebo for everolimus + placebo for anastrozole; (2) fulvestrant + everolimus + placebo for anastrozole; (3) fulvestrant, everolimus, and anastrozole. PFS is the primary outcome. There are two coprimary hypotheses: (1) Arm 2 versus Arm 1; and (2) Arm 3 versus Arm 1, each will be tested at α = 0.025 (2-sided). Arm 2 versus Arm 3 is a secondary comparison. Sample size computations are based on accrual of 33 patients per month for 24 months resulting in a computed accrual total of 792. We estimate that the final analysis will occur 24 months after the last patient is accrued, an average followup of 36 months at the time of the final analysis with an expected trial duration of 4 years.

*SWOG-CTI manages the non-federally funded components of SWOG under The Hope Foundation. The study is supported by AstraZeneca and Novartis Pharmaceuticals Corp.
2014 San Antonio Breast Cancer Symposium

**Publication Number:** OT2-1-02

**Average Grade:** 6.20

**Title:** A phase 2 study of neoadjuvant goserelin and letrozole for premenopausal women with estrogen receptor positive HER2 negative stage 2 and 3 breast cancer

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**Body:**

**Background:** More than half of premenopausal women with breast cancer have tumors that are estrogen receptor (ER) positive. Despite this, there has been limited utilization of neoadjuvant endocrine therapy in that setting.

**Trial Design:** A Phase 2 study in premenopausal women (Age>18) with tumor size>2cm and locally advanced tumor, ER positive (Allred 6-8), HER2 negative, breast cancer who are not considered candidates for breast conservation surgery (BCS) or are borderline for BCS. Patients will be treated with a LHRH-A in combination with letrozole for a period of 4 weeks. At the end of 4 weeks, patients will undergo biopsy of the tumor for Ki67 assessment along with a serum estradiol (E2) level to confirm adequate hormone suppression.

Patients will then fall into one of 3 groups:

1. Postmenopausal (E2<10pg/ml) and Ki67 ≤ 10%
   These patients will remain on letrozole for 16-18 weeks followed by definitive surgery. Patients with a PEPI-0 and continued estradiol suppression will be offered surgical oophorectomy, continued letrozole and no chemotherapy (CT).

2. Postmenopausal (E2<10pg/ml) and Ki67>10%
   These patients will be designated endocrine therapy resistant and be offered neoadjuvant CT. Patients who decline neoadjuvant CT should be offered immediate surgery.

3. Premenopausal (E2 ≥ 10pg/ml)
   There is a failure of the LHRH-A to fully suppress estradiol levels at one month, treatment decisions will be individualized.

**Specific aims**

1. To determine the pathological complete response (pCR) rate to CT in patients with a Ki67>10% at one month, despite a fully suppressed estradiol level.

2. To determine the PEPI-0 rate in patients whose estradiol is fully suppressed at both 4 weeks and 16 weeks and whose 4 week tumor Ki67<10%.

3. In patients with a PEPI-0 tumor to determine the acceptability of management with surgical oophorectomy with continued oral letrozole and no CT.

**Statistical methods:**

The study design was chosen assuming the expectation for the efficacy of neoadjuvant CT in premenopausal women with ER+ breast cancer is low, with a pCR rate in the range of 5%. Our hypothesis is that patients with tumors resistant to endocrine therapy will be enriched for sensitivity to CT to a level typical of patients with ER negative disease (20%). Thus, the trial was designed so that at a 0.1 significance level, there would be a 90% chance of detecting a pCR rate >20% when the true pCR rate >5%. A 90% binomial confidence interval (CI) for the true pCR rate will be constructed. We anticipate approximately 20% of patients will have a 4 week Ki67 > 10%. A sample of 235 eligible patients will be required to obtain 35 with a 4-week post treatment Ki67 value >10 % and estradiol level <10 pg/ml who are willing to switch to neoadjuvant CT.

We anticipate that 75% of patients will have a 4-week Ki67 <10% along with estradiol <10pg/ml. Based upon the sample size needed, there would be 175 patients who complete 16 weeks of neoadjuvant endocrine therapy. We estimate at least 20% of the patients with a Ki67 <10% at 4 weeks will be in the PEPI 0, Stage 0/1 group after surgery. A 90% binomial CI will be constructed for the proportion of these who choose to forego CT.

**Accrual:** Open June 2014. Target=235.
Title: A pilot and phase II study of entinostat and anastrozole/tamoxifen in women with triple negative breast cancer (TNBC) to evaluate biomarkers and surrogates for response

Saranya Chumsri¹, Gauri Sabnis¹, Nancy Tait¹, Jane Lewis¹, Emily Bellavance¹, Susan Kesmodel¹, Steven Feigenberg¹, Katherine RH Tkaczuk¹, Peter Ordentlich³, Martin J Edelman² and Angela H Brodie¹. ¹University of Maryland Greenebaum Cancer Center, Baltimore, MD; ²University of New Mexico Cancer Center, Albuquerque, NM and ³Syndax Pharmaceuticals, Inc, Waltham, MA.

Body: Background: Triple negative breast cancer (TNBC) is an aggressive form of breast cancer that lack ER, PR, and HER2. TNBC is inherently resistant to endocrine therapy (ET) such as aromatase inhibitor (AI) and tamoxifen. Previous studies suggested that the loss of ER/PR expression in TNBC is due to epigenetic silencing. We have demonstrated histone deacetylase inhibitor (HDACi), entinostat, can induce expression of functional ER and render TNBC sensitive to ET both in vitro and in vivo (Sabnis et al. Cancer Research 2011). Furthermore, the combination of entinostat and AI can induce prolonged tumor regression and significantly reduce lung metastasis in vivo.

Trial Design: This is a single arm phase I/II study of entinostat in combination with ET. There are 2 cohorts in this study: cohort 1 – operable stage I-II TNBC, cohort 2 – metastatic and unresectable locally advanced TNBC. For ET, anastrozole will be used in postmenopausal women and tamoxifen in premenopausal women. The treatment regimen is: entinostat started on day 1 followed by ET on day 4. In cohort 1, paraffin embedded tissue from core needle biopsy and surgical specimens will be collected. In cohort 2, biopsies before and after 15-29 days of treatment are required.

Eligibility Criteria: Female age ≥ 18 with ECOG PS ≤ 2 and TNBC defined as ER and PR < 1% with no HER2 overexpression. Cohort 1 includes pts with operable stage I-II and cohort 2 includes pts with metastatic and unresectable locally advanced with ≤ 2 prior lines of chemotherapy who have accessible tumors for biopsies, excluding bone only metastasis.

Specific Aims: To determine ER expression as well as the percentage change in proliferation index (Ki67) after treatment. Moreover, safety and tolerability of the combination given either in neoadjuvant or metastatic setting will be evaluated. Response is defined as ≥ 50% reduction in Ki67 AND ≥ 1% ER-positivity OR pathologic complete response in the post-treatment surgical specimens.

Statistical Methods: For the pilot phase, a 3+3 cohort design is employed. The Simon’s two-stage design is used in the phase II. The total sample size for the phase II is 32 with 12 and 20 pts in the first and second stage respectively. The study will be terminated if there is no response among the first 12 pts. If ≥ 1 response in these 12 pts is observed, then 20 more pts will be accrued. The combination will be considered promising, if ≥ 4 out of 32 pts have a response. This design yields 87% power. The probability of early stopping and declaring that this combination has no sufficient activity is 0.54, if the true success rate is 5% and 0.07 if the true response rate is 20%. If the true proportion of pts with Ki67 reduction combined with ER up-regulation is 0.20, the probability of concluding that the drug has sufficient activity is 0.87 and 0.07 if the true proportion is 0.05. To allow about 10% inevaluable, the phase II trial will accrue 35 eligible pts. Therefore, the total target accrual is 41 pts.

To date, there are a total of 7 pts enrolled. Accrual is currently ongoing. Please contact ntait@umm.edu for further information.
**Title:** Alliance A011106: ALTernate approaches for clinical stage II or III Estrogen Receptor positive breast cancer NeoAdjuvant TrEatment (ALTERNATE) in postmenopausal women: A phase III study

Cynthia X Ma, Vera Suman, A Marilyn Leitch, Souzan Sanati, Katherine DeSchryver, Gary W Unzeitig, Paul Haluska, Mark Watson, Olwen Hahn, Jo Anne Zujewski, Kelly Hunt, Eric P Winer, Cliff A Hudis and Matthew J Ellis.

**Body:**

Neoadjuvant endocrine therapy (ET) in patients (pts) with locally advanced estrogen receptor positive (ER+) breast cancer (BC) improves breast conservation rate, and importantly offers an opportunity for individualized assessment of tumor responsiveness to ET to guide subsequent treatment. Previous neoadjuvant ET trials demonstrated that pathologic tumor size (pT), axillary lymph node status (N), and tumor Ki67 value at surgery predicted risk of relapse. Preoperative endocrine prognostic index (PEPI) was therefore developed to assign risk scores based on these factors. Modified PEPI 0, defined by pT1-2 N0 Ki67< 2.7%, was associated with extremely low risk of recurrence without adjuvant chemotherapy. In addition, high Ki67 post 2-4 weeks (wks) of neoadjuvant ET identified resistant tumors with poor outcome.

ALTERNATE trial is a three-arm phase III neoadjuvant/adjuvant ET trial designed to achieve two primary objectives. The first is to prospectively validate that modified PEPI 0 predicts > 95% relapse-free survival (RFS). The second is to determine whether fulvestrant (F), or fulvestrant (F) plus anastrozole (A), is superior to A in inducing a higher rate of modified PEPI 0. Secondary objectives include assessing RFS for pts with endocrine resistant tumor, defined by Ki67 > 10% at 4 or 12 wk, disease progression, or modified PEPI non-0, and pathologic responses of resistant tumors to neoadjuvant chemotherapy. Correlative studies include degrees of Ki67 suppression and ER level post treatment. In addition, ongoing research sequencing studies of DNA and RNA will be performed to contrast sensitive vs resistant tumors.

During the first phase of the trial, 1200 pts are randomized 1:1:1 to the F, A or F/A. This provides an 82% chance, 1-sided alpha 0.025 chi-square test to detect at least 10% difference in modified PEPI 0 rate comparing F or F/A with A. While waiting for result of analysis, the A arm will continue for the 2nd phase enrollment. Only the F-containing arm(s) superior over A will be continued to the 2nd phase.

During the 2nd phase, an additional 540 pts in each arm is estimated to obtain 317 pts with PEPI score 0. This will have a 90% chance, with a one-sided alpha=0.025 nonparametric Brookmeyer-Crowley type one sample survival test, rejecting that 5 year RFS rate is 95%. The maximum sample size is 2820 pts.

Eligible pts include postmenopausal women with newly diagnosed clinical stage II or III ER+ (Allred score 6-8) HER2- BC. Tumor biopsy is required at baseline, 4 wk, and surgery for central Ki67 analysis. Treatment decision is individualized based on 4 wk Ki67 and modified PEPI score. Pts with Ki67 > 10% at 4 wk are switched to chemotherapy. Pts with a modified PEPI score of 0 are recommended not to receive chemotherapy, but continue the assigned ET for 1.5 years followed by 3 years of A.

This trial is currently accruing pts through CTSU. This research is supported in part by NCI BIQSFP, and grants from Breast Cancer Research Foundation, Genentech, and the Investigator-Sponsored Study Program of AstraZeneca. The Clinical Trials.gov Identifier: NCT01953588.
Title: Prospective multicenter study evaluating the effect of impaired tamoxifen metabolization on efficacy in breast cancer patients receiving tamoxifen in the neo-adjuvant or metastatic setting - The CYPTAM-BRUT 2 trial

Kathleen Van Asten\(^1\), Lynn Jongen\(^1\), Anne-Sophie Dieudonné\(^1\), Annelenee Lintermans\(^1\), Chantal Blomme\(^2\), Olivier Brouckaert\(^2\), Diether Lambrechts\(^3\), Hans Wildiers\(^4\), Marie-Rose Christiaens\(^5\), Dirk Timmerman\(^2\), Ben Van Calster\(^6\), Jan Decloedt\(^7\), Patrick Berteloot\(^8\), Didier Verhoeven\(^9\), Markus Joergert\(^10\), Khalil Zaman\(^11\), Vincent Dezentjé\(^12\) and Patrick Neven\(^2\).

\(^1\)KU Leuven, Oncology, Leuven, Belgium; \(^2\)University Hospitals Leuven, Leuven, Belgium; \(^3\)KU Leuven, Laboratory of Translational Genetics, Leuven, Belgium; \(^4\)University Hospitals Leuven, Leuven, Belgium; \(^5\)University Hospitals Leuven, Leuven, Belgium; \(^6\)KU Leuven, Development and Regeneration, Leuven, Belgium; \(^7\)AZ Sint-Blasius, Gynecology, Dendermonde, Belgium; \(^8\)AZ Sint-Maarten, Gynecology and Obstetrics, Duffel, Belgium; \(^9\)AZ Klinia, Medical Oncology, Brasschaat, Belgium; \(^10\)Cantonal Hospital, St-Gallen, Switzerland; \(^11\)University Hospital CHUV, Breast Center, Lausanne, Switzerland and \(^12\)Leiden University Medical Center, Leiden, Netherlands.

Body: Background
Tamoxifen is commonly used to treat and prevent hormone receptor positive breast cancers. This drug is metabolized into more active metabolites by liver enzymes such as cytochrome P450 (CYP) enzymes. Endoxifen is considered to be the principal active metabolite of tamoxifen. As CYP enzymes are highly polymorphic in humans, endoxifen plasma levels are modulated by the patient’s genotype. It, however, is not yet clear if lowered endoxifen plasma levels have an effect on tamoxifen efficacy. This is the first prospective study where the association between endoxifen plasma concentrations, multiple CYP-genotypes and clinical outcome in postmenopausal patients treated with tamoxifen is investigated.

Trial Design
CYPTAM-BRUT 2 is a prospective multi-center open label, single-arm, non-randomized observational study. Postmenopausal women with measurable, estrogen receptor positive breast cancer receiving tamoxifen as neo-adjuvant or as first-line metastatic treatment are included in this study. The objective treatment response and clinical benefit are observed to investigate the efficacy of 20 mg tamoxifen daily. Patients are allowed to have started tamoxifen before inclusion but not more than three months. Further, if more than twelve months have passed after completion of the adjuvant therapy prior endocrine therapy in the adjuvant setting is allowed. Patients receiving neo-adjuvant tamoxifen will be assessed no more than four months after starting with tamoxifen.

The primary endpoint is a statistical association between steady-state endoxifen plasma concentrations and the objective response rate (ORR) after 3-6 months of tamoxifen, under the assumption that the relationship is linear with an odds ratio (OR) of 1.49 per 10 nmol/L. Using available data on endoxifen concentrations, this OR is chosen to reflect an improvement from 10% ORR in the lowest endoxifen quartile to 30% in the highest endoxifen quartile when the overall ORR is around 18%. To have 90% power at a 5% significance level, 180 patients have to be included into the study. The main secondary study endpoint is the relation between endoxifen plasma concentrations and clinical benefit (CR+PR+SD at 6 months). The study has to include 270 patients to detect a statistically significant association with endoxifen with 90% power at a 5% significance level, assuming an OR of 1.28 per 10 nmol/L. This OR is chosen to reflect an improvement of clinical benefit at 6 months from 30% in the lowest endoxifen quartile to 50% in the highest endoxifen quartile (overall clinical benefit around 39%). For both endpoints the RECIST criteria are used. Other endpoints are progression-free survival, tolerability of tamoxifen treatment and the association between CYP2D6 genotype and clinical outcome.

Patient accrual
Patients from 22 participating centers in Belgium and Switzerland are included in this trial. In May 2014, the predefined sample size of 270 patients was reached. Follow-up of the last patients will continue until all required data are obtained (i.e blood samples and response evaluation).
Title: E2112: A randomized phase III trial of endocrine therapy plus entinostat/placebo in patients with hormone receptor-positive advanced breast cancer

Roisin M Connolly1, Fengmin Zhao2, Kathy D Miller3, Amye J Tevaarwerk4, Lynne I Wagner5, Min-Jung Lee6, Judy Murray1, Robert Gray2, Richard L Piekarsz7, Jo Anne A Zujewski7 and Joseph A Sparano8. 1Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD; 2Dana-Farber Cancer Institute, Boston, MA; 3Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; 4University of Wisconsin Carbone Cancer Center, Madison, WI; 5Northwestern University Feinberg School of Medicine, Chicago, IL; 6National Cancer Institute, Bethesda, MD and 7Cancer Therapy Evaluation Program (CTEP), National Cancer Institute, Bethesda, MD.

Body: Background:
Epigenetic alterations in the genome, including abnormal DNA methylation and histone hypoacetylation, initiate and promote cancerous changes. A potential mechanism of resistance to endocrine therapy in breast cancer involves changes in gene expression secondary to epigenetic modifications, which might be modulated with the use of histone deacetylase (HDAC) inhibitors such as entinostat. Results from ENCORE 301, a phase II study evaluating the addition of entinostat to the steroidal aromatase inhibitor (AI) exemestane in patients with hormone receptor (HR)-positive advanced breast cancer who had experienced disease progression on a non-steroidal AI (NSAI), showed a significant improvement in progression-free survival (PFS), the primary endpoint, and overall survival (OS), an exploratory endpoint. Based on the OS results, entinostat has been designated a Breakthrough Therapy by the FDA when used in combination with exemestane in HR-positive advanced breast cancer.

Trial design:
E2112 is a multicenter randomized double-blind placebo-controlled phase III study (NCT02115282) enrolling patients with advanced HR-positive, HER2-negative breast cancer who have experienced disease progression on a NSAI (n=600). Patients will receive exemestane 25mg po daily and entinostat/placebo 5mg po every week (28 day cycle). Staging every 12 weeks.

Eligibility Criteria:
Postmenopausal women and men (≥ 18 years), ECOG performance status 0-1, locally advanced/metastatic invasive adenocarcinoma of the breast: ER/PR-positive, HER2-negative, measurable or non-measurable (20% cap) disease. Disease progression after NSAI use in the metastatic setting OR relapse while on or within ≤ 12 months of end of adjuvant NSAI therapy. Prior CDK inhibitor or everolimus permitted, but not exemestane or fulvestrant. One prior chemo permitted in metastatic setting.

Specific Aims:
Both PFS (defined by central review) and OS are primary endpoints, and the study is designed to show an improvement in either PFS or OS. Secondary endpoints include: Safety and tolerability, objective response rate, changes in lysine acetylation status in peripheral blood mononuclear cells, patient-reported symptom burden and treatment toxicities, adherence.

Statistical Methods:
One-sided type 1 error 0.025 split between two hypotheses tests: 0.001 for PFS test and 0.024 for OS. PFS is tested in the first 360 pts, 88.5% power to detect 42% reduction in the hazard of PFS failure (median PFS 4.1 to 7.1 months); OS is tested in all 600 pts, 80% power to detect 25% reduction in the hazard of death (median OS 22 to 29.3 months)

Present and Targeted Accrual: This study was activated in March 2014 and accrual is anticipated to complete in 40 months.

Contact Person: Dr. Roisin Connolly, Email: rconnol2@jhmi.edu

Funding: ECOG-ACRIN Cancer Research Group (ECOG-ACRIN), Cancer Therapy Evaluation Program (CTEP) at the National Cancer Institute, and Syndax Pharmaceuticals.
Title: A phase II study with orteronel as monotherapy in patients with androgen receptor (AR) expressing metastatic breast cancer (MBC)

Denise A Yardley¹, Suzanne F Jones², Nancy W Peacock³, Mythili Shastry², Robyn R Young⁴, Andre M Kallab⁵ and Howard A Burris III¹. ¹Sarah Cannon Research Institute and Tennessee Oncology, PLLC, Nashville, TN; ²Sarah Cannon Research Institute, Nashville, TN; ³Tennessee Oncology, PLLC, Nashville, TN; ⁴Center for Cancer and Blood Disorders, Fort Worth, TX and ⁵Northeast Georgia Medical Center, Gainesville, GA.

Body: Background: Androgen Receptor (AR) signaling is a new target present in and under evaluation in all breast cancer subtypes. AR expression is noted in 70%-90% of primary breast tumors, up to 75% of all breast cancer metastases and is associated with resistance to endocrine therapy. Orteronel, an oral, selective, nonsteroidal inhibitor of androgen synthesis, is being developed as an endocrine therapy for hormone-sensitive cancers. In preclinical studies, orteronel suppresses sex hormone levels in blood and hormone-dependent malignant tissue. This study will evaluate the safety and efficacy of orteronel in the treatment of AR+ MBC.

Study Objectives: This phase II trial is designed to determine the response rate and disease control rate (CR+PR+SD at 6 mo) following treatment with orteronel in pts with AR+ MBC. Two groups will be evaluated: Cohort 1 will include pts with AR+, triple negative (TN) (ER-/PR-/HER2-) MBC, and Cohort 2 will include pts with AR+, ER+ and/or PR+ MBC (may be HER2+ or HER2-). Secondary objectives include evaluation of PFS and OS, safety and tolerability, as well as evaluation of changes in serum levels of total and free testosterone, sex hormone binding globulins (SHBG), adrenocorticotropic hormone (ACTH), dehydroepiandrosterone sulfate (DHEA-S), cortisol and estradiol during orteronel treatment. As an exploratory objective, archived tumor tissue will be analyzed for the presence of phosphatidylinositol 3-kinase (PIK3CA) mutations and loss of phosphatase and tensin homolog (PTEN).

Eligibility: Pts ≥18 years with AR+ (≥10% staining by immunohistochemistry) MBC, either TN or ER+ and/or PR+, are eligible. TNBC pts must have had 1-3 prior chemotherapy regimens in the advanced setting and ER+ and/or PR+ pts must have had 1-3 prior hormonal therapies and ≥1 chemotherapy regimen in the advanced setting. HER2+ pts must have received ≥2 lines of HER2-directed therapies. Additional eligibility criteria include: ECOG PS 0-2; adequate bone marrow and organ function, including left ventricular ejection fraction of ≥50%.

Trial Design: Six pts will be enrolled and treated with orteronel in the lead-in phase to confirm safety and tolerability. In the absence of any safety concerns, subsequent pts will be enrolled to either Cohort 1 or Cohort 2 (described above). Cohort 1 will contain 31 pts with AR+ TNBC and Cohort 2 will contain 55 pts with AR+ ER+ and/or PR+ MBC. All pts will receive 300 mg orteronel PO BID over a 4 week cycle. Pts will be evaluated with CT scans every 2 cycles and treatment will continue until disease progression or unacceptable toxicity. Response rate and disease control rate will be presented as the point estimate along with 95% confidence interals calculated using both asymptotic normal approximation and exact binomial methods. Simon’s two-stage design will be applied using alpha=0.10 and power=0.80 for each cohort. Blood samples will be collected at baseline, on Cycle 2 Day 1, Cycle 4 Day 1, and at the end of treatment to evaluate serum total and free testosterone, SHBG, ACTH, DHEA-S, cortisol, and estradiol levels. Archival tumor samples will be collected for exploratory PTEN and PIK3CA biomarker evaluations. This trial is currently enrolling and has a total accrual goal of 86 pts.
**Title:** A phase II open label study of everolimus in combination with anti-estrogen therapy in hormone receptor-positive HER2-negative advanced breast cancer

Denise A Yardley\(^1\), Mythili Shastry\(^2\), Laura M DeBusk\(^2\), Howard A Burris III\(^1\) and John D Hainsworth\(^1\). \(^1\)Sarah Cannon Research Institute and Tennessee Oncology, PLLC, Nashville, TN and \(^2\)Sarah Cannon Research Institute, Nashville, TN.

**Body:** **Background:** Hormone receptor positive (HR+) breast cancer, defined as estrogen receptor and/or progesterone receptor positive, accounts for 70% of invasive breast cancers. While most HR+ tumors initially respond to anti-estrogen therapy, resistance, linked to crosstalk between cell signaling and signal transduction pathways, subsequently develops in almost 100%. Preclinical data has shown that the PI3K/Akt/mammalian target of rapamycin (mTOR) pathway is activated in long-term estrogen-deprived and aromatase inhibitor-resistant breast cancer cells. It is hypothesized that in this group of endocrine-resistant patients (pts), resistance to anti-estrogen therapy is driven by the PI3K/Akt/mTOR pathway and hence the inhibition of this pathway may reverse resistance. This study will evaluate the efficacy of adding everolimus (an inhibitor of mTOR) to anti-estrogen therapy in pts with HR+ metastatic breast cancer (MBC) who have progressed on anti-estrogen therapy.

**Study Objectives:** This multi-center, open-label Phase II trial is designed to determine the efficacy (primary endpoint is PFS), safety and tolerability of everolimus in combination with anti-estrogen therapy in pts with HR+, HER2-negative MBC with disease progression on prior anti-estrogen therapy. An exploratory objective of this study is to evaluate the prognostic value of the VeriStrat assay in this population. VeriStrat testing has been used to predict treatment efficacy in clinical trials of non small cell lung cancer, head and neck squamous carcinoma, and MBC.

**Eligibility:** Pts \(\geq\) 18 years with HR+, HER2-negative breast cancer that is either unresectable, locally recurrent, or metastatic are eligible. Pts must have progressed on anti-estrogen therapy (recurrence while on/within 12 months of treatment for early stage breast cancer, or progressed while on/within 1 month of treatment for advanced/MBC), but anti-estrogen therapy is not required to be the last therapy prior to enrollment. No washout for anti-estrogen therapy is required, and \(\leq\) 1 previous chemotherapy regimen is allowed. Post menopausal or pre-/ peri-menopausal women on tamoxifen are eligible and ovarian function suppression is permitted. Prior treatment with a mTOR inhibitor is not allowed. Additional eligibility requirements include: measurable or evaluable disease, ECOG \(\leq\) 2, adequate bone marrow and organ function and an adequate lipid profile (fasting serum cholesterol \(\leq\) 300 mg/dL or \(\leq\) 7.75 mmol/L and fasting triglyceride \(\leq\) 2.5 x ULN).

**Trial Design:** Everolimus will be administered at a dose of 10 mg PO daily in combination with the FDA prescribed doses of the most recent anti-estrogen therapy on which the pt progressed. Treatment cycles will be 4 weeks, with response evaluations by CT scans every 2 cycles. Pts will be treated until disease progression or unacceptable toxicity occurs. With an alpha=0.5, a sample size of 42 will provide 80% power to detect improvement in median PFS. Blood samples for the VeriStrat assay will be collected at baseline, on Cycle 3 Day 1, at disease progression and at the first follow-up visit post disease progression. Archival tumor tissue samples will be collected for comprehensive genomic profiling. This trial has a total accrual goal of 46 pts.
**Title:** tnAcity: A phase II/III trial of nab-paclitaxel (nab-P) plus either gemcitabine (Gem) or carboplatin (Carbo) vs Gem/Carbo as first-line treatment for patients with triple-negative metastatic breast cancer (TNMBC)

Denise A Yardley, Robert E Coleman, Pierfranco Conte, Joyce O'Shaughnessy, Javier Cortes, Stefan Glück, Adam Brufsky, Jean-Marc A Nabholtz, Li Li, JulieAnn Miller, Debora Barton, Nadia Harbeck and on behalf of the tnAcity Investigators.

**Body:** Background: TNMBC has an aggressive clinical course and poor prognosis. In phase II trials, nab-P–based therapy has demonstrated efficacy and tolerability as first-line therapy, in patients with MBC. The international triple-negative Albumin-bound paclitaxel combination treatment study (tnAcity) will evaluate 2 nab-P regimens (with either Gem or Carbo) and compare the selected regimen with Gem/Carbo as first-line treatment for TNMBC.

**Trial design:** All patients must have pathologically confirmed TNBC (TN defined as < 1% estrogen receptor and progesterone receptor expression by immunohistochemistry (IHC) and 0 - 1+ human epidermal growth factor receptor 2 expression by IHC or confirmed negative by fluorescence in situ hybridization) with measurable metastatic disease. Eligibility criteria include no prior cytotoxic therapy for MBC, prior adjuvant or neoadjuvant anthracycline use unless not indicated by physician or other appropriate regimens were administered, and no history or current evidence of brain metastasis. Prior neoadjuvant or adjuvant chemotherapy must have been completed ≥ 6 months before randomization (≥ 12 months if using taxane-, Gem-, or platinum-containing regimen). Patients will be enrolled internationally in North America, Europe, Latin America, and Australia. In the phase II portion, 240 patients will be randomized to receive nab-P 125 mg/m² plus Gem 1000 mg/m² (arm A), nab-P 125 mg/m² plus Carbo area under the curve (AUC) 2 (arm B), or the control regimen of Gem 1000 mg/m² plus Carbo AUC 2 (arm C), all given intravenously on days 1 and 8 of a 21-day cycle. The phase II portion will identify the nab-P combination to be evaluated in the phase III portion based on efficacy and safety (Table 1). In the phase III portion, 550 patients will receive the selected nab-P regimen (A or B) or Gem/Carbo. The primary endpoint of the phase III portion is progression-free survival (PFS) by independent radiological assessment; secondary endpoints include overall response rate (ORR), overall survival (OS), disease control rate, duration of response, and safety (Table 1). Monotherapy is allowed in the event of hypersensitivity or toxicity to 1 of the treatment components. The trial design provides ≈ 90% power to detect a hazard ratio of 0.70 for PFS with a 2-sided 5% significant level. Current enrollment is 52 patients. Clinical trial NCT01881230.

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**Key Endpoints**

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Title: Phase I/II clinical trial on the combination of carboplatin, eribulin and E7449

Virginia Kaklamani¹, Ruth o’Regan², Kari Wisinski³, Kent Hoskins⁴, James Wade⁵ and William Gradishar¹. ¹Northwestern University, Chicago, IL; ²Emory University, Atlanta, GA; ³University of Wisconsin, Madison, WI; ⁴UIC, Chicago, IL and ⁵CancerCare Specialists of Central Illinois, Dacatur, IL.

Body: The combination of carboplatin and eribulin has been found to be effective in triple negative breast cancer patients. A recently conducted neoadjuvant clinical trial showed a pathologic complete response (pCR) of 45% with four cycle of carboplatin and eribulin. Recent preclinical data from EISAI suggest that there is synergy between carboplatin, eribulin and E7449 (an oral PARP inhibitor) in BRCA deficient and PTEN mutated xenograft models.

Loss of Heterozygosity (LOH) is DNA alterations which develop through double strand DNA breaks. The homologous recombination (HRD) Score is calculated by the number of LOH regions greater 15Mb in length and less than an entire chromosome present in the genome. A high HRD score is present in BRCA deficient tumors and predicts for sensitivity to platinum-based chemotherapy. We propose a phase I/II clinical trial on the combination of carboplatin, eribulin and E7449. As part of the phase I clinical trial HRD will be performed and correlated to response to therapy. In the phase II clinical trial women with BRCA related breast or ovarian cancer, triple negative breast or ovarian cancer with high HRD score or PTEN deficient will be enrolled at a dose level found during the phase I clinical trial. Tissue will be requested to test for PARP inhibition. The primary endpoint of the phase II clinical trial is response rate. A total of 30 patients will be enrolled to the phase II clinical trial.
2014 San Antonio Breast Cancer Symposium

Publication Number: OT2-2-03
Average Grade: 5.75

Title: MERIBEL study: Efficacy of eribulin in first line of taxane-resistant patients with HER2 negative metastatic breast cancer

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Body: Brief background discussion:
Eribulin is a nontaxane microtubule dynamics inhibitor. It has shown a significant benefit in overall survival in patients with metastatic breast cancer (MBC) that underwent treatments in multiple previous lines (Cortes et al. 2009). However, little is known about the activity of the drug in patients with taxane-resistant early advanced disease. This trial is designed to determine the safety and efficacy of Eribulin as single first line agent in the treatment of patients with HER2(-) MBC deemed resistant to taxanes.

Trial design:
This study is a Phase IIa, multicenter, open-label, single-arm in patients with HER2 (-) metastatic breast cancer clinical trial. The scheme consists in the administration of 1.23 mg/m2 of Eribulin (equivalent to 1.4mg/m2 eribulin mesylate) as single agent the days 1 and 8 of each 21 days of the cycle, until progression of illness, unacceptable toxicity level or investigator’s criteria. The study was approved by the competent authorities and Ethics committees from 14 participating centers in Spain and Portugal.

Eligibility criteria:
The principal inclusion and exclusion criteria were: (1) Patients with MBC that have not received prior chemotherapy for Advanced disease; (2) Previous chemotherapy for early stages (I-IIIB) including taxanes (paclitaxel and/or docetaxel) for at least 4 cycles; (3) Less than 36 months* between the last cycle with taxanes and metastatic relapse; (4) Evaluable disease under RECIST criteria; (5) Absence of HER2 over-expression (IHQ of 0/1 or negative FISH/CISH); (6) Absence of neurotoxicity > G1. (* Amended to 48 months (Llombart et al. ASCO 2013).

Specific aims:
The main objective is to determine the efficacy of Eribulin as single first line agent in the treatment of patients with HER2-negative MBC and taxanes resistant criteria. Secondary objectives are: Estimate the tolerance to the treatment; assess the clinical response of the study treatment by RECIST criteria; correlate hormone receptor status (+/-) with clinical response and tolerability; identify the relationship between presence of visceral disease and response to therapy.

Statistical Methods:
The primary endpoint is time to progression (TTP). Secondary endpoints include the safety of treatment, clinical benefit rate, objective response, progression-free survival and overall survival. A total of 60 patients were predefined for the primary outcome. The sample size is based on a one-arm survival design. We test the null hypothesis that true TTP is 3.7 versus the alternative hypothesis (H1) that TTP is 5.5 months. An interim analysis with 30 patients completed has been planned. The study will be stopped for futility or efficacy if conditional power is less than 20% or more than 80%, respectively. Type I and II error assumed were 5% one-sided and 16%, respectively.

Present accrual:
Currently, 16 women have been recruited for the trial since study start in July 2013. The expected end of accrual will be on March 2015.

Contact information for people with a specific interest in the trial:
Maria Campos (maria.campos@medsir.org)
Scientific director
Medica Scientia Innovation Research (MedSIR ARO).
A randomized phase II neoadjuvant study of sequential eribulin followed by FAC/FEC-regimen compared to sequential paclitaxel followed by FAC/FEC-regimen in patients with operable breast cancer not overexpressing HER-2

Ricardo H Alvarez1, Nuhad K Ibrahim1, Joe Ensor1, Kimberly Koenig1, Mariana Chavez-MacGregor1, Ana M Gonzalez-Angulo1, Jill Schwartz Gomez1, Aurora G Madrigal1, Savitri Krishnamurthy1, Abigail S Caudle1, Simona F Shaitelman1, Gary J Whitman1, Lee J Murray1, Daniel J Booser1, David Cox2, James M Reuben1 and Vicente Valero1. 1University of Texas MD Anderson Cancer Center, Houston, TX and 2Eisai Inc, Woodcliff Lake, NJ.

Body: Background:
PST is a major component of the multidisciplinary management for locally advanced and operable breast cancer. The sequence of a taxane- followed by anthracycline-based regimen has been our standard of care for almost 20 years. Eribulin is a non-taxane, synthetic analogue of halichondrin B with distinct mechanism of action as microtubule dynamics inhibitor.

Trial Design:
Randomized, single institution, open-label, phase II study. Patients received four cycles of eribulin 1.4 mg/m² on day 1 and 8 every 21 days or 12 weekly doses of paclitaxel 80 mg/m². All patients received 4 cycles of standard doses of FAC/FEC every 21 days.

Eligibility:
Men or women 18 years of age or older with histologically confirmed primary invasive adenocarcinoma of the breast, clinical T2-T3, N0-3, M0, HER2-negative. No prior treatment for invasive carcinoma. Baseline MUGA or echocardiogram scans with LVEF of > 50%. Normal hematology, liver and kidney function laboratory studies.

Research Hypothesis:
Eribulin followed by FAC/FEC-regimen would demonstrate higher pathological complete response (pCR) rate relative to paclitaxel followed by FAC/FEC-regimen as PST for woman with operable breast cancer.

Specific Aims:
Primary objective: To estimate the pCR rate of eribulin followed by FAC/FEC-regimen versus paclitaxel followed by FAC/FEC-regimen. Secondary objectives: To evaluate the safety and rate of breast conservation surgery of eribulin followed by FAC/FEC-regimen.

Exploratory analysis: To explore Hot Spot Mutation Analysis (HSMA) and Molecular Inversion Probes (MIP) arrays can differentially predict pCR to eribulin followed by FAC/FEC-regimen versus paclitaxel followed by FAC/FEC-regimen. To determine the effect of eribulin on the circulating tumor cells (CTCs) number and epithelial and /or EMT gene expression in peripheral blood.

Statistical Methods:
The efficacy endpoint is the pCR rate. Patients are electronically randomized in a 1:1 ratio into two groups. Group A will receive eribulin followed by FAC/FEC-regimen and group B will receive paclitaxel followed by FAC/FEC-regimen. A binomial superiority two-sample test for population proportions will be used to compare the pCR rates of the two groups. An interim futility analysis is planned for this two-stage trial after the endpoint has been evaluated for the first 46 patients. If the test statistic at the end of the first stage is less than -0.876, the trials will stop for futility; otherwise, the trial will continue until 152 patients are accrued. A test statistic greater than 1.272 at the end of the second stage implies that the pCR rate for group A is significantly better. These calculations assume: an alpha error of 0.10, 80% power, a treatment arm assignment fraction of 50%, and an alpha spending function based on the O’Brien-Fleming boundary.

Present Accrual and Target Accrual:
A total of 48 patients were accrued. Target accrual is 152 patients.
2014 San Antonio Breast Cancer Symposium

Publication Number: OT2-2-05
Average Grade: 7.60

Title: Metronomic eribulin in metastatic breast cancer

Pavani Chalasani², Livingston B Robert³, Thomas A Rado³, Vijayakrishna K Gadi¹, Thomas D Kummet⁴, Jennifer M Specht¹, Alison Stopeck² and Hannah M Linden¹. ¹University of Washington, Seattle Cancer Care Alliance, Seattle, WA; ²University of Arizona Cancer Center, Tucson, AZ; ³Columbia Basin Hematology and Oncology, Kennewick, WA and ⁴Olympic Medical Cancer Center, Sequim, WA.

Body: Introduction: Breast cancer is a common and curable malignancy. In the setting of late or early recurrence, improvement in therapy is indicated as overall survival (OS) has changed little over the past few decades. Eribulin mesylate is a non-taxane microtubule dynamics inhibitor FDA approved in salvage metastatic breast cancer (MBC) treatment based on improvements in OS. Eribulin mesylate is a synthetic analogue of halichondrin B, a product isolated from the marine sponge *Halichondria okadai*. Anti-tubulin therapy is an established cornerstone of chemotherapeutics for MBC. Work at our center and others has shown a favorable therapeutic index for weekly metronomic vinorelbine. The history of other effective and tolerable agents, including paclitaxel, capecitabine, and vinorelbine, suggests that a low dose metronomic schedule of eribulin could be effective, well tolerated and result in longer time to progression (TTP), and higher quality of life during therapy. In phase I studies, Eribulin showed activity at doses well below the maximally tolerated 1.4mg/m² given 2/3 weeks, suggesting a lower metronomic schedule would not compromise efficacy. A lower dose metronomic schedule will allow responding patients to remain on treatment, resulting in longer TTP and greater use of the drug in practice.

Study Design: This is an open-label, multi-center, phase II study of eribulin for patients with MBC. HER2 positive patients may enroll with concomitant use of trastuzumab. Patients will receive eribulin 0.9 mg/m² administered intravenously over 2 to 5 minutes on Days 1, 8 and 15 of a 28-day cycle. Treatment will be continued until disease progression, unacceptable toxicity or withdrawal of patient consent.

Eligibility Criteria: Patients with MBC whose disease has progressed following 1-6 prior regimens with prior exposure to a taxane, ECOG PS of 0 – 2, measurable disease per RECIST 1.1, normal marrow and organ function. Patients with known CNS metastases must have stable disease off steroids after treatment with surgery or radiation. Exclusion criteria: mild hepatic or renal impairment, grade > 2 peripheral neuropathy, significant cardiovascular impairment or other severe or uncontrolled medical disease or psychiatric or neurologic disorder.

Specific Aims: The primary aim is to increase progression free survival (PFS). We hypothesize that metronomic dosing of eribulin will result in PFS of 4-6 months.
Secondary aims include decreasing the frequency of alopecia to less than 50%, grade 3 or 4 neutropenia to less than 30% and sensory neuropathy (all grades) to less than 25% of subjects enrolled.
An exploratory aim is to assess the role of circulating endothelial cell precursors (CEPs) and apoptotic circulating endothelial cells in predicting early response to treatment.

Statistical Methods: PFS will be measured as the time from study enrollment until the earliest date of disease progression or death. The sample size of n=60 has 99% power for the lower bound of the confidence interval to be greater than 2.2 months (the median PFS in the physician’s discretion arm of the EMBRACE trial), assuming that the true median PFS is 4 months, comparable to or an incremental improvement over the median PFS for the eribulin arm of the EMBRACE trial (3.7 months). Currently 12 of 60 expected enrollments have occurred.
Title: Phase II single arm study of liposomal doxorubicin (D), bevacizumab (A), and temsirolimus (T) for treatment of triple negative early breast cancer refractory to standard neoadjuvant chemotherapy


University of Texas MD Anderson Cancer Center, Houston, TX and University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Historically, triple negative breast cancers (TNBCs) were categorized into an ill-defined group that lacked estrogen receptors, progesterone receptors, and overexpression of human epidermal growth factor receptor 2. When TNBCs are treated with standard neoadjuvant chemotherapy (NAC), a pathologic complete response (pCR) is achieved in only 30-50% of the time. In an effort to improve outcomes for chemotherapy - refractory disease, emerging subtypes of TNBC have been identified through advances in molecular profiling leading to the discovery of distinct molecular aberrations which may be targeted. For example, mesenchymal-like TNBCs are a subset with aberrations that activate the PI3K/AKT/mTOR axis. Similar to mesenchymal-like, metaplastic breast cancers are commonly triple negative, refractory to standard therapy, and also harbor a high rate of molecular aberrations that lead to activation of the PI3K pathway. As such, metaplastic tumors may act as a morphologically identified ‘surrogate of response’ to evaluate the activity of targeted therapy regimens in mesenchymal-like TNBC. Based upon favorable results noted in a phase I trial of liposomal doxorubicin, bevacizumab, and the mTOR inhibitor temsirolimus (DAT) in metastatic metaplastic tumors, we will conduct this single arm phase II trial with neoadjuvant DAT for chemotherapy-refractory mesenchymal-like TNBC.

Trial Design: At our institution, women with TNBC who are receiving NAC will be enrolled onto a triaging protocol, where they will be randomized in a 2:1 fashion to be evaluated by a microarray-based predictive signature to determine chemotherapy sensitivity and categorized as chemotherapy-sensitive (CS) or chemotherapy-insensitive (CI). Subsequently, patients will be treated with standard anthracycline-based chemotherapy (AC or FAC) for 4 cycles. Patients with CI tumors with <80% decrease in tumor size and those with CS tumors with <10% decrease in tumor size will be deemed non-responders. Mesenchymal-like non-responders will be recommended for this single arm phase II study of DAT. Eligible patients will receive D at a dose of 30mg/m2 IV on day 1, A at 15 mg/kg IV on Day 1, and T at 25mg IV on Days 1, 8, and 15 for 4 cycles.

Eligibility Criteria: Female; > 18 years of age; newly diagnosed stage I-III triple negative breast cancer; refractory to standard NAC; LVEF > 50% by Echocardiogram or MUGA; ECOG PS 0-1

Statistical Methods: The primary endpoint is pCR following therapy with DAT. Secondary objectives will include recurrence-free survival and overall survival. Using a Simon optimal two-stage design with alpha = beta = 10%, and then setting the threshold for an acceptable pCR rate at 20%, we would enroll 12 patients into the first stage. If we see 0 patients with pCR, we would stop the study after the first stage. If we see at least one patient with a pCR we will continue to enroll 25 more patients for a total of 37 patients. We would declare the treatment worthy of further study if we see at least 4 patients with pCR out of the 37 patients. This design has a 54% probability of early termination after the first stage if the true pCR probability is 5%.

Target Accrual: 37.
A phase II study of S-1 (14 days' administration followed by 7 days' rest) for metastatic breast cancer

Akira Hirano¹, Akinori Hattori¹, Kaoru Ogura¹, Hiroaki Inoue¹, Fumie Okubo¹, Mari Kamimura¹, Shiho Sakaguchi¹, Jun Kinoshita¹, Reiko Miyamoto², Norie Jibiki², Yoshihiko Naritaka¹ and Tadao Shimizu¹.

1Tokyo Women's Medical University, Medical Center East, Tokyo, Japan and 2Tokyo Women's Medical University, Yachiyo Medical Center, Yachiyo, Japan.

Body: Background
S-1, an orally administered fluorinated pyrimidine (FP), improves overall survival in the adjuvant setting of gastric and pancreatic cancer. It is also effective for metastatic breast cancer (Yamamoto et al. Anticancer Res 30:3827-32, 2010). Although S-1 is usually administered for 28 days followed by 14 days' rest, severe adverse events are often observed by Day 15. By altering the schedule into 14 days' administration followed by 7 days' rest, discontinuation of treatment can often be avoided in daily practice. This schedule maintains the same dose intensity. Equally, an anti-tumor effect and fewer adverse events are observed in 14 days' administration followed by 7 days' rest than in 28 days' administration followed by 14 days' rest for the treatment of squamous cell carcinoma of the head and neck (Tsukuda et al. Br J Cancer 93:884-889, 2005).

In order to reduce the occurrence and severity of adverse events of S-1, we paid attention to dose reduction according to the renal function of patients. S-1 is comprised of tegafur, 5-chloro-2, 4-dihydroxy-pyridine(CDHP) and potassium oxonate. CDHP enhances the serum concentration of 5-FU by inhibiting dihydropyrimidine dehydrogenase (DPD), and it is excreted by the kidneys. Therefore, an accumulation of DPD in patients with renal dysfunction escalates the serum concentration of 5-FU and induces more severe adverse events. Thus, the dosage of S-1 should be reduced according to renal function.

Trial design
This is a phase II trial to evaluate the efficacy and toxicity of S-1 (14 days' administration followed by 7 days' rest) for metastatic breast cancer. S-1 is administered orally twice daily at a dose of 40-60 mg depending on the BSA and creatinine-clearance from Days 1 to 14, which is followed by 7 days' rest. This treatment is repeated until disease progression.

Eligibility criteria
Patients with histologically confirmed breast cancer, and recurrent and/or metastatic disease are included. Patients with HER2-positive (IHC:3+ or FISH+) tumor are excluded. Patients within 2 lines of previous chemotherapy for metastatic disease are allowed. Eligible patients are aged between 20 years and 80 years with a performance status of 0 to 2 and adequate organ function.

Specific aims
The primary endpoint is the response rate, and the secondary endpoints are disease control rate, progression-free survival, time to failure, toxicities and feasibility.

Statistical methods
The sample size was calculated using the Simon method, with a type I error of 5% and a study power of 90%. The unpromising response rate was 15% and the promising rate was 42%. The required number of patients was estimated to be 23.

Target accrual
Accrual of 25 patients was planned to produce a minimum of 23 evaluable patients. Patient accrual started in June 2014.
Title: A Phase IIb study of ANG1005, a novel, brain-penetrant taxane derivative, in HER2+ breast cancer patients with brain metastases

Betty Lawrence¹, Veronica Burdusel¹, Dimitri Fitsialos², Sana Ghani¹, Vihra Iordanova¹ and Jean-Paul Castaigne¹. ¹Angiochem Inc, Montreal, QC, Canada and ²Integrated Therapeutic Solutions Inc, Toronto, ON, Canada.

Body: Background:
Brain metastases are diagnosed in 30-55% of women with HER2+ breast cancer and represent a significant cause of morbidity and mortality in this patient population. While systemic disease can be controlled with HER2 receptor-directed therapy, treatment options for the CNS disease are limited due to the inability of most anti-cancer agents to cross the blood brain barrier (BBB). ANG1005 is a novel, targeted taxane derivative, consisting of 3 paclitaxel molecules covalently linked to a 19 amino-acid peptide that targets the low-density lipoprotein receptor-related protein 1 (LRP-1) receptor, which is highly expressed on the surface of the BBB and on various neoplastic cells. Using the LRP-1 receptor-mediated pathway, ANG1005 can cross the BBB and enter the tumor cells, where paclitaxel is cleaved off to exert its anti-tumor activity.

Trial Design:
This is an open-label, multi-center Phase II study to evaluate the efficacy, safety and tolerability of ANG1005 in patients with HER2+ breast cancer and progressive/recurrent brain metastases following whole brain radiotherapy (WBRT). Approximately 40 patients will be enrolled in the study and will receive ANG1005 at a starting dose of 600 mg/m² by intravenous infusion every 3 weeks. MRI and CT scans will be performed at screening and after every 2 cycles (i.e., every 6 ± 2 weeks) during treatment according to CNS RECIST 1.1 and RECIST 1.1 for intracranial and extracranial tumor evaluation, respectively. The maximum treatment duration is 1 year.

Eligibility Criteria:
Eligible patients are adults (≥18 years old) with HER2+ breast cancer with recurrent/progressive brain metastases who have received prior WBRT. Patients must have at least one radiologically-confirmed metastatic brain lesion, measurable according to CNS RECIST 1.1. Patients must be neurologically stable and have adequate blood counts, organ and bone marrow function with a KPS score ≥80. Patients with leptomeningeal disease are excluded, as are patients who have been previously treated with ANG1005 or have known allergy to paclitaxel or its components.

Specific aims:
The primary endpoint is to evaluate the intracranial objective response rate (iORR). Additional endpoints include: median intracranial progression free survival (PFS), overall PFS at 3, 6 and 12 months, 6-month overall survival rate, intracranial ORR by 2-D modified RANO criteria, overall ORR, extracranial ORR and duration of response, plasma pharmacokinetic profile, and safety and tolerability.

Statistical Methods:
The primary objective is to determine the efficacy of ANG1005 as measured by iORR. Sample size calculation is based on Exact test statistical method with testing null hypothesis: iORR equals historical control iORR versus alternative hypothesis H1: iORR > historical control iORR with one sided alpha=0.05. A sample size of 40 has 92% power to reject iORR being 5%, assumed the study has an iORR of 20%.

Study Accrual:
Target accrual is 40 patients. The study is open for recruitment and currently no patients have been enrolled.

Contact Information:
For more information on this trial, please contact Betty Lawrence at Angiochem Inc. (blawrence@angiochem.com). Clinical trial information: NCT02048059.
Title: SGN-LIV1A: A phase 1 trial evaluating a novel antibody-drug conjugate in patients with LIV-1-positive breast cancer

Andres Forero\textsuperscript{1}, Howard Burris III\textsuperscript{2}, Patricia LoRusso\textsuperscript{3}, Jennifer Specht\textsuperscript{4}, Kathy Miller\textsuperscript{5}, Monica Mita\textsuperscript{6}, Minetta C Liu\textsuperscript{7}, Shanu Modi\textsuperscript{8}, Lajos Pusztai\textsuperscript{9}, Django Sussman\textsuperscript{10} and Ana Kostic\textsuperscript{10}. \textsuperscript{1}University of Alabama, Birmingham, AL; \textsuperscript{2}Sarah Cannon Research Institute, Nashville, TN; \textsuperscript{3}Karmanos Cancer Institute, Detroit, MI; \textsuperscript{4}Seattle Cancer Care Alliance, University of Washington, Seattle, WA; \textsuperscript{5}Indiana University Simon Cancer Center, Indianapolis, IN; \textsuperscript{6}Cedars-Sinai Medical Center, Los Angeles, CA; \textsuperscript{7}Mayo Clinic, Rochester, MN; \textsuperscript{8}Memorial Sloan Kettering Cancer Center, New York, NY; \textsuperscript{9}Yale University School of Medicine, New Haven, CT and \textsuperscript{10}Seattle Genetics, Inc, Bothell, WA.

Body: Background

First identified as an estrogen-inducible gene in a breast cancer cell line, LIV-1 is a multispan transmembrane protein of the solute-carrier family 39 with putative zinc transporter and metalloproteinase activity. As a downstream target of STAT3, it promotes the epithelial-to-mesenchymal transition that is important in the malignant progression to metastasis. LIV-1 is expressed in a number of cancers with the highest prevalence and level of expression in breast, prostate, and melanoma. Additionally, LIV-1 has been linked with malignant progression to metastasis and associated with lymph node involvement in breast cancer. Normal tissue expression is predominantly limited to hormonally regulated tissues, including breast and prostate. In metastatic breast cancer tissue samples, the current validated immunohistochemistry assay for LIV-1 has detected moderate to high expression in 82% of samples tested, with 88% positivity in hormone receptor-positive tumors and 73% in triple-negative tumors.

SGN-LIV1A is an antibody-drug conjugate (ADC) composed of a humanized anti-LIV-1 monoclonal antibody conjugated to the microtubule-disrupting agent monomethyl auristatin E (MMAE) via a protease-cleavable linker. In vitro, SGN-LIV1A shows target-specific internalization and cytotoxic activity against LIV-1-positive neoplastic cell lines. Significant dose-dependent tumor regression was demonstrated in mouse xenograft models.

Methods

The primary objective of this first-in-human phase 1, open label, multicenter study is to evaluate the safety and tolerability, and to identify the maximum tolerated dose of SGN-LIV1A using a 3+3 dose-escalation study design. Pharmacokinetics, immunogenicity, and antitumor activity will also be evaluated (ClinicalTrials.gov #NCT01969643). Enrollment to this US-based trial began in late 2013. Eligible patients are adult females who have hormone receptor-positive/HER2-negative or triple-negative metastatic breast cancer. Tumor tissue must be positive for expression of LIV-1 per central assessment. Patients must have received at least 2 prior cytotoxic regimens in the metastatic setting and have measurable disease per RECIST v1.1. Pre-existing neuropathy ≥ Grade 2 is not permitted.

SGN-LIV1A is administered intravenously every 3 weeks at protocol-defined doses starting at 0.5 mg/kg. Patients who achieve an objective response or stable disease per RECIST v1.1 are eligible to continue treatment until disease progression. At the completion of dose escalation, expansion cohorts may be opened to enroll patients with specific breast cancer subtypes to further define safety and antitumor activity. Tumor biopsies are being obtained at baseline and after one cycle of treatment to evaluate the role of LIV-1 expression, target saturation, and other tumor-specific measurements in the antitumor activity of SGN-LIV1A.
Title: A prospective study of glycoprotein 88 GP-88 blood test in healthy women with Gail model risk <=1.66 undergoing screening for breast cancer (BC) with mammography (MM)

Katherine HR Tkaczuk¹, Cristina Campassi¹, Susan Kesmodel¹, Emily Bellavance¹, John Olson¹, Elizabeth Nichols¹, Steven J Feigenberg¹, Binbin Yue², David Hicks² and Ginette Serrero². ¹University of Maryland Greenebaum Cancer Center, Baltimore, MD and ²A & G Pharmaceutical, Columbia, MD.

Body: Background: In the US, the majority of BC is diagnosed by screening XRAY mammography (MM) but up to ~20% of BC are undetected by MM. The development of reliable, blood-based BC screening test to increase the sensitivity and specificity of currently existing BC screening methods such as MM.

Rationale: GP88 is expressed & secreted by BC cells & is not expressed by normal mammary epithelial cells. Two retrospective randomized multi-site trials (one training study & one validating study with about 300 cases each) have demonstrated that elevated GP88 expression in ER+ IDC was statistically correlated with a 4-fold increase in the risk of 5-yr disease recurrence. Multivariate analysis showed that GP88 as a risk predictor was independent from PR expression, tumor size, grade, lymph node status & disease stage. The quantitative GP88 EIA developed to determine the amount of GP88 in biological fluids was developed at A&G. The assay is highly specific for GP88 & both sensitive & linear over a wide dynamic range, i.e. detection of GP88 concentrations from 0.1 to 20ng/ml. A baseline (28.4 ± 5 ng/ml) was established for healthy volunteers (HV). In BC pts a statistically significant increase of serum GP88 was seen for early stage pts (40.7 ± 16 ng/ml; p=0.007). Additionally, a stratification of pts according to their clinical outcomes shows that pts having no evidence of disease (NED) have serum GP88 levels within the range of HV. These data suggest that pts with breast tumors express & secrete high levels of GP88.

Objectives: 1. To determine prospectively GP-88 blood levels in HV at average risk of developing BC who are undergoing screening MM & in women with biopsy-confirmed BC. 2. To establish the statistical distribution of GP88 serum levels in these women by baseline BIRAD classification (1-6). 3. To determine if baseline GP88 level is predictive of change in BIRADS classification from baseline to 12-mos follow-up. 4. To determine if baseline GP88 level is predictive of the appearance of BC at 12 mos follow-up in HV who were cancer-free at baseline.

Inclusion Criteria: Female, aged >=40 yrs old, presenting to UM Breast Center for screening MM or for diagnostic MM or diagnostic workup and/or biopsy due to BIRADS 0 MM at an outside facility <= to 12 wks; Gail model assessment of risk of developing BC<=1.66.

Study procedures: Serum levels of GP88 in subjects with average BC risk factors will be measured prospectively at baseline; 3-6 mos & 6-12 mos. & correlated with BIRADS reading of the screening MM, BIRADS 1 or 2 (benign), BIRADS 3 (short term MM follow-up) & BIRADS 4 or 5 (suspicious and tissue diagnosis needed immediately). GP88 blood level will be correlated with pathologic results of breast biopsies performed on subjects with suspicious BIRADS (4 & 5) MM & final pathologically confirmed diagnosis of BC as BIRADS 6.

Statistical Considerations The total number of pts will be 725 & screened up to 1400 subjects. Study is UM IRB approved & just started accruing. Funding is provided by Maryland Industry Partnership Grant (MIPS)& Avon Grant No. 02-2013-018. Contact: Ntait@umm.edu.
Title: Testing BRCA 1/2 mutation using next generation sequencing (BRCANGS)

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Body: Testing BRCA 1/2 mutation is important for patients with breast cancer, and Sanger sequencing is a standard method to identify BRCA 1/2 mutation. Next generation sequencing (NGS) is a high-throughput parallel sequencing that can provide genetic information with high accuracy. NGS is a faster and cost-effective method to detect gene mutations compared to Sanger sequencing. This study is a prospective non-randomized observational study which evaluates the clinical role of NGS testing for BRCA 1/2 compared to Sanger sequencing. Eligibility criteria are women aged 19 to 80 years, patients with breast or ovarian cancer history in 2nd degree family members, male breast cancer, bilateral breast cancer, patient with breast cancer under 40 year of age, simultaneous breast and ovarian cancer, patients with epithelial ovarian cancer, and breast cancer with other simultaneous extramammary malignancy. Primary endpoint of the study is the overall accuracy of NGS compared to Sanger sequencing. Secondary endpoints are sensitivity, specificity, false negative, and false positive rates of NGS compared to Sanger method. Target accrual of the pilot study is 12 patients, and further extension of patients accrual is scheduled after the analysis of the pilot study. This study is approved by institutional review board of Severance Hospital. The study is conducted and planned by Prof. HS Park, Yonsei University College of Medicine, Seoul, Korea and supported by Korea Breast Cancer Foundation. ClinicalTrials.gov identifier is NCT02151747.
2014 San Antonio Breast Cancer Symposium

Publication Number: OT2-5-01
Average Grade: 4.60

Title: Brain metastases in breast cancer network Germany (BMBC, GBG 79): Multicentric, retro- and prospective collection of patient data and biomaterial from breast cancer patients with brain metastases

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Body: Background: The incidence of brain metastases in breast cancer patients is rising and has become a major clinical challenge in the last years. So far, limited therapeutic options and insights into the biology of brain metastases exist. Most reports include patients with brain metastases from different tumor entities and few studies so far analyzed exclusively patient data or tumor samples from breast cancer patients. In this context, an open, multicenter registry and biobank should be helpful for analysis of a large number of breast cancer patients with brain metastases. Therefore, we initiated the Brain Metastases in Breast Cancer Network Germany (BMBC; GBG79).

Trial design and eligibility criteria: Patients with brain metastases from breast cancer are eligible. Registration of patient data is allowed retrospectively and prospectively. To enable an easy access, an internet-based database was chosen ("MedCodes" by the German Breast Group).

Specific aims: Documented data cover incidence, symptoms, number and localization of brain metastases, histopathology, imaging methods, outcome, therapy, and quality of life measurement. Tissue of brain metastases and primary tumors will be collected for translational research projects. Planned analyses include treatment patterns in Germany, patient outcome, as well as validation of prognostic scoring systems in a multicenter setting and in the context of new targeted therapies. Translational research projects investigate the impact of glycosylation, resistance mechanisms against HER2-targeted therapies, role of the blood brain barrier, evaluation of markers of radioresistance and specific genomic alterations associated with brain tropism of breast cancer cells. The registry and biobank should be also open for cooperation with other groups.

Present accrual and target accrual: The accrual target is app. 1000 patients in the database and app. 400 tissues of brain metastases from German centers. The study was opened for documentation in April 2014 with more than 50 participating centers as of June 2014.
A phase 2 randomized, double-blind, placebo-controlled trial of hormone therapy ± radium-223 dichloride in HER2-negative hormone receptor–positive breast cancer patients with bone metastases

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Background: Bone metastases (mets) are frequent in patients (pts) with metastatic breast cancer (MBC). Radium-223 dichloride (Ra-223) is a first-in-class alpha-emitter that selectively targets bone mets, which in a phase 2a pilot study of advanced breast cancer pts with bone-dominant disease (n=23), reduced baseline bone biomarker levels with favorable safety (Coleman et al. Breast Cancer Res Treat 2014). This study will evaluate efficacy and safety of Ra-223 versus placebo (pbo) in HER2-negative hormone receptor–positive breast cancer pts with bone mets receiving single-agent hormone therapy.

Trial Design: Eligible pts will be randomized 1:1 to Ra-223 50 kBq/kg IV or matching pbo every 4 weeks for 6 cycles. Additionally, all pts will receive concurrent single-agent hormone therapy and best supportive care for the entire study. Randomization will be stratified by geographic region, previous lines of hormone therapy for MBC, and number of prior skeletal events. Pts will be assessed at each visit for efficacy and safety, and followed until occurrence of symptomatic skeletal event (SSE), radiologic progression, death, or withdrawal.

Main Eligibility Criteria: Eligible pts are pre- or postmenopausal women with histologically or cytologically confirmed estrogen receptor–positive, HER2-negative, bone-dominant MBC with ≥2 radiologically confirmed bone mets and ≥1 or 2 prior skeletal events (ie, external beam radiotherapy for bone pain, pathologic bone fracture, spinal cord compression, orthopedic surgery). Pts must have had ≥1 line of hormone therapy for MBC; be taking bisphosphonates or denosumab for ≥3 months prior to study entry; be eligible for endocrine treatment (one of selective estrogen receptor modulators [SERMs], nonsteroidal aromatase inhibitors [NSAIs], steroidal AIs, or estrogen receptor downregulators); and have radiologically evaluable disease using RECIST 1.1, asymptomatic or mildly symptomatic disease on Brief Pain Inventory, ECOG score 0-1, and adequate hematologic, renal, and liver function. Exclusion criteria include history or presence of visceral mets, history or presence of brain mets or leptomeningeal disease, prior or current need for chemotherapy for metastatic disease, and untreated spinal cord compression.

Endpoints: The primary endpoint is SSE-free survival. Secondary endpoints are radiologic progression-free survival (rPFS), overall survival, time to opiate use for cancer pain, time to pain progression, time to cytotoxic chemotherapy, and acute and long-term safety. Exploratory endpoints include time to first on-study SSE, bone biomarker assessments, bone-specific rPFS, resource utilization, and time to visceral mets onset.

Statistical Methods: Target enrollment is 227 pts. Time-to-event variables will be analyzed using a log-rank test, accounting for trial stratification factors. Kaplan-Meier estimates and survival curves will be presented for each treatment group. Safety analyses will be descriptive.

First patient first visit expected in Sept 2014.
Title: A two-cohort, phase II, cardiac safety study of pertuzumab, trastuzumab, and neoadjuvant anthracycline-based chemotherapy in patients with HER2-positive, locally advanced, inflammatory, or early breast cancer

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Body: Background: In HER2-positive early breast cancer (BC), neoadjuvant pertuzumab (P) + trastuzumab (T) + docetaxel (D) significantly increased pathologic complete response (pCR) rates compared with T+D (Gianni L, et al. Lancet Oncol 2012); high pCR rates were also seen with P+T in combination with anthracycline- and non-anthracycline-based regimens (Schneeweiss A, et al. Ann Oncol 2013). Neoadjuvant P+T + chemotherapy was well tolerated, with little increased toxicity associated with the addition of P. Although there was a higher rate of cardiac adverse events (AEs), left ventricular systolic dysfunction (LVSD), and left ventricular ejection fraction (LVEF) declines in P+T-containing arms compared with arms without P, these events were generally asymptomatic, reversible, and patients (pts) recovered with no sequelae. No data are available for neoadjuvant P-based regimens that include paclitaxel (Ptx) and dose-dense doxorubicin plus cyclophosphamide (ddAC), despite these being widely used in (neo)adjuvant BC therapy. This study will evaluate the cardiac safety of two anthracycline-based neoadjuvant regimens in combination with P+T. Following surgery, all pts will receive adjuvant P+T.

Trial design: In this non-randomized, open-label, phase II study, two parallel cohorts of pts receive different P-based neoadjuvant treatment regimens. In Cohort A, pts receive four 2-weekly cycles of ddAC, followed by 12 doses of weekly Ptx, plus four 3-weekly (q3w) cycles of P+T from the start of Ptx. In Cohort B, pts receive four q3w cycles of 5-fluorouracil, epirubicin, and cyclophosphamide, followed by four q3w cycles of D, plus four q3w cycles of P+T from the start of D. Following surgery, both cohorts receive 13 cycles of P+T.

Eligibility: Pts with HER2-positive, locally advanced, inflammatory, or early BC (size >2 cm or >5 mm and node-positive) are eligible if they have a baseline LVEF ≥55% and an ECOG performance status ≤1. Among the exclusion criteria are prior incisional biopsy or excision of the primary tumor, prior systemic or radiation therapy for cancer, and prior chemopreventive agents. Pts are also excluded if they have poorly controlled hypertension, angina requiring medication, a history of congestive heart failure, serious or uncontrolled cardiac arrhythmia requiring treatment, or myocardial infarction within 6 months prior to enrollment.

Aims: The primary objective is cardiac safety during the neoadjuvant period, assessed by incidence of symptomatic LVSD and LVEF changes. Secondary objectives include cardiac safety in the adjuvant and post-treatment periods, incidence of all AEs, pCR rate, event-free survival, invasive disease-free survival, and overall survival.

Statistical methods: No statistical hypothesis testing will be performed. Safety and efficacy results will be summarized descriptively. A sample size of 200 pts per cohort is expected to provide sufficient data to evaluate the cardiac safety of each regimen with acceptable precision (exact Clopper-Pearson 95% CIs) around the expected rates.

Accrual: The first patient in is planned for August 2014.
Title: A Phase 3 randomized, double-blind trial comparing PF-05280014 + docetaxel and carboplatin vs. trastuzumab + docetaxel and carboplatin for neoadjuvant treatment of operable HER2+ breast cancer

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Body: Background: PF-05280014 is being developed as a potential biosimilar to trastuzumab. PF-05280014 demonstrated similarity to trastuzumab in nonclinical evaluations. In a Phase I trial in healthy subjects, pharmacokinetic (PK) characteristics and safety profile of PF-05280014 were similar to those of trastuzumab. The goal of this Phase 3 trial is to demonstrate that the efficacy and safety of PF-05280014 + docetaxel and carboplatin are similar to those of trastuzumab sourced from the EU (trastuzumab-EU) + docetaxel and carboplatin in the neoadjuvant treatment of women with HER2-positive operable breast cancer.

Trial design: In this randomized, double-blind trial, subjects will be randomized (1:1) with stratification by primary tumor size (<5 cm or ≥5 cm), estrogen receptor (ER) status, and progesterone receptor (PR) status to PF-05280014 + docetaxel and carboplatin or trastuzumab-EU + docetaxel and carboplatin. PF-05280014 or trastuzumab (8 mg/kg in Cycle 1; 6 mg/kg thereafter over 90 min) will be administered followed by docetaxel (75 mg/m²) and carboplatin (target area under the curve [AUC]: 6 mg/mL/min; 30- to 60-minute infusion) every 3 weeks for 6 treatment cycles. The primary objective is to compare the percentages of patients with Cycle 5 Ctrough (trastuzumab serum trough concentration) >20 µg/mL in the neoadjuvant setting. Secondary objectives include measures of tumor control, safety, immunogenicity, PK, and to explore the relationship between drug exposure and pathologic complete response (pCR).

Eligibility criteria: Female subjects with known ER and PR status ≥18 years with confirmed HER2 overexpressing breast cancer and a plan for definitive surgical resection and neoadjuvant chemotherapy, Eastern Cooperative Oncology Group status 0 or 1, normal left ventricular ejection fraction and normal laboratory values are eligible. Key exclusion criteria are bilateral or inflammatory breast cancer; prior treatment, including chemotherapy, endocrine therapy, biologic therapy, radiation or surgery (except diagnostic biopsy); other concomitant active malignancy or history of malignancy in the past 5 years or presence of known distant metastases. All subjects must provide informed consent.

Specific aims: The goal of this Phase 3 trial is to demonstrate that PF-05280014 in combination with docetaxel and carboplatin has similarity in PK (trough level) and comparable efficacy and safety versus trastuzumab-EU + docetaxel and carboplatin in subjects with operable HER2-positive breast cancer in the neoadjuvant setting.

Statistical methods: This study tests whether percentage of subjects with steady state (Cycle 5) Ctrough >20 µg/mL of PF-05280014 is similar to that of trastuzumab-EU, using a noninferiority margin of -12.5% tested with α=0.025 (one-sided). Assuming the percentages of subjects reaching steady state is 95% with trastuzumab-EU and 93% with PF-05280014, 188 subjects (94/arm) will be needed to achieve 85% power.

Target accrual: 220 subjects.
Title: An open-label, multicentre, phase IIIb study with intravenous administration of pertuzumab, subcutaneous trastuzumab, and a taxane in patients with HER2-positive metastatic breast cancer (SAPPHIRE)

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Body: Background: The primary goals of treatment for patients (pts) with metastatic breast cancer (mBC) are maximising survival and preserving the quality of life. Intravenous (IV) trastuzumab has proven clinical benefits in pts with human epidermal growth factor receptor 2 (HER2)-positive mBC. Pertuzumab also targets HER2 through an independent epitope to that of trastuzumab. Addition of pertuzumab to the established combination of trastuzumab and docetaxel has shown improved efficacy with acceptable toxicity in mBC (Swain et al. Lancet Oncol 2013;14:461-71); they are considered standard of care. Subcutaneous (SC) and IV trastuzumab formulations have shown comparable efficacy but SC administration is preferred by pts for reducing duration of clinic visits (Pivot, et al. Lancet Oncol 2013;14: 962–970). The combination of IV pertuzumab and SC trastuzumab has not been studied. The aim is to assess safety, tolerability, and efficacy of combining IV pertuzumab with SC trastuzumab and a taxane, as first-line therapy in pts with HER2-positive mBC.

Trial Design: SAPPHIRE is an open-label, multicentre, Phase IIIb study. Pts will receive IV pertuzumab every 3 weeks with a loading dose of 840 mg and subsequent doses at 420 mg combined with SC trastuzumab at 600 mg/5 mL every 3 weeks and a taxane (docetaxel, paclitaxel, or nab-paclitaxel; regimen determined by the investigator). Treatment will continue until disease progression, unacceptable toxicity, or pts withdraw consent, whichever occurs first. The study is expected to run for 42 months.

Eligibility: Pts aged ≥18 years old with histologically or cytologically confirmed HER2-positive [immunohistochemistry (IHC) positive at 3+ or in situ hybridisation-positive (ISH+)] mBC with at least one measurable lesion and/or non-measurable disease according RECIST version 1.1 and ECOG performance status (PS) 0-2 are eligible.

Specific Aims: The primary objective is to assess the safety and tolerability of combining IV pertuzumab with SC trastuzumab and investigator’s choice taxane chemotherapy. The secondary objectives are to assess the efficacy of the first-line combination of pertuzumab, trastuzumab, and taxane chemotherapy and second-line treatments for mBC after disease progression.

Statistical Methods: Primary safety analyses will report incidence and severity of adverse events (AEs)/serious AEs, AEs leading to premature discontinuation of study treatment and evidence of cardiac dysfunction. Secondary safety analyses will include exposure and duration of study treatment, ECOG PS and laboratory data. Secondary efficacy analyses will report the overall response rate, progression-free survival, event-free survival and overall survival. Continuous data will be summarised using mean, median, range, standard deviation, and standard error. Discrete data will be summarised using frequency counts and percentages. Time-to-event analyses will be based on Kaplan-Meier methodology.

Accrual: The planned 50 pts will be recruited from 13 Australian Centres. The study started in December 2013 with 24 pts now enrolled. Clinicaltrials.gov#NCT02019277.
A phase 2 study of eribulin in combination with pertuzumab and trastuzumab for advanced or recurrent human epidermal growth factor receptor 2 (HER2)-positive breast cancer (SONG-02)

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Body: Background: Pertuzumab, an anti-HER2 humanized monoclonal antibody that inhibits receptor dimerization, has a mechanism of action that is complementary to that of trastuzumab, and the combination of pertuzumab plus trastuzumab plus taxanes, when used as first-line treatment for HER2-positive metastatic breast cancer, significantly prolonged progression-free survival (PFS). However, in the second and later line treatment of HER2-positive advanced or recurrent breast cancer, it has not settled whether it should be treated with pertuzumab plus trastuzumab plus chemotherapy or with trastuzumab plus chemotherapy. Eribulin mesylate is a non-taxane microtubule dynamics inhibitor that has been proved to prolong the overall survival of advanced or recurrent breast cancer patients compared with the treatment of physician's choice. The benefit of eribulin in combination with trastuzumab for patients with locally recurrent or metastatic HER2-positive breast cancer has been reported. However, the efficacy and safety of eribulin in combination with pertuzumab and trastuzumab for advanced or recurrent HER2-positive breast cancer patients has not been reported. The purpose of this study is to evaluate the efficacy and safety of eribulin in combination with pertuzumab and trastuzumab as second and later line therapy for patients with advanced or recurrent HER2-positive breast cancer.

Trial Design: This is a multicenter single arm phase 2 study for advanced or recurrent HER2-positive breast cancer patients who have experienced progression with anti-HER2 therapy. Patients received eribulin mesylate 1.4mg/m² administered via intravenous (IV) infusion over 2 to 5 minutes on Days 1 and 8 each 21-day cycle and pertuzumab 840mg/kg IV and trastuzumab 8mg/kg IV over 90 minutes on Day 1 of Cycle 1. Thereafter eribulin mesylate 1.4mg/m² and pertuzumab 420mg/kg and trastuzumab 6mg/kg was infused each 21-day cycle until disease progression or the appearance of toxic effects that could not be effectively managed. The primary endpoint is PFS of the combination therapy, based on local assessment of response using RECIST 1.1 criteria. Secondary endpoints are overall response rate (ORR), safety and tolerability. In addition, we examine PFS and safety according to the most recent treatment regimen. The study was conducted in accordance with the Declaration of Helsinki (2008), and the protocol and informed consent forms were submitted for approval to institutional review boards by the primary investigators. All patients provided written informed consent before undergoing any study-related procedures.

Statistical Method: All efficacy analyses were based primarily on the full analysis set (FAS), which included all patients who received over 1 dose(s) of study treatment. The PFS and ORR were calculated 95% confidence intervals (CIs). Treatment of 39 evaluable patients with the identified phase 2 doses will detect this difference with a power of 80% and alpha=5% (one-sided test). Accounting for a 10% invaluable rate and lead-in patients, a total of 43 patients will be enrolled on the study. Clinical trial information UMIN000014107.
Title: Phase I, open-label study evaluating the safety and tolerability of LJM716, BYL719 and trastuzumab in patients with metastatic HER2+ breast cancer

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Body: Background: Breast cancers that overexpress the human epidermal growth factor receptor 2 (HER2+) are driven by HER2/HER3/PI3K signaling. Single agent therapy against this pathway is only modestly effective due to redundant mechanisms of pathway activation and regulatory feedback loops. Drug combinations designed to directly inhibit multiple nodes simultaneously have proven synergistic in preclinical models. Single agents and doublets targeting HER2, HER3, and PI3K have thus far proven safe in clinical testing. Methods: We are conducting a phase I clinical trial of LJM716, a fully human monoclonal antibody against HER3, plus BYL719, a small molecule alpha-specific PI3K inhibitor, and trastuzumab, an approved human monoclonal antibody against HER2, in patients (pts) with HER2+ metastatic breast cancer (MBC). There will be dose-escalation and dose-expansion phases, with correlative serum and tumor tissue studies. The primary objective is to determine the MTD of BYL719 with fixed dose LJM716 and trastuzumab in pts with HER2+, PIK3CA mutated MBC; determination of MTD will utilize the Continual Reassessment Method (CRM). LJM716 will be administered at 20mg/kg and trastuzumab at 2mg/kg, both intravenously weekly; starting dose of BYL719 will be 250mg orally daily, with dose escalation to MTD. Secondary objectives are to describe safety (CTCAE 4.0) and preliminary efficacy (RECIST v1.1) of the combination and to describe immunogenicity of LJM716. Exploratory objectives are to assess pre-treatment (tx) genomic alterations predictive of response or resistance to the tx; to assess proteomic pharmacodynamics markers of effective HER2, HER3 and PI3K inhibition, and to correlate cell-free DNA mutant allele fraction with clinical response. For these correlative studies, all pts will undergo serial plasma collection for cell-free DNA, and 10 pts will undergo pre-tx, on-tx, and at-progression biopsies. Eligible pts for dose-escalation and expansion are required to have ECOG 0-1, HER2+ MBC (immunohistochemistry 3+ or in-situ hybridization ≥ 2.0), and prior treatment with pertuzumab and ado-trastuzumab emtansine. For dose-escalation, pts may have measurable or non-measurable disease and somatic PIK3CA mutations are required. For dose-expansion, pts must have measurable disease and there will be two cohorts: one for wild-type and the other for mutant PIK3CA tumors. Accrual beginning June 2014 will include an anticipated 18 pts for dose-escalation and 30 pts for the expansion. Contact modis@mskcc.org for further information.


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Title: A phase 2 single-arm study to evaluate the clinical activity, safety and tolerability of enzalutamide (ENZA) with trastuzumab in patients with advanced human growth factor receptor 2 (HER2)-positive breast cancer

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Body: Background
Androgen receptor (AR) expression is observed in ~60% of patients (pts) with HER2+ breast cancer1. In vitro, ENZA inhibits proliferation of AR+/HER2+ cell lines and enhances the activity of trastuzumab. ENZA also inhibits proliferation of trastuzumab resistant HER2 positive cells2.

Methods
Any amount of AR expression (local or central) is allowed, submission of tissue is mandatory. Patients must have measurable disease per RECIST 1.1, and have received 1 to 4 prior lines of anti-HER2 therapy in the advanced / metastatic setting. Brain imaging is required to exclude patients with CNS metastases. Pts with a seizure history are excluded.
Women with metastatic or locally advanced HER2+, AR+, and ER-/PgR- breast cancer will receive daily ENZA (160 mg) continuously and trastuzumab (6 mg/kg) administered every 21 days, until disease progression (NCT02091960). The primary endpoint (EP) is clinical benefit rate (CBR) where benefit is defined as complete or partial response (CR or PR) or stable disease (SD) ≥24 weeks according to RECIST 1.1 criteria. Additional EPs include safety and tolerability, and the relationship between AR expression and ENZA activity. If the CBR is ≥3 in 21 evaluable pts, the sample size will increase to 66 pts.

The primary EP will be analyzed in pts with centrally confirmed AR expression (≥10% nuclear staining by IHC), who have received at least one dose of ENZA, and have ≥1 post-baseline tumor assessment. The null hypothesis (H0), that the true CBR is 10%, will be tested against a 1-sided alternative. This Simon’s two-stage design yields 90% power when the true response rate is 25% with a 1-sided type 1 error rate of 5%. Enrollment is expected to continue through 2016.

References:
MotHER: A US registry for women with breast cancer who have received trastuzumab, pertuzumab in combination with trastuzumab, or trastuzumab emtansine (T-DM1) during pregnancy or within 7 months prior to conception

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Body: Background: Trastuzumab (Herceptin) and pertuzumab (Perjeta) are HER2-targeted monoclonal antibodies, and T-DM1 (Kadcyla) is a HER2-targeted antibody–drug conjugate. These molecules are classified as FDA Pregnancy Category D, indicating evidence of fetal harm; there have been postmarketing reports of oligohydramnios in women who received trastuzumab during pregnancy; in non-clinical studies of pertuzumab, oligohydramnios, delayed renal development, and embryo-fetal death occurred in pregnant cynomolgus monkeys. Furthermore, maytansine, the parent molecule of DM1, has demonstrated embryotoxicity in mice. The MotHER pregnancy registry was established in 2008 as an FDA postmarketing commitment to evaluate the effects of trastuzumab on pregnancy outcome. Pertuzumab and T-DM1 were included in the registry following their respective FDA approvals in 2012 and 2013.

Study design: MotHER is a US pregnancy registry and uses a prospective, observational cohort design. Enrollment is voluntary and can be initiated by the patient or her healthcare provider. Women are followed up until pregnancy outcome; infants are followed up through the first year of life.

Eligibility criteria: Pregnant women ≥18 years of age and resident in the US are eligible if they have received at least one dose of trastuzumab, pertuzumab in combination with trastuzumab, or T-DM1 for breast cancer during pregnancy or within 7 months prior to conception. Enrollment must be initiated before pregnancy outcome is known.

Specific aims: The primary outcome measures are pregnancy outcomes and the incidence of pregnancy complications, such as oligohydramnios, congenital anomalies, and fetal/infant functional deficits. Information on potential birth defects, noted either at birth or during pediatric follow-up, is evaluated and classified by a birth defect evaluator/clinical geneticist.

Statistical methods: The study is descriptive and not designed for formal hypothesis testing; event rates with 95% confidence intervals will be calculated.

Accrual: There is no pre-specified sample size. Data collection is ongoing, from 2009 to 2019 for trastuzumab, from 2012 to 2022 for pertuzumab plus trastuzumab, and from 2013 to 2023 for T-DM1. Fourteen women have enrolled as of January 31, 2014.
**Title:** A phase II, randomized study of T-DM1 versus T-DM1 plus short induction with docetaxel in first line treatment for locally advanced or metastatic HER2+ breast cancer (SOLTI-1203)

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**Body: Background:** This study is founded upon the hypothesis that the addition of a short induction phase with docetaxel to the treatment with T-DM1 can improve efficacy with respect to T-DM1 administered as a single agent, in patients with advanced HER2 positive breast cancer who have not received previous treatment for the advanced disease. The rational for this approach is based on the results of the phase II randomized study of T-DM1 versus docetaxel trastuzumab, in which the administration of T-DM1 to the patients resulted in a statistically significant and clinically relevant improved progression free survival (PFS). One important observation with respect to this comparative study is that, in the T-DM1 arm, a higher number of patients progressed during the first six months than in the docetaxel trastuzumab arm. The existence of T-DM1 resistant patients, who progress during the first 6 months, highlights the need to explore new combinations with cytotoxic agents to induce an early response.

**Study design:** This is a phase II, prospective, randomized, open label, research study with two groups designed to assess the efficacy of T-DM1 together with short induction with docetaxel (SI docetaxel) versus T-DM1monotherapy treatment, in patients recently diagnosed with locally advanced or metastatic, progressive or recurrent, HER2+ breast cancer, who have not received previous chemotherapy for the advanced disease. The randomization will be carried out at a 1:1 ratio to one of the following two groups: 1.Monotherapy treatment regimen with T-DM1 at a dose of 3.6 mg/kg every 3 weeks until treatment finalization, 2.T-DM1 at a dose of 3.6 mg/kg on Day 1 and every 3 weeks until treatment finalization, together with docetaxel 60 mg/m² on Day 1 and every 3 weeks for a total of 4 treatment cycles.

**Eligibility criteria:** Female adults recently diagnosed with progressive or recurrent, locally advanced or metastatic HER2+ breast cancer, who have not received previous chemotherapy for the advanced disease.

**Endpoints:** The primary endpoint is to compare the early efficacy of the combination of T-DM1 together with SI docetaxel versus monotherapy treatment with T-DM1 measured as the PFS rate at 4 months. Secondary endpoints include comparison of the ORR, CBR, DR and OS of the combination versus monotherapy treatment. Exploratory endpoints consist to assess if the PAM50 intrinsic subtypes (HER2-enriched in comparison with the rest) predict benefit of T-DM1 plus SI docetaxel or T-DM1 as monotherapy treatment, in terms of ORR and PFS.

**Statistical methods:** The considerations for the sample size are based on obtaining a statistical power of 80 % to be able to detect a difference in the rate of PFS at 4 months of 8% (83% in group A and 91% in group B), with a bilateral significance level of 5% and supposing a patient withdrawal rate of 10%. According to these calculations, the sample size is estimated at 236 patients. The analysis of the primary endpoint will be based on the Kaplan Meier estimate analysis.

**Target accrual:** Approximately 40 European sites from Spain, Portugal and Austria will be participating. The FPI is planned in October 2014.
Title: Combination immunotherapy with trastuzumab and the HER2 vaccine E75 (nelipepimut-S) in high-risk HER2+ breast cancer patients to prevent recurrence

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Body: Background: In an adjuvant phase II trial, the HER2-derived nelipepimut-S (E75) + GM-CSF vaccine (Neuvax) has been shown to reduce breast cancer recurrence. Preclinical testing of the combination of trastuzumab (Tz) and nelipepimut-S has shown synergistic cytolysis against HER2 expressing cancer cells. In pilot phase II data in HER2+ patients (pts), 55 pts dosed with CD8-eliciting HER2 derived peptide vaccines sequentially after treatment with Tz resulted in no recurrences at 36 months median follow-up compared with a 16% recurrence rate in 34 randomized controls treated with Tz without vaccine (p=.012). Based on these data, we have designed a trial to evaluate the ability of the combination of Tz and the E75 vaccine concurrently to prevent recurrence in pts with high-risk, HER2+ breast cancer.

Trial Design: This study will be a multicenter, prospective, randomized, single-blinded, phase II trial evaluating adjuvant Tz + NeuVax (E75+GM-CSF) vs. Tz + GM-CSF alone in high-risk HER2+ (IHC 3+ and/or FISH ≥2.2) breast cancer pts. High-risk pts include: 1) those that did not achieve a pathologic complete response (pCR) after neoadjuvant chemotherapy and HER2-targeted therapy or 2) those treated with upfront surgery that are node positive (≥ 4+ LN or 1-3+ LN if hormone receptor negative). Pts must be HLA-A2/A3+ to be eligible (E75 is HLA-A2/A3-restricted) with ECOG performance status 0-1. Pts will be enrolled after completing standard of care multi-modal therapy but prior to the 3rd dose of Tz maintenance therapy (monotherapy). Pts will be randomized 1:1 to receive either NeuVax or GM-CSF alone which will be administered as six monthly intradermal inoculations concurrently with Tz therapy. Pts will then receive four booster inoculations of either NeuVax or GM-CSF every 6 months. The primary efficacy endpoint is to compare disease-free survival (DFS) between treatment arms. Secondary objectives will include evaluation of local and systemic toxicity, distant recurrence free survival, and in vivo/in vitro immunologic responses. From previously published experience with Tz, we expect a recurrence rate of 20% in Tz (plus GM-CSF) treated pts and anticipate that the combination of Tz with E75+GM-CSF will reduce this recurrence rate to 5%. In order to show statistical difference between these recurrence rates, we plan to enroll 50 pts per treatment arm (100 total) with a type I error rate of 5% and 80% power to detect the primary endpoint. Trial accrual is anticipated to begin in September of 2014, with a two year period for trial enrollment followed by a three year follow-up period.

Conclusion: We hypothesize that combination adjuvant immunotherapy with Tz and NeuVax will result in a greater reduction in breast cancer recurrence than Tz therapy alone. We have designed a prospective, randomized, single-blinded, phase II trial evaluating the efficacy of this immunotherapy combination in high-risk HER2+ breast cancer pts to test this hypothesis.

Contact Information: This trial is funded by a DoD grant to EAM with matching funds from Galena Biopharma and is being conducted with the assistance of the academic CRO, Cancer InCITe, LLC.
**Title:** HERmione: A Phase 2, randomized, open label trial comparing MM-302 plus trastuzumab with physician’s choice of chemotherapy plus trastuzumab, in anthracycline naive HER2-positive, locally advanced/metastatic breast cancer, previously treated with pertuzumab and trastuzumab emtansine (T-DM1)

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**Body:** Background: Despite improvements in treatment with newly approved HER2-targeted therapies such as pertuzumab and trastuzumab emtansine (T-DM1), HER2-positive metastatic breast cancer (MBC) remains a serious and life-threatening disease. MM-302 is an antibody drug conjugated liposomal doxorubicin targeted to HER2. The present Phase 2 study was developed based upon data from a recently reported Phase 1 study (SABCS 2013, Munster, et al., #P4-12-29) in 47 heavily pretreated HER2-positive MBC patients treated with MM-302 alone or in combination with trastuzumab, with more benefit being seen in patients without prior anthracycline exposure. In that study, MM-302 appeared to have an acceptable safety profile and, importantly, no clinically significant declines in left ventricular ejection fraction (LVEF) were observed.

Trial design: HERmione is a randomized Phase 2, two-arm, open-label trial designed to evaluate if MM-302 can address an unmet medical need in treating anthracycline naive patients with HER2-positive metastatic breast cancer who have previously received pertuzumab and T-DM1 in the locally advanced or metastatic setting. Patients are randomly assigned 1:1 to MM-302 (30 mg/m², IV q3w) plus trastuzumab (6 mg/kg, IV q3w) or a chemotherapy of physician’s choice (chosen from vinorelbine, capecitabine, or gemcitabine) plus trastuzumab (6 mg/kg, IV q3w). Patients will be stratified according to geographic region, presence of visceral disease, and ≥ 4 prior chemotherapy-containing regimens for metastatic disease.

Eligibility criteria: HER2-positive locally advanced or MBC (HER2 confirmed by central lab); prior trastuzumab in any setting, prior pertuzumab and T-DM1 exposure in the incurable locally advanced or metastatic setting; unlimited prior lines of therapy; ECOG 0-1, and LVEF ≥ 50%. Patients with prior anthracycline exposure in any setting are excluded. Central nervous system metastases are permitted, if stable and without symptoms or steroids for 4 weeks after treatment. Patients with significant cardiac dysfunction or risk of cardiac dysfunction are excluded.

Specific aims: The primary efficacy endpoint is independently assessed progression free survival (PFS) with tumor response evaluated based on RECIST version 1.1. Secondary endpoints include Investigator Assessed PFS, Overall Survival (OS), 6 and 12 month OS rate, Time to Treatment Failure, Objective Response Rate, Duration of Response, Clinical Benefit Rate, safety and toxicity, Patient Related Outcomes and pharmacokinetic exposure. Safety assessments include all adverse events, abnormal laboratory values, cardiac events, and LVEF decline.

Statistical methods: A total sample size of 250 subjects will be enrolled to observe 191 PFS events for 90% power to detect a Hazard Ratio of 0.625. The MM-302 arm will be compared to the control arm on the primary endpoint of PFS using a stratified log-rank test at a one-sided 0.025 level.

Accrual status: Recruitment is planned to start in July 2014 at approximately 60 sites in the United States and Western Europe.
**Title:** IMPACT: IMaging PAtients for Cancer drug selecTion – Metastatic breast cancer (MBC)

Frederike Bensch¹, Adrienne Brouwers¹, Andor Glaudemans¹, Johan de Jong¹, Erik de Vries¹, Winette van de Graaf², Eline Boon², Wim Oyen², Lioe-Fee de Geus-Oei², Eric Visser², Eric van Helden³, Willemien Menke-van der Hoeven van Oordt³, Henk Verheul³, Otto Hoekstra³, Jim Janssen³, Marc Huisman³, Sjoerd Elias⁴, Carl Moons⁴, Liesbeth de Vries¹ and Carolien Schröder¹. ¹University Medical Center Groninge, Netherlands; ²Radboud University Medical Center Nijmegen, Netherlands; ³VU Medical Center, Amsterdam, Noord-Holland, Netherlands and ⁴University Medical Center, Netherlands.

**Body:**

**Background:** Therapy of newly identified MBC is largely based on estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status. Optimal receptor information should be up-to-date and preferably from the whole body, given receptor conversion over time and intra-patient tumor heterogeneity. Novel molecular imaging by means of $^{18}$F-fluoroestradiol (FES)- and $^{89}$Zr-trastuzumab-PET/CT is a non-invasive, patient friendly way to obtain such information. Comprehensive prospective data comparing novel molecular imaging, metastasis biopsy and blood biomarkers, are needed to assess clinical utility for optimal therapy guidance and response prediction.

**Trial design:** The IMPACT-MBC trial (NCT01957332), is a multicenter prospective cohort study, supported by the Dutch Cancer Society-Alpe d’HuZes, in which $n=200$ newly diagnosed MBC patients will be entered. Prior to start of treatment patients will undergo i) standard MBC work up including bone scan, diagnostic CT and $^{18}$F-fluorodeoxyglucose(FDG)-PET/CT, ii) a metastasis biopsy, for standard (immuno)pathology and DNA sequencing, iii) $^{18}$F-FES- and $^{89}$Zr-trastuzumab-PET/CT to assess whole-body metastatic ER and HER2 status, and iv) blood sampling (CTCs, ctDNA, germline DNA, $^{89}$Zr-radioactivity measurements).

Treatment advice will be based on standard work up and experimental PET scans. Tumor response is assessed by a 2 week $^{18}$F-FDG-PET/CT (experimental) and an 8 week diagnostic CT (standard; primary outcome).

**Eligibility criteria:** All newly diagnosed non-rapidly progressive MBC patients with measurable or clinical evaluable (bone only) disease can be enrolled, regardless of primary tumor ER and HER2 status. Patients should be eligible for systemic therapy, but not require immediate start of chemotherapy. A histological biopsy of a metastatic lesion should be safely obtainable. Excluded are pregnant or lactating women and patients with a prior allergic reaction to immunoglobulins.

**Specific aims:** i) To assess the (added) clinical utility of $^{18}$F-FES- and $^{89}$Zr-trastuzumab-PET/CT, in the setting of MBC at first presentation, in relation to other diagnostics, ii) to assess the relation of experimental $^{18}$F-FES-, $^{89}$Zr-trastuzumab- and 2 week $^{18}$F-FDG-PET/CT with (progression free) survival and iii) to assess the cost-effectiveness of the experimental PET/CT scans.

**Statistical methods:** IMPACT-MBC aims to model the predictive value of several tests (novel molecular imaging, biopsy and blood biomarkers) in combination, by means of multivariable regression-model based techniques, combined with state-of-the-art methods for estimating the added value of novel tests to existing information (e.g. NRI, IDI). All these analyses will be employed both on (predicting responsiveness on) a patient- and metastasis level.

**Present accrual and target accrual:** The IMPACT-MBC trial was opened for accrual at the University Medical Center (UMC) Groningen, in August 2013. Accrual rate is as anticipated 2-3 patients/month/center. The two other participating centers, Radboud MC Nijmegen and VUmc Amsterdam recently opened. It is anticipated that accrual of patients will be finalized in 2016.
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Title: MINT I: Multi-Institutional Neo-adjuvant Therapy, MammaPrint Project I

Charles E Cox¹, Peter Blumencranz², Ruben Saez³, Robert Wesolowski⁴, William Dooley⁵, Lisette Stork-Sloots⁶, Femke de Snoo⁶, Sarah Untch⁷ and Eli Avisar⁷. ¹University of South Florida, Tampa; ²Morton Plant Hospital, Clearwater; ³Plano Cancer Institute, Plano; ⁴Ohio State University; ⁵University of Oklahoma; ⁶Agendia Inc, Irvine, CA and ⁷Miller School of Medicine, University of Miami, Miami, FL.

Body: Background:
Women with locally advanced breast cancer (LABC) are often treated with neo-adjuvant chemotherapy to reduce the size of the tumor prior to surgery, to enable breast conserving surgery and to observe the clinical effect of therapy in real time. Studies have shown that the 25–27% of individuals who have a pathologic complete response (pCR) to neoadjuvant therapy have a survival advantage of 80% in 5 years, which is double the expected survival of the remaining patients without pCR. If patients who are likely to show a pCR could be identified prior to initiation of therapy, it would enable more informed treatment decisions – patients likely to respond would be served well by current neoadjuvant chemotherapy protocols, while those unlikely to respond may be better suited to innovative new strategies for drug discovery [von Minckwitz et al. JCO 2006]. Genomic assays, which are widely used to provide prognostic and predictive information in early breast cancer, have the potential to provide information on the likelihood of a patient with LABC responding to neo-adjuvant therapy [Glück et al. BRCRT2013].

Trial design:
MINT I is a prospective study designed to test the ability of molecular profiling, as well as traditional pathologic and clinical prognostic factors, to predict response to neo-adjuvant chemotherapy in patients with LABC. MammaPrint risk profile, BluePrint molecular subtyping profile, TargetPrint estrogen receptor (ER), progesterone receptor (PR) and HER2 single gene readout, and TheraPrint Research Gene Panel will be analyzed on a fresh tumor specimen using the whole genome array. Patients will receive neo-adjuvant chemotherapy pre-specified in the protocol. Response will be measured centrally. pCR is defined as the absence of invasive carcinoma in both the breast and axilla at microscopic examination of the resection specimen, regardless of the presence of carcinoma in situ.

Eligibility:
The study will include women ≥18 years with histologically-proven invasive breast cancer T2 (≥3.5cm)-T4, N0M0 or T2-T4N1M0, adequate bone marrow reserves and normal renal and hepatic function who signed an IRB approved informed consent.

Objectives:
The objectives of the study are to:
1. Determine the predictive power of MammaPrint and BluePrint for sensitivity to neo-adjuvant chemotherapy as measured by pCR.
2. Compare TargetPrint ER, PR and HER2 with local and centralized IHC and/or CISH/FISH assessment.
3. Identify correlations between TheraPrint and response to neo-adjuvant chemotherapy.
4. Identify and/or validate predictive gene expression profiles of clinical response or resistance to neo-adjuvant chemotherapy.
5. Compare BluePrint with IHC-based subtype classification.

Statistical methods:
Standard statistical tests such as the Pearson Chi-square test will be used to characterize and evaluate the relationship between chemoresponsiveness and gene expression patterns.

Accrual:
A total of 226 eligible patients will be enrolled from multiple institutions. To date (June 06, 2014), 103 patients have been enrolled.
**Title:** Prospective observational study of clinical outcomes for the NanoString® technologies Prosigna™ test by the West German Study Group


**Body:**

**Background:** Current diagnostic tools for breast cancer (BC), including screening, diagnostic breast imaging and biopsy, do not provide information about subtype classification. However, the subtype classification is crucial for the assessment of the recurrence risk and for the choice of optimal adjuvant treatment. A vast amount of data from recent research, clinical trials, and peer reviewed publications support the value of intrinsic subtyping based on gene expression analyses to assess prognosis and treatment options for patients with early BC.

**Trial design:** The multicenter (n=8), prospective study examines whether the Prosigna™ test influences physician and patient adjuvant treatment selection over and above currently used prognostic factors. It also examines the impact of the test results on patients’ reported outcomes including their decisional conflict status, anxiety levels, and functional status.

Prosigna™ is a standardized test measuring the expression levels of 50 classifier genes in formalin-fixed, paraffin-embedded tissue samples and provides a subtype classification based on the fundamental biology of individual patient’s tumor (intrinsic subtyping), as well as a prognostic score (risk of recurrence (ROR) score) that predicts the probability of cancer recurrence over 10 years.

**Eligibility criteria:** Postmenopausal women with resected N0, estrogen-receptor-positive (ER) (by immunohistochemistry (IHC); >1% of stained tumor cells is considered positive), HER2-negative (IHC and/or in-situ fluorescence hybridization by local laboratory), early-stage invasive BC (T1-T2, pN0 (i+/mol+), M0). Patients must be able to give consent and must be eligible for treatment with adjuvant chemotherapy (Ctx) (ECOG performance status ≤ 1).

Patients with non-invasive (e.g. Paget’s disease, DCIS), ER-negative or HER2-positive BC must not be included. Metastatic disease and contraindications for adjuvant Ctx (age, ECOG >1, significant comorbidities) are exclusion criteria. Patients unable to complete patient reported outcome surveys will be excluded.

**Specific aims:**

**Primary endpoint** is the proportion of patients whose choice of treatment is changed as a result of receiving the Prosigna™ result.

**Secondary endpoints** include the proportion of patients whose choice of treatment is changed stratified by ROR groups (low, intermediate, high) and who have cancer recurrence risk proximal to the cutoff points between risk strata (± 5% from cutoff point). Investigators’ confidence in treatment recommendations before/after Prosigna™ results and change in patients’ decisional conflict status, anxiety levels, and functional status, stratified by cancer recurrence risk strata, will be investigated. Rate and severity of treatment-related adverse events stratified by whether patient received adjuvant Ctx and agreement in molecular subtyping between Prosigna™ and local IHC will be evaluated.

**Statistical methods:** Sample size of 200 produces a one-sided 95% lower-limit CI with a distance from the sample proportion to the lower limit that is equal to 0.05 when the sample proportion is 0.25.

Present and target accrual: A total of 200 patients will be included, current accrual is n=111 as of 3rd June 2014.
Title: ADAPT - Adjuvant Dynamic marker-Adjusted Personalized Therapy trial optimizing risk assessment and therapy response prediction in early breast cancer

Ulrike Nitz¹,², Oleg Gluz¹,², Raquel von Schumann¹,², Daniel Hofmann¹, Ronald E Kates¹, Sherko Kuemmel³, Michael Braun⁴, Claudia Schumacher⁵, Benno Nuding⁶, Bahriye Aktas⁷, Helmut Forstbauer⁸, Nicolai Maass⁹, Mahdi Rezai¹⁰, Stefan Kraemer¹¹, Mathias Warm¹², Rachel Wuerstlein¹,¹³ and Nadia Harbeck¹,¹³.¹

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Body: Background: Early therapy response is currently not regarded for further treatment decisions as standard of care in the treatment of breast cancer (BC). Predictive markers for the success of a certain therapy could support the physician’s choice of adequate and beneficial therapies by simultaneous reduction of unnecessary toxicity. Proliferation markers as Ki-67 seem to be a suitable tool, as dynamic changes of proliferation (as result of induction therapy) have been shown to be most important for outcome of neoadjuvant chemotherapy prediction in patients with pCR in distinct BC subtypes (luminal B, TNBC, HER2+).

Methods: Trial design: ADAPT combines early assessment of prognosis by conventional markers (e.g. molecular classification, nodal status) with dynamic measurement of proliferation changes during a 3-week induction therapy, using baseline diagnostic core biopsy and a second biopsy after induction therapy. ADAPT consists of an umbrella trial and five different sub-trials (HR+/HER2-, HR+/HER2+, HR-/HER2+, HR-/HER2-, Elderly) and is set up as prospective, multi-center, controlled, non-blinded, randomized phase II/III trial.

- HR+/HER2-: endocrine therapy (ET) vs. chemotherapy (4xPac q2w – 4xEC q2w vs. 8xNab-Pac q1w – 4xEC q2w) + ET, depending on risk classification/early response.
- HER2+/HR+: T-DM1 vs. T-DM1 + ET vs. trastuzumab + ET.
- HER2+/HR-: Trastuzumab + Pertuzumab ± Paclitaxel q1w.
- TN: Nab-Paclitaxel + Gemcitabine vs. nab-Pac + Carboplatin.
- Elderly: 2xMyocet + Cyclophosphamide q3w, depending on cPR/cCR or NC/toxicity the treatment will be continued for two more cycles or changed to 6xPac q1w.

Adaptation/change in therapy regimens can be made by interim analysis after n=130 in each sub-trial. Eligibility criteria: Histologically confirmed unilateral primary invasive BC with known HR-/HER2-status (central pathology) for allocation to the respective sub-trial. Pts requiring chemo- or targeted (anti-HER2) therapy must have adequate laboratory values and organ function and must have no contraindications for the planned treatment.

Primary endpoints: Evaluation of dynamic test for outcome prediction/prospective comparison of 5yr EFS in responders (intermediate risk (RS 12-25) / good response to short-term ET in HR+/HER2- or pts with pCR in HER2+/TN BC) compared to low risk HR+/HER2- (RS≤11, N0-1) pts (control group).

Statistical methods: Assumption across sub-protocols: adjuvant CTx can be spared in HR+/HER2- or pCR be achieved in HER2+/TN in expected 1200 (HR+/HER2-) or 170 (HER2+/TN) pts, respectively. Outcome will be compared to the control group (expected n=640 HR+/HER2- pts: low risk (by RS), i.e. no CTx). Assuming 94% 5yr survival in control group, one-sided test of non-inferiority at 95% CI will have 80% power for survival non-inferiority margin of 3.2% (i.e. 90.8% survival).

Present and target accrual: By June 2014, 73 active sites have recruited 1820 pts for ADAPT HR+/HER2-. Target accrual is 4000 pts. 190 of 380 pts were successfully randomized for ADAPT HER2+/HR+. ADAPT HER2+/HR- has included 17 of 220 pts and ADAPT Triple Negative has recruited 150 of 336 pts.
Title: Improving outcomes in triple-negative breast cancer (TNBC) using molecular triaging and diagnostic imaging to guide neoadjuvant therapy

Stacy Moulder-Thompson¹, Wei Yang¹, Naoto T Ueno¹, Joe Ensor¹, Vicente Valero¹, Ricardo Alvarez¹, Jennifer Litton¹, Rashmi Murthy¹, Nuhad Ibrahim¹, Banu Arun¹, Beth Mittendorf¹, Kelly Hunt¹, Funda Meric-Bernstam¹, Helen Piwnica-Worms¹, Rosalind Candelaria¹, Debu Tripathy¹ and Fraser Symmans¹. ¹University of Texas MD Anderson Cancer Center, Houston, TX.

Body: BACKGROUND:
Known disparities exist in patients with TNBC treated using neoadjuvant chemotherapy (NACT) with 30-50% having excellent response to treatment (pCR/RCB-I) and good survival prognosis, while 50-70% demonstrate marked residual disease (RCB-II-III) with significantly worse prognosis. Lack of response early into NACT also indicates a low chance (5%) of achieving pCR. Thus, it is important to develop diagnostic platforms predictive of pCR, in order to direct patients with responsive disease toward standard NACT and non-responsive disease toward experimental therapies within clinical trials.

TRIAL DESIGN
This study will determine the impact of predicting response to NACT using both molecular and imaging diagnostics and will determine if offering a clinical trial of selected targeted therapy will impact outcomes (as measured by pCR and RCB) in predicted non-responsive disease. An algorithm that incorporates pre-defined genomic signatures will determine predicted sensitivity to chemotherapy (JAMA, 2011; 305:1873-81). All patients will undergo biopsy of the primary tumor for molecular analyses, but will then be randomized 2:1 to know these results (Arm A) versus not (Arm B). All patients will begin anthracycline-based NACT with diagnostic imaging to assess response after 4 cycles. Patients who fit molecular/imaging criteria for non-responsive disease will be offered a clinical trial based upon molecular profiling (Arm A) or based upon physician/patient choice (Arm B). Patients who fit criteria for responsive disease in either arm will continue with taxane based NACT.

ELIGIBILITY CRITERIA
INCLUSION: Candidate for biopsy of the primary tumor site; tumor size ≥ 1.5 cm diameter; TNBC by standard pathologic assays; ≥18 years of age; LVEF ≥ 50%; adequate organ and bone marrow function
EXCLUSION: Stage IV disease; history of invasive cancer within 5 years; excisional biopsy of the primary tumor; biopsy site changes that limit response assessment; medically unfit for chemotherapy; prior anthracycline; ≥grade II neuropathy; Zubrod performance status of >2; history of serious cardiac event

PRIMARY AIM
Primary Aim: to prospectively determine the impact of implementation of a research platform that includes molecular (genomic) testing from a primary tumor biopsy to predict response, and diagnostic imaging to assess response to standard NACT in patients with localized invasive TNBC. Secondary Aims: compare rates of clinical trial enrollment between study arms, compare DFS, integrated “prospective-retrospective” biomarker analysis, correlative science studies to identify therapeutic targets for resistant disease

STATISTICAL METHODS AND TARGET ACCRUAL
Success will be defined as an improvement in the rate of excellent pathologic response (pCR/RCB-I) from 50%-->64% using the triaging platform. A maximum of 360 patients will be randomized 2:1 to the experimental arm vs. the control arm using a group sequential design with one-sided O’Brien-Fleming boundaries, with up to two equally spaced binding interim tests for both futility and superiority and one final test, having an overall Type I error .05 and power .80 to detect a response rate improvement from a null rate of .50 to a target value of .642.
Title: NEOPAL: A randomized phase II study comparing RCB response to neoadjuvant chemotherapy or letrozole-palbociclib in PAM50 defined postmenopausal luminal breast cancer

Paul H Cottu¹, François Duhoux¹, Jérôme Lemonnier², Hervé Bonnefoi³, Anne Vincent Salomon¹, Bernard Asselain¹ and Suzette Delaloge⁴. ¹Institut Curie, Paris, France; ²UNICANCER, Paris, French Polynesia; ³Institut Bergonie, Bordeaux, France and ⁴Institut Gustave Roussy, Villejuif, France.

Body: Background: HR-positive BC heterogeneously sensitive to neoadjuvant chemotherapy (NEC), with pCR rates varying from 2 to 20% at most. The alternative systemic primary therapy in ER+ breast cancer is neoadjuvant endocrine therapy (NET). Clinical response with NET is roughly 50%. It has also been suggested that NET may yield a higher rate of breast conserving surgery than NEC in ER+ disease, although pCR remains under 10% in both instances. No definite pathological criteria for response to NET has been defined so far. Within this context, the PAM 50 signature has been evaluated on numerous series of pts and has been shown to highly correlate with Residual Cancer Burden (RCB – Symmans, JCO 2007) and pCR in patients treated with NEC, as well as with the PEPI score in patients treated with NET (Ellis 2011). Palbociclib + Letrozole has been shown as a very active association as first-line metastastic treatment of HR+ Her2- BC pts on both response rates and progression free survival (Paloma-1 study). This combination could represent an interesting alternative to CT in carefully selected Luminal BC pts.

Trial design and statistical methods: Open-label, randomized, parallel, multicenter, exploratory phase II study, comparing sequential standard NEC (3 FEC 100 followed by 3 Docetaxel 100) and a same duration letrozole + palbociclib combination as neoadjuvant treatment of stage II-IIIA PAM 50 defined Luminal A-Node+/Luminal B breast cancer. Medical treatment will be followed by adequate surgery and complementary chemotherapy and radiation therapy as clinically indicated. All pts will receive adjuvant chemotherapy. Randomizations are equally balanced between the 2 arms and stratified based on T2 versus T3-T4 and PAM 50 luminal A vs luminal B. A Fleming 2-step statistical design will be used in the experimental arm with an intermediate futility analysis. The target accrual is 60 pts evaluable for RCB in both arms.

Eligibility criteria: Newly diagnosed pts with ER+, Her2-negative, stage II-III breast cancer will be tested for PAM50 signature. Only luminal A N+ or Luminal B (PAM50 ROR (Prosigna™) centralized evaluation) pts will be eligible and randomized. Pts must be post-menopausal women, aged > 18 years, bear operable unilateral invasive BC, and not be candidate or uncertain for breast conservation.

Specific aims: The main objective of this trial is to evaluate the ability of each treatment strategy to provide RCB 0-I at surgery. Secondary end-points are: Clinical/radiological response rates in each treatment arm (RECIST 1.1), safety (CTC-AE V4.0), relative dose intensity of each drug in both arms, positive and negative predictive values of PAM50 ROR-defined status, assessment of several biomarkers as potential predictors of clinical and pathological response, rates of BCS, with regard to the initially planned surgery, all in both arms

Target accrual: This study has started in autumn 2014. 132 pts will have to be included (estimation of 10% risk of non evaluable pts). As about 10% of PAM50 evaluable pts will be classified as non luminal, and taking into account potential technical failures, about 180 pts will be screened.
Title: Predicting hormonal therapy response in breast cancer using diffuse optical spectroscopic imaging (DOSI): Ongoing clinical study

Thomas D OSullivan1, Anais Leproux1, Alice M Police1, Dorota Wisner2, Christine McLaren1, Wen-Pin Chen1, Albert E Cerussi1, Min-Ying Su1 and Bruce J Tromberg1. 1University of California, Irvine, CA and 2University of California, San Francisco, CA.

Body: Background: The goal of this multi-site prospective study is to validate a safe, painless imaging method to measure the change in breast density caused by hormonal chemotherapy treatments such as tamoxifen. Recent studies have demonstrated that hormonal therapies are more effective at reducing risk in women who exhibit >10% reduction in breast density compared to women who had little or no density change, suggesting that breast density is a predictor of tamoxifen effectiveness. Current methods to measure breast density include MRI and mammography, however frequent applications of these modalities are limited due to cost and x-ray exposure, respectively. Alternatively, we are testing an imaging method that uses safe near-infrared light to measure breast tissue physiology called diffuse optical spectroscopic imaging (DOSI).

Trial Design and Eligibility: The primary aim of the study is to determine whether the percentage change in the DOSI measurement of water correlates with the change in the MRI measurement of breast density after 18 months of treatment in the contralateral normal breast of breast cancer patients receiving tamoxifen. Other DOSI-derived parameters such as lipid content and hemoglobin concentration will be examined in secondary aims. Two groups of women are being recruited for the study: Pre-menopausal subjects receiving tamoxifen (treatment group) and pre-menopausal subjects not receiving chemoprevention agents (control group). Participants are measured with DOSI and non-contrast MRI before, and 6, 12 and 18 months after beginning tamoxifen. Eligible subjects are pre- and peri-menopausal females older than 21 years of age who have not and do not intend to receive chemotherapy, radiation, or surgical cancer treatment to the imaged breast, and are not pregnant or nursing. Study sites include the University of California, Irvine and San Francisco campuses.

Statistical Methods: At a 5% significance level, the pre-determined power of the study is sufficient to detect the difference between the treatment and control groups by measuring the percentage change in the DOSI measurement of water correlates with the change in the MRI measurement of breast density after 18 months of treatment in the contralateral normal breast of breast cancer patients receiving tamoxifen. Other DOSI-derived parameters such as lipid content and hemoglobin concentration will be examined in secondary aims. Two groups of women are being recruited for the study: Pre-menopausal subjects receiving tamoxifen (treatment group) and pre-menopausal subjects not receiving chemoprevention agents (control group). Participants are measured with DOSI and non-contrast MRI before, and 6, 12 and 18 months after beginning tamoxifen. Eligible subjects are pre- and peri-menopausal females older than 21 years of age who have not and do not intend to receive chemotherapy, radiation, or surgical cancer treatment to the imaged breast, and are not pregnant or nursing. Study sites include the University of California, Irvine and San Francisco campuses.

Accrual Update: Out of the target accrual of 36, 11 subjects (6 treatment and 5 control group) have been enrolled to date. Enrollment is open until 11/30/2015.
Title: PROMIS: PRospective study Of MammaPrint in breast cancer patients with an Intermediate recurrence Score (PROMIS)

Hatem Soliman¹, Sarah Untch² and Lisette Stork². ¹Moffitt Cancer Center, Tampa and ²Agendia Inc, Irvine, CA.

Body: Background:
Gene expression profiling in breast cancer offers the potential to improve prognostic accuracy, treatment choice, and health outcomes in women diagnosed with early-stage breast cancer. Numerous gene-profiling assays are now available, which can be applied to a single tumor specimen to provide physicians with a more complete basis for treatment decisions.

• MammaPrint is a 70-gene profile to estimate whether patients are at high or low risk of developing metastases within the first 10 years after curative surgery.
• BluePrint is an 80-gene molecular subtyping profile that discriminates between three breast cancer subtypes: Luminal, HER2, and Basal.
• TargetPrint provides a quantitative measurement of estrogen receptor (ER), progesterone receptor (PR), and HER2.
• Oncotype DX measures expression of five reference genes and 16 cancer-related genes, quantifying the risk of distant recurrence in patients with ER+ early breast cancer who are treated with adjuvant hormonal therapy.

Trial design:
PROMIS is a prospective study that will investigate the additional value of MammaPrint, BluePrint and TargetPrint in women with an intermediate Oncotype DX score. An initial CRF – capturing baseline patient characteristics, pathology information, Oncotype DX score and the recommended treatment plan – will be completed before receiving the MammaPrint result. A second CRF – capturing the actual treatment – will be completed within 4 weeks after receiving the MammaPrint result.

Eligibility: The study will include women aged ≥18 years with histologically proven invasive stage I-II, node negative or node positive (N1), hormone receptor positive, HER2 negative breast cancer, who received an Oncotype DX intermediate score (18-30) and who signed informed consent.

Objectives:
Primary objective:
Assess the impact of MammaPrint on chemotherapy + endocrine versus endocrine alone treatment decisions in lymph node negative, hormone receptor positive, HER2 negative breast cancer patients, who received an Oncotype DX intermediate score (18-30)

Secondary objectives:
• Assess the impact of MammaPrint on chemotherapy + endocrine versus endocrine alone treatment decisions in lymph node positive (N1), hormone receptor positive, HER2 negative breast cancer patients, who received an Oncotype DX intermediate score (18-30)
• Assess the distribution of MammaPrint Low and High Risk in patients with an intermediate recurrence score
• Assess concordance of TargetPrint ER, PR and HER2 results with Oncotype DX ER, PR and Her2 and with locally assessed IHC/FISH ER, PR and HER2
• Compare clinical subtype based on IHC/FISH ER, PR, HER2 and Ki-67 (if available) with BluePrint molecular subtype

Statistical methods:
A sample size of 820 lymph node negative, hormone receptor positive, HER2 negative breast cancer patients is required to detect a 20% overall treatment change (5% significance and 90% power). A McNemars test will be performed for the comparison of the two proportions treated (before and after), both expressed as a percentage.

Accrual: A total of 385 out of 820 have been enrolled from multiple institutions.

Contact information: Clinicaltrials.US@agendia.com
Clinical trial registry number: NCT01617954.
Title: ASTER 70s UNICANCER phase III trial: Can a genomic prognosticator help tailoring adjuvant systemic treatment for luminal breast carcinoma in elderly women?

Body: The benefit of adjuvant chemotherapy (CT) added to hormonal therapy (HT) compared with HT alone remains debated for women >70 with ER+ HER2- breast cancer (BC). Selection of valid indications might be improved by the use of better prognosticator. This trial compares the impact of both strategies on overall survival (OS) according to Genomic Grade (GG). Following surgery, ∼2,000 patients (Pts) will have a GG performed centrally on FFPE specimens by RT-PCR. Those with a high risk (high or equivocal GG) will be randomized to HT alone vs CT+HT. Pts with a low GG will be followed as an observational cohort.

OS (all deaths) is the primary endpoint. Secondary objectives include competing events, cost-effectiveness and Q-TWiST analysis, geriatric dimension, willingness and health-related quality of life including specific ELD15. Translational research will focus on prognostic biomarkers and pharmacogenetics.

Statistical design: sample size based on 4-year OS benefit favouring CT (87.5 vs 80%; HR 0.60); bilateral test α=0.05, β=0.20; 129 events expected in 700 randomized Pts enrolled over 4 years.

From 04/12-05/14, 67 centres in France and Belgium have included 990 Pts aged 70-92. Only 31 GG evaluations were not performed (tumour blocks not available, 14; consent withdrawal or central pathology review discordance, 7 each; treatment choice, 3). In the main recruiting site, the study was not proposed to 20% of pre-screened Pts mostly because of team choice (50%) and inclusion criteria (25%). Amongst those informed, 66% accepted to participate. Median time to get GG information was 17 days (11-25) from sending tumour sample to providing the information to patient.

Of 932 cases with GG report, 374 (40%), 187 (20%) and 362 (39%) were low, equivocal and high GG respectively; 9 tests (1%) failed for technical reasons. The proportion of high-risk tumours (high/equivocal GG 59%) is similar to that observed in general BC populations (40% to 60%) and only 21 of high-risk cases were not randomized (consent withdrawal, 6; treatment choice, 5; laboratory values, 4; tumour phenotype not confirmed or distant metastasis, 3 each).

With 75% of target recruitment in < 2 years, we confirm the feasibility of such innovative multicentre program in an usually underserved population. This might help to better select adjuvant strategy in the elderly BC population and to avoid jeopardising any benefit if stymied by uncontrolled side effects.
A randomized controlled trial comparing primary tumour resection plus systemic therapy with systemic therapy alone in metastatic breast cancer (JCOG1017 study; PRIM-BC)

Tadahiko Shien¹, Hiroji Iwata², Kenichi Nakamura³, Takayuki Kinoshita⁴, Fumikata Hara⁴, Tomomi Fujisawa⁵, Norikazu Masuda⁶, Kenichi Inoue⁷, Taro Shibata³ and Haruhiko Fukuda⁸. ¹Okayama University Hospital; ²Aichi Cancer Center Hospital, Nagoya, Japan; ³JCOG Data Center; ⁴Shikoku Cancer Center; ⁵Gunma Prefectural Cancer Center, Ota, Gunma, Japan; ⁶Osaka National Hospital; ⁷Saitama Cancer Center and ⁸National Cancer Center Hospital.

Body: A brief background discussion: The efficacy and indication of primary tumour resection for breast cancer patients with distant metastases are under debate. There were many retrospective analysis reports indicating the survival benefit of it. However, the first results of two randomized studies reported in SABCS 2013 could not demonstrate the survival benefit. Nevertheless, the results were not conclusive because the systemic therapy was not uniform (e.g. molecular target therapy) and the diagnostic procedures of metastases was different from the widely accepted guidelines.

Trial design: Our trial is being conducted to confirm the superiority, in terms of the overall survival, of surgery plus systemic therapy over systemic therapy alone in stage IV patients who are not refractory to primary systemic therapy (PST).

Eligibility criteria: The inclusion criteria for the study are as follows: untreated patients with histologically confirmed invasive breast cancer with one or more measurable distant metastatic lesions diagnosed by radiological examination.

Specific aims: All patients receive PST according to the ER and HER2 status of the primary breast cancer after the first registration. After three months, the patients without disease progression are randomized to the primary tumour resection plus systemic therapy arm or the systemic therapy alone arm. After randomization and surgery in the former arm, or after randomization in the latter arm, the same systemic therapies are continued until progression of diseases and next appropriate regimens are started after that.

Statistical methods: The primary endpoint is the overall survival, and the secondary endpoints are proportion of patients without tumour progression at the metastatic sites, yearly local recurrence-free survival, proportion of local ulcer/local bleeding, yearly primary tumour resection-free survival, adverse events of chemotherapy, operative morbidity, and serious adverse events.

Sample size for randomized patients was determined to attain at least 80% of power to detect a 6 months difference with one-sided alpha of 0.05.

Present accrual and target accrual: The patient recruitment was started in May 2011. Enrolment of 410 patients for randomization is planned over a 5-year recruitment period. More than two hundred twenty patients were already enrolled until May 2014.
Title: A prospective, randomized trial of sentinel lymph node biopsy versus no additional staging in patients with T1-T2 invasive breast cancer and negative axillary ultrasound

Natalia S Tucker1, William E Gillanders1, Timothy Eberlein1, Rebecca Aft1, Julie Margenthaler1, Feng Gao1, Catherine Appleton1, Imran Zoberi1, Ademuyiwa Foluso1 and Amy Cyr1. 1Washington University, School of Medicine, St Louis, MO.

Body: Background: The American College of Surgeons Oncology Group Z0011 prospective randomized trial demonstrated no local control or survival advantage with more extensive axillary surgery, even in the setting of known axillary disease. These results convincingly showed that axillary surgery provides little, if any, therapeutic benefit. Given that axillary surgery is not associated with local control or survival benefit, the current role of sentinel lymph node (SLNB) is limited to staging the axilla (in other words, SLNB provides staging information but is not therapeutic).

Objectives: In this randomized, controlled non-inferiority trial we aim to determine the utility of axillary ultrasound (AUS) as a pre-operative staging modality for patients with clinically node-negative invasive breast cancer with the hope that it will be a minimally invasive replacement for SLNB.

1. Primary Objective: To assess whether axillary recurrence rates for patients randomized to Arm 1 (no SLNB) is equivalent to axillary recurrence rates for patients randomized to Arm 2 (SLNB).
2. Secondary Objective: To assess disease-free survival in Arm 1 vs. Arm 2.
3. Tertiary Objective: To assess overall survival in Arm 1 vs. Arm 2.

Study Design: A randomized non-inferiority trial comparing no further axillary staging versus SLNB in women with clinical T1-T2, N0 M0 breast cancer and negative AUS. Following a negative AUS, four hundred sixty women will be randomized in a 1:1 fashion to no further axillary staging or SLNB. Subjects will be monitored for local-regional and distant recurrence per NCCN guidelines.

Eligibility Criteria: To be eligible the patient must be 18-y.o. or older; female; have tissue-diagnosis of invasive breast cancer; cT1-2N0M0 cancer; negative AUS; ECOG \( \leq 2 \); available for follow-up; candidate for SLNB. A patient is ineligible if pregnant or lactating; has concurrent invasive bilateral breast malignancies or multicentric disease or is considered a poor surgical candidate.

Statistical Methods:
Power analysis: A power analysis was performed to determine the accrual goal. The power analysis is based on axillary recurrence, our primary endpoint. We expect an axillary recurrence rate of 1% in patients undergoing SLNB. By assuming a noninferiority limit of 2% difference, our sample size will allow us 80% power at 1-sided 0.1 significance level to assure such a noninferiority.

Data analysis: Longer-term formal data analysis for the study will be performed following the intent-to-treat (ITT) principle. Demographic and clinical characteristics of the sample, as well as efficacy, complication rates and loss to follow-up will be summarized using descriptive statistics. The balance of demographic and baseline clinical characteristics between two arms will be compared using t-test, Mann-Whitney rank-sum test, or Chi-square test as appropriate. The differences in OS and DFS between treatment arms will be compared using log-rank test.
**Title:** The LORD trial: A randomized, non-inferiority trial, between active surveillance versus standard treatment in patients with low risk ductal carcinoma in situ

Lotte E Elshof¹, Konstantinos Tryfonidis², Leen Slaets², A Elise van Leeuwen-Stok³, Nicolas Dif², Victoria P Skinner¹, Claudette E Loo¹, Gonneke Warnars¹, Eveline Bleiker¹, Ruud M Pijnappel⁴, Nina Bijker²; Emiel JTh Rutgers¹ and Jelle Wesseling¹.

¹Netherlands Cancer Institute – Antoni van Leeuwenhoek, Amsterdam, Noord-Holland, Netherlands; ²European Organisation for Research and Treatment of Cancer, Brussels, Belgium; ³BOOG Study Center, Amsterdam, Noord-Holland, Netherlands; ⁴University Medical Centre, Utrecht, Netherlands and ⁵Academic Medical Center, Amsterdam, Noord-Holland, Netherlands.

**Body: Background**

The goal in treating patients with ductal carcinoma in situ (DCIS) is to prevent the development of invasive breast cancer (iBC). However, a substantial number of DCIS lesions will never form a health hazard, particularly if it concerns slow-growing, low-grade DCIS. The conventional treatment of low-grade DCIS is similar to that of early-stage iBC, i.e. breast-conserving surgery (BCS) often followed by radiotherapy (RT), or mastectomy, and possibly hormonal therapy (HT). This implies that many women might be unnecessarily going through intensive treatment resulting in a decrease in quality of life and an increase in health care costs, without any survival benefit.

**Trial Design**

LORD is a randomized, international, multicenter, phase III non-inferiority trial. Patients will be randomized between active surveillance and standard treatment according to local policy. Active surveillance comprises monitoring by annual mammography and treatment if there is progression to iBC or higher grade DCIS. Standard treatment encompasses BCS only, BCS + RT, or mastectomy, possibly followed by HT, and follow-up by annual mammography.

**Eligibility criteria**

- Women ≥49 years
- Referral to the hospital solely based on microcalcifications detected by population-based or opportunistic screening mammography
- Unilateral, pure DCIS grade I based on multiple vacuum assisted biopsies
- No prior history of iBC or DCIS

**Specific Aims**

Our aim is to investigate whether active surveillance of low-grade DCIS is as safe as the current standard treatment, and to study the effects on quality of life of these women by saving them from intensive treatment. The primary end-point is time to ipsilateral iBC (iiBC). Secondary end-points include rate of iBC at final pathology after standard treatment, radical intervention rate, and biopsy rate during follow-up. Tissue will be collected for genomic profiling and proteomics to detect iBC risk patterns in low-grade DCIS. Furthermore we aim to develop an online informational aid for clinicians and patients to facilitate the dissemination and understanding of information on low-grade DCIS and its treatment.

**Statistical Methods**

We apply a special type of non-inferiority design, where the alternative hypothesis corresponds to "minor inferiority". We assume that the iiBC-free rate in the standard arm at 5 years equals 97.6%. The hazard ratio under the null hypothesis is 3.4 and the hazard ratio under the alternative hypothesis is 2.1, corresponding to iiBC-free rates in the experimental arm of 92.0% and 95.0% respectively. With a one-sided log-rank test for non-inferiority with α=0.05 and β=0.2, 124 events are needed for which 1842 low-grade DCIS patients need to be randomized during an accrual period of 5.5 years, and the accrual period will be followed by a further follow-up period of 7.5 years.

**Present Accrual and Target accrual**

The LORD trial is endorsed by the Dutch Breast Cancer Research Group (BOOG 2014-04) and the European Organisation for Research and Treatment of Cancer (EORTC 1401). Participating sites will be identified mid-2014. Patient accrual is expected to start early 2015.
Title: Mammographic breast density and magnetic resonance (MR) imaging in women with mammographically occult breast cancer

Chun Jennifer¹, Freya Schnabel¹, Shira Schwartz¹, Jessica Billig¹, Jennifer Gillman¹ and Linda Moy¹. ¹NYU Langone Medical Center, New York, NY.

Body: Background: Recent data suggests a correlation between mammographically-occult breast cancer (MOBC) and increasing breast density. There is a dearth of information on the assessment of the amount of fibroglandular tissue (FGT) with contiguous MR images through both breasts and background parenchymal enhancement (BPE) in women with MOBC. The purpose of this study was to evaluate the relationship between mammographic breast density (BD), BPE, and FGT in women with MOBC and mammographically evident breast cancer (MEBC).

Methods: The Breast Cancer Database at our medical center was queried for all women who were newly diagnosed with breast cancer between 2010 and 2014. Variables included age, BD, BPE, FGT, tumor characteristics. Statistical analyses included Pearson’s Chi Square Tests.

Results: Out of a total of 1781 women, there were 110 (6%) with MOBC and 1671 (94%) with MEBC. The median age of the cohort was 59 years. Majority of the patients had early stage disease (72%) and invasive ductal carcinoma (61%). There was a higher proportion of women with DCIS with MEBC (22%) compared to women with MOBC (16%) and a higher proportion of invasive lobular carcinoma in women with MOBC (17%) compared to the women with MEBC (10%). Increased mammographic BD was significantly associated with mammographically occult breast cancer (p<0.001). However, BPE (p=0.29) and FGT (p=0.28) were not associated with MOBC. In the MOBC group, there was a higher proportion of women with dense breasts on mammography (74%) compared to women with dense breasts on MRI (48%).

Conclusions: For women who develop mammographically occult breast cancer, there are currently no guidelines regarding recommendations for future screening. Increased breast density reduces the sensitivity of mammography, which compromises the ability to detect meaningful findings. Our study shows that MRI may be less affected by obstacles of sensitivity that is usually associated with mammography. Our results support the use of MRI in women with dense breasts who are at higher risk of developing MOBC. By better understanding the patient population who are more likely to be diagnosed with mammographically-occult breast cancer, we can more effectively select patients who should be screened with breast MRI.

Table 1. Mammographic breast density, BPE, and FGT in study population

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>TOTAL (N=1781)</th>
<th>%</th>
<th>MOBC (N=110)</th>
<th>%</th>
<th>MEBC (N=1671)</th>
<th>%</th>
<th>P-VALUES</th>
</tr>
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<tbody>
<tr>
<td>MAMMOGRAPHIC BREAST DENSITY</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predominantly fatty</td>
<td>94</td>
<td>6</td>
<td>3</td>
<td>3</td>
<td>91</td>
<td>6</td>
<td>p&lt;0.001</td>
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<tr>
<td>Scattered fibroglandular</td>
<td>587</td>
<td>38</td>
<td>25</td>
<td>23</td>
<td>562</td>
<td>40</td>
<td></td>
</tr>
<tr>
<td>Heterogeneously dense</td>
<td>715</td>
<td>47</td>
<td>67</td>
<td>61</td>
<td>648</td>
<td>46</td>
<td></td>
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<tr>
<td>Extremely dense</td>
<td>135</td>
<td>9</td>
<td>14</td>
<td>13</td>
<td>121</td>
<td>9</td>
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<td>BACKGROUND PARENCHYMAL ENHANCEMENT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Minimal</td>
<td>74</td>
<td>16</td>
<td>15</td>
<td>24</td>
<td>59</td>
<td>15</td>
<td>p=0.29</td>
</tr>
<tr>
<td>Mild</td>
<td>225</td>
<td>49</td>
<td>30</td>
<td>48</td>
<td>195</td>
<td>49</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>116</td>
<td>25</td>
<td>13</td>
<td>21</td>
<td>103</td>
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<tr>
<td>Marked</td>
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<td>10</td>
<td>5</td>
<td>8</td>
<td>42</td>
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<tr>
<td>FIBROGLANDULAR TISSUE</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predominantly fatty</td>
<td>91</td>
<td>21</td>
<td>7</td>
<td>12</td>
<td>84</td>
<td>23</td>
<td>p=0.28</td>
</tr>
<tr>
<td>Scattered fibroglandular</td>
<td>160</td>
<td>37</td>
<td>24</td>
<td>41</td>
<td>136</td>
<td>37</td>
<td></td>
</tr>
<tr>
<td>Heterogeneously dense</td>
<td>121</td>
<td>28</td>
<td>20</td>
<td>34</td>
<td>101</td>
<td>27</td>
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<td>Extremely dense</td>
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<td>8</td>
<td>14</td>
<td>49</td>
<td>13</td>
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</tr>
</tbody>
</table>
Value of breast MRI for preoperative axillary assessment of breast cancer patients

Sean C Dupont1, Judy C Boughey1, Tanya L Hoskin1, Katrina N Glazebrook1 and Tina J Hieken1. 1Mayo Clinic, Rochester, MN.

Body: Background: Many newly diagnosed breast cancer patients undergo preoperative breast MRI which includes visualization of some degree of the axilla. The impact of patient characteristics and tumor biology on the fidelity of MRI imaging of the axilla in these cases is not well-studied. We sought to examine the correlation between nodal findings on breast MRI and pathologic nodal status in newly diagnosed breast cancer patients.

Methods: With IRB approval, we identified 1868 consecutive breasts with invasive cancer in 1803 patients undergoing primary operation with axillary surgery from 1/2010-7/2013. Patients undergoing neoadjuvant chemotherapy (348) were excluded leaving 1510 breasts with cancer in 1455 patients. Preoperative MRI was performed in 763 patients (52%). We evaluated patient, imaging and pathology data.

Results: Patients evaluated with MRI were median age 57 years. The majority of tumors were T1 (63%) and T2 (28%). MRI identified suspicious axillary nodes in 240 cases (31%), of which 123 (51%) were node positive at operation. Suspicious axillary findings on MRI predicted both nodal status and pN stage at operation (both p<0.0001). The sensitivity, specificity, accuracy, positive (PPV) and negative predictive value (NPV) of MRI for nodal disease across tumor types is shown.

<table>
<thead>
<tr>
<th>Pathologic Node Positive</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>NPV (95% CI)</th>
<th>PPV (95% CI)</th>
<th>Accuracy (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Cases (n=763)</td>
<td>234 (31%)</td>
<td>52.6 (46-59.1)</td>
<td>77.9 (74.1-81.3)</td>
<td>78.8 (75-82.1)</td>
<td>51.3 (44.8-57.7)</td>
</tr>
<tr>
<td>Approximated Biologic Subtype (missing=24)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER+/HER2- (n=609)</td>
<td>194 (32%)</td>
<td>51.0 (43.8-58.2)</td>
<td>80.0 (75.8-83.7)</td>
<td>77.8 (73.4-81.5)</td>
<td>54.4 (46.9-61.7)</td>
</tr>
<tr>
<td>HER2+ (n=69)</td>
<td>20 (29%)</td>
<td>65.0 (40.9-83.7)</td>
<td>71.4 (56.5-83)</td>
<td>83.3 (68-92.5)</td>
<td>48.1 (29.2-67.6)</td>
</tr>
<tr>
<td>ER-/HER2- (n=61)</td>
<td>17 (28%)</td>
<td>58.8 (33.5-80.6)</td>
<td>75.0 (59.4-86.3)</td>
<td>82.5 (66.6-92.1)</td>
<td>47.6 (26.4-70)</td>
</tr>
<tr>
<td>p value</td>
<td>0.74</td>
<td>0.43</td>
<td>0.33</td>
<td>0.56</td>
<td>0.73</td>
</tr>
</tbody>
</table>

Diagnostic performance did not vary significantly based on patient body mass index (BMI) or approximated biologic subtype, but specificity was better for patients >age 50 (p=0.007) and sensitivity and PPV both were worse for grade 1 vs grade 2/3 tumors.

Node positivity rate at operation was 48% (51/106) for patients with a solitary abnormal node on MRI and 54% (72/134) when >1 suspicious node was seen (p=0.39). However, multiple vs solitary MRI suspicious nodes correlated with ≥3 positive nodes at operation (40 [30%] vs 19 [18%, p=0.03) and pN2/pN3 disease (29 [22%] vs 12 [11%, p=0.03).
Conclusion: Axillary lymph node findings on MRI for breast cancer predict nodal status and disease volume in invasive breast cancer patients. Tumor biologic subtype did not affect performance characteristics of preoperative MRI, but tumor grade did influence the sensitivity and PPV of MRI. When MRI is performed in the evaluation of newly diagnosed breast cancer, axillary findings inform pathologic nodal stage at operation regardless of tumor subtype.
2014 San Antonio Breast Cancer Symposium

Publication Number: P1-01-04
Average Grade: 5.00

Title: Evaluation of tumor response after neoadjuvant chemotherapy in breast cancer patients: Correlation between dynamic contrast-enhanced MRI and pathologic tumor cellularity

Hak Hee Kim, Won Kyung Kim, Joo Hee Cha, Hee Jung Shin, Eun Young Chae and Woo Jung Choi. 1Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea.

Body: Purpose: The objective of the study was to analyze whether the measurement of changes of the tumor on magnetic resonance imaging (MRI), could be applied to predict pathologic response after neoadjuvant chemotherapy.

Methods: Between January 2013 and October 2013, 155 patients (age range, 31-74 years; mean age, 47.6 years) with pathologically proven breast cancer who underwent neoadjuvant chemotherapy followed by surgery were retrospectively enrolled. Pre-and post-treatment measurements and changes in tumor size and dynamic curve pattern of MRI were analyzed. Histological regression was scored on the residual overall tumor cellularity based on Miller-Payne grading system.

Results: The change of maximal diameter (\( \rho = 0.681, p < 0.001 \)), angio-volume of tumor (\( \rho = 0.683, p < 0.001 \)) and washout ratio of dynamic curve (\( \rho = 0.607, p < 0.001 \)) measured on MRI showed strong correlation with histological regression. Among them, the correlation factor was highest in the change of angio-volume. The change of plateau ratio of dynamic curve showed moderate correlation (\( \rho = 0.574, p < 0.001 \)) with histological regression. However, the change of persistent ratio of dynamic curve showed weak correlation (\( \rho = 0.206, p = 0.011 \)).

Conclusions: The change of maximal diameter, angio-volume of tumor and washout ratio of dynamic curve measured on MRI showed strong correlation with residual cellularity in lesions after neoadjuvant chemotherapy and could be applied to predict pathologic response.
Title: Effects of preoperative MRI on rate of ipsilateral and contralateral recurrence of breast cancer

Amanda L Amin¹, Irene B Helenowski¹, Thomas E Kmiecik¹, Shruti R Zaveri¹, Nora M Hansen¹, Kevin P Bethke¹ and Seema A Khan¹. ¹Northwestern University, Chicago, IL.

Body: Introduction: Preoperative MRI of the breast is the most sensitive imaging modality in the detection of multifocal or multicentric breast cancer, as well as simultaneous contralateral breast cancer. The aim of this retrospective review was to evaluate the effect of preoperative MRI on local control for patients with breast cancer.

Methods: The Enterprise Data Warehouse of Northwestern Medicine was searched for women who underwent breast conserving surgery for ductal carcinoma in situ (DCIS) or primary invasive breast cancer in the interval of 2004-2010. The use of preoperative MRI, and the clinical and therapeutic details of the patients thus identified were extracted by direct review of the electronic medical record. A breast event was defined as a local recurrence in the treated breast more than six months after completion of treatment (ipsilateral) or a new breast cancer in the untreated breast (contralateral). Differences in the frequency of all events (local and distant), for ipsilateral breast events, and for contralateral breast events was evaluated with Cox proportional hazards model, adjusting for patient age, tumor size, nodal status, the presence of triple negative disease, and the use of radiotherapy and systemic therapy.

Results: In our cohort of 1097 patients, 526 had preoperative MRI and 571 had no MRI. The patients who had preoperative MRI were younger (59 vs. 66 years, p<0.0001), were more commonly premenopausal (37.8% vs. 27.3%, p=0.0004), were more likely to present with palpable tumors (34.8% vs. 26.6%, p=0.004), were more likely to have invasive lobular disease (16% vs. 11.6%), and less likely to have DCIS (16.5% vs. 29%, p=0.001 for differences in histologic pattern). Mean tumor size was equivalent in the two groups (17.5 and 17.3 mm), but nodes were more frequently positive in the MRI group (23.9% vs. 19.1%, p=0.045). Triple negative tumors were more frequent in the MRI group (14.1% vs. 7.5%, p=0.0003). Mean follow up was 51.5 months in the MRI group and 59.4 months in the no MRI group (p<0.0001). The number of events was 49 in the MRI group and 68 in the no MRI group. The Cox hazard ratio (HR) for all events (adjusted for follow-up duration and factors described in the Methods) was equivalent between the two groups (HR 0.90, 95% CI 0.59-1.36, p=0.61). The HR for ipsilateral (HR 0.93, 95% CI 0.57-1.51, p=0.76) and contralateral events (HR 1.22, 95% CI 0.57-2.62, p=0.61) was equivalent between the two groups.

Conclusions: In analyses adjusted for important prognostic features, the use of preoperative breast MRI was not associated with a reduced hazard of any breast cancer event, or of in-breast events (ipsilateral or contralateral). However, the MRI group had a more adverse tumor and patient profiles; a propensity score analysis will be performed, to further adjust for these differences. These findings add weight to the position that routine use of preoperative MRI for all breast cancer patients is not beneficial.
Title: Quantitative breast DCE-MRI risk biomarkers and breast tumor immunohistochemistry phenotypes: A preliminary assessment

Shandong Wu¹, Margarita L Zuley¹,², Brenda F Kurland¹, Rachel C Jankowitz¹,², Jules Sumkin¹,² and David Gur¹. ¹University of Pittsburgh, Pittsburgh, PA and ²UPMC Magee-Womens Hospital, Pittsburgh, PA.

Body: Purpose
Fibroglandular tissue (FGT) and background parenchymal enhancement (BPE) estimated from breast dynamic contrast enhanced MRI (DCE-MRI) have been correlated with breast cancer risk. We performed a preliminary study assessing in a small cohort of breast cancer patients the relationship between FGT and BPE both quantified from cancer-unaffected breasts and breast tumor’s immunohistochemistry phenotypes, including testing of estrogen (ER), progesterone (PR), and human epidermal growth factor receptor 2 (HER-2/neu) receptors.

Materials and Methods
We retrospectively identified 51 breast cancer patients who were diagnosed with unilateral breast cancer from 2009-2011. Six out of the 51 were excluded for analysis due to missing data in ER, PR, or Her-2 testing in their pathology reports. The 45 cancer patients (mean 47.3±7.6 YO; 24 IDC, 4 ILC, 16 mixed of IDC and DCIS, and 1 papillary carcinoma) consist of 25 premenopausal and 20 postmenopausal women. Breast DCE-MRI scans acquired at time-of-diagnosis were analyzed using fully automated computer algorithms. From the contralateral cancer-free breast, four quantitative variables were computed for each patient: the absolute volumes of FGT and BPE (i.e., |FGT| and |BPE|) and their relative amount over the whole-breast volume (i.e., FGT% and BPE%). BPE were assessed over the FGT region of the breast, and separately from each of the three sequential DCE post-contrast-subtracted sequences (i.e., SUB1, SUB2, and SUB3). Multivariable logistic regressions were performed to assess the quantitative FGT/BPE variables for distinguishing/predicting the status (+/-) of ER, PR, and HER-2 testing. Area under the curve (AUC) of the receiver operating characteristic (ROC) was used to evaluate the regression models’ discriminative performance. Odds ratios (ORs) were reported with 95% confidence intervals (CIs).

Results
In this cohort, the most common phenotypes were ER+/PR+ (14/45 each HER2+ and HER2-) and triple negative (9/45). Premenopausal status was associated with tumors that were HER-2+ (AUC=0.66; p=0.04), ER+ (AUC=0.73; p=0.02), and PR+ (ACU=0.66; p=0.04). FGT% (AUC=0.74; p=0.017; OR=5.3 [95% CI: 1.3, 21.1]) and BPE%-SUB3 (AUC=0.69; p=0.02; OR = 1.4 [95%CI: 1.1, 1.9]) were discriminative of HER-2 status; after adjusting for menopausal status, FGT% remained the association (AUC=0.77; p=0.04; OR=4.4 [95% CI: 1.1, 18.5]) but BPE%-SUB3 did not (p=0.06). BPE%-SUB1 was discriminative of PR status (AUC=0.70; p=0.03; OR=2.3 [95%CI: 1.1, 4.8]) but did not contribute to predict when adjusting for menopausal status (AUC=0.75; p=0.06). All FGT and BPE variables were not associated (p>0.05) with ER status.

Conclusions
In this preliminary study, FGT% and BPE% were associated with HER-2 and PR status, and none of FGT and BPE was associated with ER status, of breast tumor.

Clinical Relevance
Quantitative MRI variables of FGT and BPE that are predictive of breast cancer risk are related to breast tumor immunohistochemistry phenotypes. While FGT and BPE cannot replace the testing of these phenotypes, they may contribute to assess the risk of molecular subtypes of breast cancer, with potential implications for improving breast cancer screening strategies.
Title: Quantitative assessment of early- and delayed DCE-MRI background parenchymal enhancement in breast cancer risk prediction

Shandong Wu¹, Wendie A Berg¹,², Margarita L Zuley¹,², Brenda F Kurland¹, Rachel C Jankowitz¹,², Jules Sumkin¹,², Robert M Nishikawa³ and David Gur¹. ¹University of Pittsburgh, Pittsburgh, PA and ²UPMC Magee-Womens Hospital, Pittsburgh, PA.

Body: Purpose

Background parenchymal enhancement (BPE) estimated from breast dynamic contrast enhanced MRI (DCE-MRI) has been correlated with breast cancer risk. Multiple time-point post-contrast-subtracted sequences are acquired in typical clinical breast DCE-MRI protocols. We quantitatively assessed BPE using fully automated computer algorithms and investigated the relationship between BPE quantified from three time-point post-contrast sequences and breast cancer risk prediction.

Materials and Methods

A retrospective case-control study was performed using breast DCE-MRI scans from 102 patients (mean 47.2±7.3 YO) who underwent either open surgical biopsy or core biopsy from 2009-2011: 51 women had unilateral breast cancer and 51 were age- and date-of-MRI matched controls with a unilateral biopsy-proven benign. For each MRI scan, three post-contrast-subtracted sequences (i.e., SUB 1, SUB 2 and SUB 3) acquired over a total of 7 minutes were analyzed. BPE was quantified from each of the sequences using fully automated computer algorithms on the breasts contralateral to the cancers and the contralateral (negative) breasts of the controls. For each sequence, two quantitative BPE measures were generated: the absolute BPE volume (|BPE|) and its relative amount over the whole breast volume (BPE%). Volumetric absolute and relative amounts of fibroglandular tissue (|FGT| and FGT%) were also automatically quantified, from the pre-contrast sequence. BI-RADS breast density assessment was retrieved from the mammography report (< 6 months) prior to cancer diagnosis (for cases) or MRI (for controls). Multivariable conditional logistic regression was performed to assess BPE measures, quantified respectively from SUB 1, SUB 2, and SUB 3, as predictors of breast cancer risk.

Results

Breast cancer risk odds ratios (ORs) were reported by |BPE| and BPE% (Table 1), after adjustment for breast density, |FGT|, and FGT%. Breast density did not correlate with risk for breast cancer in this study cohort: OR for BI-RADS density alone was 0.75 (95% CI: 0.35, 1.59; p=0.5). OR was 1.14 (95% CI: 0.72, 1.81; p=0.6) for |FGT| alone and 0.70 (95% CI: 0.19, 2.52; p=0.6) for FGT% alone.

Table 1: Breast cancer risk odds ratios (ORs) with 95% confidence intervals [CIs] for BPE quantified from SUB 1, SUB 2, and SUB 3, respectively.

<table>
<thead>
<tr>
<th>BPE from SUB1</th>
<th>BPE from SUB2</th>
<th>BPE from SUB3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BPE</td>
<td>2.0 (95% CI: 1.1-3.7; p=0.02)</td>
</tr>
<tr>
<td>BPE%</td>
<td>3.6 (95% CI: 1.3-10.1; p=0.01)</td>
<td>2.8 (95% CI: 1.3-6.2; p=0.01)</td>
</tr>
</tbody>
</table>

Conclusions

Increased BPE quantified from DCE-MRI are predictive of breast cancer risk, independent of measures of breast density and FGT. BPE estimated from three time-point SUB sequences has a similar predictive effect of breast cancer risk, while an early sequence (e.g., SUB 1) appears to have a larger magnitude of effect than that of a delayed sequence (e.g., SUB 3).

Clinical Relevance

Quantified BPE in breast DCE-MRI has potential for use as a biomarker of breast cancer risk and may be included to improve breast cancer risk prediction. A single post-contrast sequence (i.e., SUB 1) may be adequate for use to estimate breast cancer risk using breast MRI in the context of breast cancer screening for high-risk women.
Title: Changes in breast density with mammography and breast MRI among high-risk postmenopausal women

Parijatham S Thomas, Mary Beth Terry, Xiaotao Guo, Binsheng Zhao, Richard Ha, Swathi Namburi, Tong Xiao, Dawn Hershman, Susan Refice and Katherine D Crew. Columbia University Medical Center, New York, NY.

Body: Background: Mammographic density (MD) represents one of the strongest predictors of breast cancer risk. Tamoxifen, a selective estrogen receptor modulator (SERM) and proven chemopreventive agent, causes reductions in MD which correlate with subsequent breast cancer risk. Current methods for measuring MD, such as the Cumulus technique, are labor intensive and not fully automated, which limits utility in the clinical setting. Breast MRI is being used with increasing frequency in high-risk women for breast cancer screening and yields 3-dimensional views of breast volume. Breast MRI fibroglandular tissue volume (MRIV) has been correlated with MD in cross-sectional studies, but less is known about changes in breast density over time on MRI and its relation to breast cancer risk. One study reported a significant reduction in breast MRIV but not MD with the SERM, raloxifene, in high-risk women. We implemented a novel MRI segmentation method to measure MRIV compared to MD in 20 high-risk postmenopausal women that received high-dose vitamin D supplementation for 1 year.

Methods: Twenty high-risk postmenopausal women [defined as a 5-year breast cancer risk per the Gail model of ≥1.67%, lobular or ductal carcinoma in situ (LCIS/DCIS)] were assigned to a 1-year intervention of vitamin D3 20,000 IU or 30,000 IU weekly. Other eligibility criteria included baseline MD ≥25% (as assessed qualitatively by BIRADS category) and no current SERM use. Women underwent a digital mammogram and breast MRI at baseline and 12 months. MD was evaluated by the Cumulus technique and MRIV was measured using a novel segmentation method on noncontrast T1-weighted images from breast MRIs. The method incorporated two well-established image analysis techniques, the region-based active contour model and watershed algorithm, into a unified framework. Specific anatomical landmarks were introduced into the algorithm to consistently identify breast boundaries on longitudinal MRI slices. For fibroglandular tissue segmentation, an algorithm combining region-based active contours and level-set approach was used to allow for easy initialization and fast speed. Correlation coefficients and paired t-tests were used to compare MD and MRIV and changes from baseline to 12 months.

Results: From Nov 2007 to Jan 2011, 20 postmenopausal women were enrolled and 16 were evaluable at 12 months. Mean baseline breast density measurements were lower for MD compared to MRIV, 12.89% (SD 11.99) vs. 26.64% (SD 6.95), respectively. After 1 year of high-dose vitamin D, there was no significant change in MD or MRIV, mean absolute changes of -0.14% vs. -1.12%, respectively. We did not observe a significant correlation between MD and MRIV density measurements (correlation coefficient= -0.31, p=0.08).

Discussion: We observed greater changes over time in breast density on serial breast MRI compared to mammography, however, no significant changes in MD or MRIV was noted after a 1-year intervention of vitamin D. Longer follow-up may be required in order to detect changes in breast density with non-hormonal interventions.
**Title:** Optimization of magnetic resonance imaging predictive performance by breast cancer subtypes for predicting response to neoadjuvant chemotherapy

Wen Li¹, Wei-Ching Lo¹, Ella F Jones¹, David C Newitt¹, John Kornak², Lisa J Wilmes¹ and Nola M Hylton¹. ¹University of California San Francisco (UCSF), San Francisco, CA and ²UCSF, San Francisco, CA.

**Body:**

**Objective:** Change in functional tumor volume (FTV) measured by dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI), has been shown to be associated with response to neoadjuvant chemotherapy (NACT) for patients with stage II/III breast cancer. FTV reflects the vascularized volume of tumor and is calculated by applying minimum thresholds PE for the initial percent enhancement and SER for the early-to-late signal enhancement ratio following gadolinium contrast injection. In this retrospective study, we investigate the association of FTV influenced by PE and SER values with recurrence-free survival (RFS) in breast cancer subtypes defined by hormone receptor (HR) and HER2 status.

**Materials and Methods:** 64 patients with locally advanced breast cancer were imaged by DCE-MRI before treatment (MRI₁), after one cycle of adriamycin-cytoxan (AC) therapy (MRI₂), inter-regimen (MRI₃, taxane receivers only) and at the completion of chemotherapy prior to surgery (MRI₄). Because treatment length and regimen varied among patients, the MRI exam performed after all NACT and before surgery was designated as final MRI (MRI₅). FTV was calculated with varying PE (30–200% in steps of 10%) and SER (0–2 in steps of 0.2) values. A Cox proportional hazard model was used to analyze association with RFS, defined as the time between surgery and disease recurrence or last disease-free follow-up, for the following imaging predictors: early percent change in FTV (ΔFTV₂: MRI₁ vs. MRI₂), final percent change (ΔFTV₅: MRI₁ vs. MRI₅), and final FTV in MRI₅ (FTV₅). RFS association was evaluated for the full cohort and within subsets defined by tumor subtype (HR+/HER2-, HER2+, triple negative). Estimated hazard ratios per unit change in predictors, associated 95% confidence intervals (CI) and p-values were evaluated. The cutoff of p<0.05 was used to differentiate PE/SER values with higher predictive performance.

**Results:** For the full cohort, FTV₅ showed the most robust association with RFS among the three predictors, with p<0.05 across all measured combinations of PE/SER. The PE/SER combination with smallest p-value was found for PE=30% and SER=0.2, with hazard ratio 1.09 (95% CI 1.06–1.13, p<0.001). P-values less than 0.05 for the ΔFTV₂ predictor was found for the full range of SER values tested, but was mostly confined to PE in 30–110% range; ΔFTV₂ resulted in p<0.05 for very few PE/SER combinations. When analyzed by tumor subtypes, different prediction profiles were observed. FTV₂ showed p<0.05 for the lower PE range only in the HER2+ (n=15) and triple-negative (n=11) groups. A limited number of PE/SER pairs showed p<0.05 for ΔFTV₂, with the exception of the ΔFTV₂ in the triple-negative group, for which p <0.05 for many PE/SER combinations in the low PE range. **Conclusions:** This study was undertaken to explore the significance of FTV for predicting breast cancer recurrence following NACT. While this study is retrospective in nature and had small sample size, the findings nevertheless suggest that performance of imaging predictors based on FTV may be improved if threshold optimization is performed separately for the clinically-relevant subtypes defined by HR and HER2 receptor expression.
Title: A preoperative PET/MR imaging for diagnosing malignant axillary lymph nodes in women evaluated for breast cancer

Kyung Jun Yeu¹, Jeong Yeong Park¹, Jung Eun Choi¹, Su Hwan Kang¹, Eun-Jung Kong², Ihn-Ho Cho² and Soo Jung Lee¹. ¹Yeungnam University College of Medicine, Daegu, Korea and ²Yeungnam University College of Medicine, Daegu, Korea.

Body: Objective: We determined whether and how malignant node could be diagnosed preoperatively with simultaneous PET/MR imaging.

Materials and methods: Two hundred thirty-six consecutive women with breast cancer were recruited to undergo preoperative PET/MR imaging. 52 patients were excluded; 31 DCIS, 7 neoadjuvant chemotherapy and 14 previous excisional biopsy. Thus, 184 women (51.9 ±10.2 years old) were included. Axial T1w without fat saturation, contrast enhanced image and diffusion-weighted images with simultaneous acquired PET images were analyzed by nuclear medicine physicians who were blinded the histologic findings. Visual FDG avidity, long axis and cortical thickness of axillary lymph node (ALN), morphologic feature-loss of fat hilum, cortical thickening, round shape or irregular shape- and ADC value were analyzed in most suspicious ALNs.

Results: In total, 70 patients (15 patients have only micrometastasis) exhibited ALN metastasis. Mean size of breast mass was 2.1 ±1.5 cm. Macrometastatic ALN showed high FDG uptake, longer axis, thicker cortex, more frequent morphologic abnormalities, higher signal intensity at DWI and higher ADC values with statistical significance. No significant difference between micrometastatic ALN and benign ALN in PET/MR imaging. With pathologic diagnosis as the reference standard, the sensitivity, specificity and accuracy of PET for determining ALN metastasis were 84%, 58% and 68%, respectively. Those are 77%, 84% and 82% in considering both PET with morphologic change and 81%, 66% and 72% in considering PET, morphologic change and DWI, respectively.

Conclusion: PET/MR imaging techniques showed high accuracy in the preoperative evaluation of axillary status in patients with breast cancer. Additional information by DWI is unlikely to be useful in predicting metastatic ALN.
Title: The impact of a critical look at the consequences of preoperative MRI in breast cancer patients

I Ching Yeung1, Mehrzad Namazi2, Valerie Deslauriers1, Fatima Haggar2 and Angel Arnaout1,2. 1Ottawa Hospital, Ottawa, ON, Canada and 2Ottawa Hospital Research Institute, Ottawa, ON, Canada.

Body: BACKGROUND: Despite the fact that routine use of preoperative breast MRI for breast cancer has not been shown to improve oncologic outcomes, it is still an exceedingly popular test. Due to low MRI specificity, patients may be subjected to additional invasive test. There have been few studies specifically evaluating the outcomes from these additional tests and its implications on the health care system. The objective of this study was to critically evaluate the impact of performing a preoperative MRI on breast cancer patients at our institution.

METHODS: A retrospective chart review was performed on all female breast cancer patients diagnosed and awaiting surgery (2010-2012). Based on extracted data, indications for preoperative breast MRI were established for our institution (2013). Adherence to these guidelines was then assessed over the next year.

RESULTS: In 2010-2012, 1159/1674 breast cancer patients underwent a preoperative breast MRI. The MRI group was younger (p<0.0001), but not different in histologic subtype (p=0.06) or biomarker status (p=0.61). 421/1159 (36%) of MRI patients underwent at least one additional MRI induced imaging test (ultrasound, mammogram, 6 month MRI) following the MRI. 35% (415/1159) of patients underwent an additional MRI-induced biopsies, 52% of which were benign in the breast and 62% of which were benign in the axilla. Post-MRI biopsies resulted in upstaging (DCIS to invasive cancer; node negative to node positive) in 25/1159 (2%) of patients.

Preliminary data assessing local guideline adherence demonstrated that 188/349 (54%) of new breast cancer patients underwent a preoperative MRI. Locally accepted indications for MRI use included: dense breast (26%); assessment of tumor extent; (19%); lobular carcinoma (16%); assessment of locally advanced cancer (16%). 71% of MRI studies were ordered by the radiologist. 22% of patients had no obvious indication for MRI according to our local guidelines. MRI-induced biopsies occurred in 76/189 (40.2%) of patients, 60% of which were benign in the breast and 71% of which were benign in the axilla.

CONCLUSION: We have critically evaluated the impact of preoperative breast MRI on a large volume of patients. Often, MRI results did not result in significant treatment change. Barriers to guideline evidence based care implementation continue to exist in the setting of multidisciplinary breast cancer care. We must continue to work together to best counsel our patients and effectively manage our health care cost, in keeping with the Choosing Wisely Campaign Canada.
Title: Preoperative breast MRI does not affect survival outcomes in T1-2 breast cancer patients who underwent breast-conserving therapy

Jegyu Ryu¹, Sanghwa Kim¹, Jee Ye Kim¹, Hyung Seok Park¹, Seho Park¹ and Seung il Kim¹. ¹Yonsei University College of Medicine, Seoul, Korea.

Body: Purpose: The aim of the study is to evaluate the efficacy of preoperative breast MRI in T1-2 women with breast cancer. Methods: Total 1179 patients, who had T1-2 breast cancer and underwent definitive surgery between 2007 and 2010, were reviewed in this study. Preoperative breast MRI was performed in 981 women with breast cancer. We divided patients into two groups: Patients underwent preoperative MRI and breast-conserving therapy (BCT), those who did not undergo preoperative MRI but BCT. Patients underwent preoperative MRI and total mastectomy were excluded. Clinicopathological features were analyzed using Chi-square or Fisher's exact test if indicated. Survival analyses were examined using Kaplan-Meier method and log-rank test. Cox-proportional hazard model was accessed as multivariate analysis.

Results: In 873 patients who underwent BCT, 675 patients (77.3%) received preoperative MRI, and 198 (22.7%) patients did not. Clinicopathological features including T-stage, Nodal status, histologic type, progesterone receptor, histologic grade, age group (≤ 35 vs. > 35 yr), adjuvant chemotherapy, radiation, and hormone therapy were not significantly different between two groups. Patients aged 50 or younger received more preoperative MRI than those over aged 50 years (42.8 % vs. 51.5%, p=0.02). Estrogen receptor positive-tumors were more common in patients with preoperative MRI (74.9% vs. 67.2%, p=0.03). HER2 over-expression was frequently found in women without preoperative MRI (7.9% vs. 14.6%, p<0.001). In univariate analyses, there was no significant difference in recurrence-free and overall survival between patients who underwent breast MRI and those who did not (p for RFS = 0.75, p for OS = 0.48). In multivariate analyses, preoperative MRI did not influence on RFS and OS (HR for RFS = 0.82, 95%CI= 0.35-1.91, HR for OS = 1.52, 95% CI= 0.40-5.76). Age group and HER2 were not associated with RFS and OS (all p>0.05). ER was an independent prognostic factor for RFS and OS (all p<0.01).

Conclusions: Routine use of preoperative MRI in women with T1-2 breast cancer may not translate into better RFS and OS.
Title: Evaluation of second-generation photoacoustic mammography in detecting the breast cancer vasculature and hypoxic status; a preliminary study

Masahiro Kawashima¹, Iku Yamaga¹, Masae Torii¹, Mariko Tokiwa¹, Fakhrejahani Elham¹, Masako Kataoka¹, Shotaro Kanao¹, Masahiro Takada¹, Yasufumi Asao¹, Tsuyoshi Shiina¹ and Masakazu Toi¹. ¹Kyoto University, Kyoto, Japan and ²Canon Inc, Tokyo, Japan.

Body: Background: Functional imaging of tumor vasculature and oxygenation status is essential for monitoring the therapeutic response to the manipulation of abnormal vasculature. Moreover, it could be also applicable for the detection and risk assessment of breast lesion with borderline malignancy since hypoxia and angiogenesis is known to be associated with the malignant potential of precursor lesion of solid tumor. Photoacoustic mammography (PAM) is a novel optical imaging technology that can visualize the hemoglobin distribution and its oxygen saturation (SO2) noninvasively. We have previously reported a promising clinical result of a prototype model of PAM (Canon Inc., Tokyo, Japan) in breast cancer patients. However, the improvement of spatial resolution and the identification of signal origin are still big challenges when considering its application for clinical settings.

Materials and methods: We developed the second-generation model of PAM (PAM-02). This instrument has achieved the improved spatial resolution (1.3mm) and enhanced detectability by carrying a high-sensitive detector. Moreover, it is equipped with B-mode ultrasound, which enables us to identify the tumor location in PAM images more precisely. The distribution of hemoglobin within breast tissue carrying solid tumor was evaluated by using PAM-02 under the approval of the ethics committee in Kyoto University Hospital, Japan. Contralateral breast without tumor was also evaluated as a control if possible. Calculated SO2 from photoacoustic (PA) signals were illustrated by using color scale.

Results: Seventeen breast lesions from 15 patients were analyzed including 4 ductal carcinoma in situ (DCIS), 12 invasive ductal carcinoma (IDC) and one usual ductal hyperplasia. Tumor locations were successfully identified in 14 out of 17 lesions (82.3%) by B-mode ultrasound imaging. The location of 3 lesions undetectable by B-mode ultrasound imaging could be identified by comparing with corresponding MRI images. B-mode ultrasound imaging made it easy to distinguish intra-tumoral PA signals from peri-tumoral PA signals. Intra-tumoral PA signals were detectable in 68.7% of malignant lesions (11 out of 16 lesions). Peri-tumoral PA signals, which were suggested to be from feeding vessels, were detectable in 81.3% of malignant lesion (13 out of 16 lesions). In the case of benign UDH, PA signals were not detected in either intra- or peri-tumoral region. Intra-tumoral SO2 was estimated to be lower than peri-tumoral SO2 in malignant lesion. While peri-tumoral PA signals were often described as continuous vasculature, intra-tumoral PA signals often showed the spotty patterns. In addition, PA signal density was relatively higher in DCIS compared with IDC. These findings was supposed to reflect the decreased hemoglobin perfusion within solid structure of breast cancer. The minimum detectable lesion was DCIS with a diameter of 8mm.

Conclusion: Improved spatial resolution and combination with B-mode ultrasound imaging facilitate the region-specific evaluation of PAM imaging. PAM-02 was supposed to be feasible for evaluating the hypoxic status within small breast tumor and its microenvironment.
Title: Locoregional assessment by FDG PET/CT in stage II/III breast cancer patients: A multivariate analysis

Suzana C Teixeira¹, Bas B Koolen¹, Wouter V Vogel¹, Marcel P Stokkel¹, Marie-Jeanne Vrancken-Peeters¹, Vincent van der Noort¹, Emiel J Th Rutgers¹ and Renato A Valdés-Olmos¹. ¹Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; ²Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; ³Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; ⁴Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; ⁵Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; ⁶Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands and ⁷Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands.

Body: Aim: Previously we demonstrated the additional value of PET/CT in prone position for breast cancer detection. The purpose of the present study was to evaluate, with a multivariate analysis, the factors influencing detection and quantification of FDG–avid primary tumors (PT) and regional lymph node metastases (LN) detected with PET/CT in patients with stage II/III breast cancer.

Materials and methods: From August 2010 to April 2012 we included 198 patients (mean 51 yr, range 26–82), with stage II/III breast cancer. A dose of 18F–FDG between 180–240 MBq, was administered intravenously. After 60±10 minutes a PET/CT of the thorax in prone position was acquired, scanning 3.00 min per bed position and reconstructed in high–resolution using 2x2x2 mm voxels and 2 mm CT slice thickness. Subsequently, a standard PET/CT was performed in supine position from skull base to thighs, scanning 1.30 min per bed position and using a standard reconstruction with 4x4x4 mm voxels, a standard 5mm CT slice reconstruction and an extra 2 mm CT slice reconstruction. On all PET/CT images we quantitatively assessed the SUVmax of FDG–avid PTs and LNs. We qualitatively assessed tumor multifocality, PT visibility, LN detection in defined regions and the occurrence of anatomical mismatch between PET and CT. The obtained results were then evaluated with a multivariate analysis for scanning position, patient age, breast size, tumor volume, number of FDG–avid lesions, lymph node location and anatomical mismatch between PET and CT.

Results: Prone position imaging positively influenced the visualization of tumor multifocality (p<0.001), the total number of lymph nodes (p<0.001) and of axillary LNs (p<0.001). PT visibility was not significantly influenced by any of the parameters.

A higher SUVmax of the primary tumor was found solely with increased tumor volume (p=0.001) or breast size (p<0.001). The standard 5mm CT slice reconstruction of the supine PET/CT was the only factor causing an increase in anatomical mismatch between PET and CT for axillary lymph nodes (p=0.004).

Conclusion: Prone position for PET/CT influences the visualization of primary tumor multifocality and the number of FDG avid loco-regional lymph nodes. Tumor FDG-uptake appears to be most influenced by tumor volume and breast size. Both results can be important for adequate staging and subsequent breast cancer treatment.
Title: The effect on short-term progression free survival of the detection of ≥4 FDG-avid nodes or occult N3–disease in breast cancer patients with PET/CT

Suzana C Teixeira1, Bas B Koolen1, Paula HM Elkhuizen1, Vincent van der Noort1, Marie-Jeanne Vrancken-Peeters1, Marcel P Stokkel1, Emiel J Th Rutgers1 and Renato A Valdés-Olmos1. 1Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; 2Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; 3Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; 4Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; 5Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; 6Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; 7Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands and 8Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands.

Body: Aim: The risk for locoregional recurrence (LRR), after neoadjuvant chemotherapy (NAC), is based on staging before NAC as well as the final pathology after NAC. Especially the number of tumour-positive axillary nodes, which is also an important selection factor for postoperative radiotherapy, is not adequately assessed by ultrasound before NAC or axillary lymph node dissection after NAC. PET/CT has a high positive predictive value for the detection of lymph node metastases. Newly found lymph node metastasis on PET/CT incentivized us to change the radiotherapy plan for patients with primary breast cancer scheduled for NAC in our institute.

Koolen et al. reported an upstaging of 23% stage II–III breast cancer patients to the radiotherapy requiring high-risk group (≥4 FDG-avid axillary nodes or detection of occult N3–disease) due to new lymph node metastasis detected with FDG PET/CT imaging. In this study, we report the effect of this upstaging with PET/CT short-term progression free survival (PFS).

Materials and methods:
Between 2007 and 2011 a total of 278 breast cancer patients (mean age 48.9y, range 19–75y), with a tumour of at least 3 cm and without metastases, received a baseline PET/CT for staging purposes and subsequent response monitoring to NAC. The group was divided in three groups: a low-(T2N0), intermediate- (T0–2N1 and T3N0) and a high-risk group (T0–3N2–3, T3N1 and T4). We classified LRR, distant metastases and death as an "event"; including all patients in the PFS analysis of the first 3 years.

Results:
With a median follow-up (FU) of 37 months and the upstaging as depicted in table 1

<table>
<thead>
<tr>
<th>Total group before PET/CT</th>
<th>Changed After PET/CT</th>
<th>Complete after PET/CT (FU-events)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-risk: N=47</td>
<td>N=5</td>
<td>Low-risk (2) N=42</td>
</tr>
<tr>
<td>Intermediate-risk: N=144</td>
<td>N=38</td>
<td>Intermediate-risk N=106 (14)</td>
</tr>
<tr>
<td>High-risk: N=87</td>
<td>x</td>
<td>High-risk N=130 (27)</td>
</tr>
<tr>
<td>Total: N=278</td>
<td>Total: N=43</td>
<td>Total N=278</td>
</tr>
</tbody>
</table>

Table 1: The group was divided in three groups: a low- (T2N0), intermediate- (T0–2N1 and T3N0) and a high-risk group (T0–3N2–3, T3N1 and T4). The table shows the upstaging of breast cancer patients from low- and intermediate- to the high-risk group, and thus, after PET/CT, requiring radiotherapy.

: The patients not upstaged by PET/CT showed no difference in PFS between the high-risk, intermediate-risk and low-risk groups (Logrank p=0.18). Due to the migration of the 43 patients from the low- and intermediate group to the high-risk group, based on PET/CT findings, the PFS differed significantly between the risk-groups (Logrank p=0.04). No difference in loco-regional recurrence was seen between the low-risk and the high-risk group (P=0.18).

Conclusion:
After upstaging with PET/CT, into the high-risk group requiring radiotherapy, a significant difference is seen between the three risk-groups. PET/CT restaging may more adequately predict progression free survival. The detection occult lymphatic metastasis with PET/CT leads to upstaging in clinically unsuspected patients with primary breast cancer, enabling adequate radiotherapy treatment.
Impact of perflubutane-enhanced ultrasonography for evaluating malignancy grade of breast cancer

Noriko Yoshimura¹, Norio Masumoto¹, Ai Amioka¹, Keiko Kajitani¹, Hideo Shigematsu¹, Akiko Emi¹, Takayuki Kadoya¹, Tsuyoshi Kataoka¹, Rumi Haruta¹ and Morihito Okada¹. ¹Hiroshima University, Horoshima, Japan.

Body: Objectives
This study aimed to determine whether or not signal intensity caused by the contrast effects of Contrast-enhanced ultrasonography (CEUS) using perflubutane could predict malignancy grades of invasive breast cancer.

Methods
Fifty-four patients with clinical stages I – III breast cancer between April 2013 and April 2014 underwent CEUS using perflubutane. We analyzed the association between contrast-effect intensity and contrast time in CEUS and the prognostic factors based on tumor size, nodal status and immunohistochemical markers (ER, HER-2 status, nuclear grade, Ki-67) in breast cancer.

Results
Time to washout of time required to reach plateau intensity from the start of the maximum intensity was significantly associated with the Ki-67 value (p = 0.03). Also, A parameter of intratumoral blood perfusion, peak intensity (PI), was significantly associated with the Ki-67 value (p = 0.006) and ER status (p = 0.002) (Table 1), but not with tumor size (cT; p = 0.25, pT; 0.96), node status (p = 0.99), HER-2 status (p = 0.32) and nuclear grade (p = 0.61).

Table 1. Relationship between changes in temporal contrast over time as a perfusion parameter and clinicopathological factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>n(%)</th>
<th>Peak intensity</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age ± SD (y)</td>
<td>53.8 ± 13.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 50 y</td>
<td>30 (55.6)</td>
<td>65.5 ± 25.4</td>
<td>0.50</td>
</tr>
<tr>
<td>≥ 50 y</td>
<td>24 (44.4)</td>
<td>61.1 ± 21.5</td>
<td></td>
</tr>
<tr>
<td>NG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 or 2</td>
<td>19 (35.2)</td>
<td>65.8 ± 28.0</td>
<td>0.61</td>
</tr>
<tr>
<td>3</td>
<td>35 (64.8)</td>
<td>62.4 ± 21.2</td>
<td></td>
</tr>
<tr>
<td>Ki67</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 30</td>
<td>24 (44.4)</td>
<td>53.8 ± 22.4</td>
<td>0.006</td>
</tr>
<tr>
<td>≥ 30</td>
<td>30 (55.6)</td>
<td>71.4 ± 21.9</td>
<td></td>
</tr>
<tr>
<td>ER</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>13 (31.7)</td>
<td>58.2 ± 20.9</td>
<td>0.002</td>
</tr>
<tr>
<td>Positive</td>
<td>41 (68.3)</td>
<td>80.5 ± 24.6</td>
<td></td>
</tr>
<tr>
<td>HER2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>47 (87.0)</td>
<td>62.3 ± 24.0</td>
<td>0.32</td>
</tr>
<tr>
<td>Positive</td>
<td>7 (13.0)</td>
<td>71.9 ± 20.5</td>
<td></td>
</tr>
</tbody>
</table>
Also, Thirty-eight, 7, and 9 patients had luminal, HER-2-positive and triple-negative tumors, respectively. The PI values for these tumors were 56.8 ± 20.9, 71.9 ± 20.5, and 85.7 ± 23.2, respectively. And, the PI value was significantly greater in the triple-negative, than in luminal tumors (p = 0.001).

### Table 2. Relationship between signal intensity as a perfusion parameter and tumor subtypes

<table>
<thead>
<tr>
<th>Tumor subtype</th>
<th>n</th>
<th>Peak intensity</th>
<th>p *</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subtype</td>
<td></td>
<td>Mean ± SD</td>
<td></td>
</tr>
<tr>
<td>Luminal</td>
<td>38</td>
<td>56.8 ± 20.9</td>
<td></td>
</tr>
<tr>
<td>HER-2-positive</td>
<td>7</td>
<td>71.9 ± 20.5</td>
<td>0.09</td>
</tr>
<tr>
<td>Triple-negative</td>
<td>9</td>
<td>85.7 ± 23.2</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Furthermore, PI significantly correlated with the Ki-67 value (Spearman r = 0.54, P = 0.00002).

**Conclusions**

These findings indicated that PI has excellent predictive value for grade malignancy in breast cancer and might help to determine appropriate therapeutic strategies.

**Key points**

- Contrast-enhanced ultrasonography (CEUS) enables the real-time evaluation of detailed hemodynamics in breast cancer.
- Peak intensity (PI) was significantly associated with Estrogen Receptors and Ki-67 assessed by immunohistochemistry.
- PI significantly correlated with the Ki-67 value, indicating that PI reflects the grade of proliferative activity in tumors.
- Analyses of contrast-effect intensity will be applied to evaluate grades of malignancy and determine treatment strategies.
Title: An analysis of lesions diagnosed on screening breast ultrasound in women with dense breasts: Four years of data

Jean M Weigert¹, ¹Hospitals of Central Connecticut, New Britain, CT and ²Mandell and Blau MD's PC, New Britain, CT.

Body: Objective: To analyze the type of cancers and high risk lesions diagnosed in women with normal mammograms with dense breasts with the addition of bilateral breast ultrasound and determine whether these lesions make an impact on clinical outcomes. Methods: Four years of ultrasound data from two sites with five offices in Connecticut was analyzed. The type of lesion including size, nuclear and histologic grade, ER/PR/Her2 status, node status, patient age and risk factors was reviewed. Results: A total of 532 Ultrasounds with Birads 4 or 5 were reported with 46 cancers or high risk lesions. There were 14 Invasive Ductal Carcinoma, 10 Invasive Lobular Carcinoma, 8 Mixed type, 1 Mucinous, 1 Tubular, 6 Ductal carcinoma in situ, 3 Atypical Ductal Hyperplasia with papilloma and 3 Lobular Carcinoma in situ. Of the invasive cancers and DCIS, 9 were nuclear grade 1, 25 nuclear grade 2 and 7 nuclear grade 3. They ranged in size from .3 to 8 .0 cm and the patient age was 45-77 years. Four patients had positive metastatic lymph nodes. 3 were in tumors that were nuclear grade 3 and one with micro metastasis was in a nuclear grade 2 tumor. 29 cancers had known hormonal status of which 27 were ER/PR+ and 2 were ER+/ PR-. 5 patents had a family history of breast cancer and 2 patients had prior history of their own breast cancer, one had uterine cancer. In the third and fourth year of screening usg, 4 cancers were diagnosed in patients who had a negative usg either one year or two years prior. They ranged in size from .4 to 1.2 cm and were all node negative.

Discussion: In our first four years of tracking patients with dense breasts, the addition of bilateral breast ultrasound diagnosed a variety of cancers and high risk lesions. These were not visible on screening mammography and were not palpable. There were 4 with node positive disease diagnosed in high nuclear grade tumors. Most patients had no additional risk factors other than dense breasts. In the third and fourth year, cancers were found in patients who had a prior screening usg. Their mammograms remained negative. The cancers were small and node negative.

Conclusion: The successful treatment of breast cancer is based on several variables including type of cancer, grade, size at diagnosis, and stage at diagnosis. We do not know at what point these lesions diagnosed with screening breast usg would have

<table>
<thead>
<tr>
<th>Lesion Type</th>
<th>Number</th>
<th>Average Size</th>
<th>Sentinel Node</th>
<th>Family History</th>
<th>Prior Self Cancer</th>
<th>Prior Screening Usg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive Ductal Grade 1</td>
<td>4</td>
<td>1.2 cm</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Invasive Ductal Grade 2</td>
<td>5</td>
<td>1.7 cm</td>
<td>1 micro-met</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Invasive Ductal Grade 3</td>
<td>5</td>
<td>1.4 cm</td>
<td>3 macromet</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Invasive Lobular Grade 1</td>
<td>2</td>
<td>0.7 cm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Invasive Lovular Grade 2</td>
<td>8</td>
<td>1.5 cm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Mixed IDC/ILC Grade 1</td>
<td>1</td>
<td>n/a</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mixed IDC/ILC Grade 2</td>
<td>6</td>
<td>1.2 cm</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Mixed IDC/ILC Grade 3</td>
<td>1</td>
<td>1.3 cm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DCIS Grade 2</td>
<td>5</td>
<td>1.3 cm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>DCIS Grade 3</td>
<td>1</td>
<td>1.9 cm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>LCIS</td>
<td>3</td>
<td>n/a</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>ADH /Papilloma</td>
<td>3</td>
<td>0.5 cm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tubular</td>
<td>1</td>
<td>0.4 cm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mucinous</td>
<td>1</td>
<td>8.0 cm</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
been clinically evident, either on mammography or physical examination. One might consider that the treatment outcome would be altered if the lesions were larger and with more positive lymph nodes.
Title: Retrospective comparison of sensitivity and positive predictive value (PPV) of contrast enhanced spectral mammography (CESM) to contrast enhanced breast MRI (BMRI) in 50 malignant breasts

Luna Li¹, Lydia Liao¹, Pauline Germaine¹, Elizabeth Tinney¹, Kristin Brill² and Karen Hendershott². ¹Cooper University Hospital Women's Imaging, Voorhees, NJ.

Body: Objective: Contrast enhanced spectral mammography (CESM) is a new study using contrast for early breast cancer detection. It utilizes dual energy digital mammographic technology to detect contrast enhanced cancer that may be invisible on conventional mammogram. Limited studies have shown that adding CESM to diagnostic workup adjunct with mammogram and breast ultrasound does increase sensitivity for breast cancer detection. More studies are needed to compare the sensitivity and positive predictive value (PPV) of CESM to BMRI to further define the role of CESM in breast cancer diagnosis.

Methods: This study involved 50 malignant breasts in 48 women retrospectively chosen from of 960 patients in our institution during the period of October 2012 to March 2014. Both CESM and BMRI were done for each patient within 30 days. The cancer diagnoses were confirmed by tissue diagnoses. The number of malignant lesions was quantified in each breast. The size of lesions was classified into three categories based on standard of breast cancer stages: 1 (0.2cm - <= 2cm), 2 (2cm<lesion<=5cm), 3(>5cm). The enhancement intensity of both lesions and background has been quantified based on a scale of 0-3. Statistical significance was analyzed using T test for mean size of index cancer and mean score of enhancement intensity of background and lesions on CESM and BMRI. Sensitivity and PPV were calculated for both CESM and BMRI. Morphology consistence was calculated on both studies.

Results: Both CESM and BMRI have sensitivity of 100% for breast cancer detection. CESM has a PPV of 98% versus 93% for BMRI. No statistical significance was identified on mean size of index cancer (3.7cm for CESM and 3.8cm for BMRI). The enhancement intensity of background and lesions is significantly higher on CESM than on BMRI (p<0.01). The smallest cancer can be detected by both CESM and BMRI is less than 0.5 cm. Morphology consistence was 46/50 (92%). Of the 50 breasts, there was one false-positive finding at CESM mammography, and four false-positive findings at MR imaging.

Conclusions: Our study indicates that CESM and BMRI have comparable high sensitivity on breast cancer detection. CESM has a higher PPV than BMRI that may indicate a better specificity (no significant difference due to the small sample size). Significantly less background enhancement intensity on CESM than on BMRI reflect an increased specificity. More studies need to be conducted for further evaluation.
Title: Contrast-enhanced ultrasound for evaluation of therapeutic efficacy of radiofrequency ablation for primary breast cancer

Toshikazu Ito¹, Jyunji Okayama¹, Kumiko Uji¹ and Masaaki Izukura¹. ¹Rinku General Medical Center, Osaka, Izumisano, Japan; ²Rinku General Medical Center, Osaka, Izumisano, Japan; ³Rinku General Medical Center, Osaka, Izumisano, Japan and ⁴Rinku General Medical Center, Osaka, Izumisano, Japan.

Body:
To evaluate therapeutic efficacy of percutaneous ultrasound (US) guided radiofrequency (RF) ablation therapy for primary breast cancer using contrast-enhanced US.

The study was approved by the institutional ethics committee, and written informed consent was obtained. Between January 2012 and April 2014, 25 patients with biopsy-confirmed T1 breast cancer underwent RF ablation therapy in one institution. We examined 25 patients with 25 T1 breast cancers by contrast-enhanced MRI/CT, and contrast-enhanced US before and after RF ablation therapy. US guided RF ablation was performed using a 17-gauge internally cooled electrode (Cool-Tip™, Valleylab, Boulder, CO, USA) under general anesthesia. After injection 0.015mL/kg body weight of Sonazoid®(perflubutane), contrast-enhanced US was performed at a low mechanical index (0.17-0.24). Therapeutic success was defined as a lack of contrast enhancement by contrast-enhanced MRI, US and non-viable cancer tissues by US guided vacuum assisted biopsy (VAB) or core needle biopsy (CNB).

Contrast-enhanced US and MRI four weeks or more after RF ablation therapy showed ablation zones and adequate tumor necrosis in all 25 cancer lesions treated. Contrast-enhanced US made it possible to see that tumor vessels of treated lesions had disappeared after treatment. Contrast enhanced MRI showed a lack of stain with ring-like enhancement of ablation zone and non-viable tissues were seen by VAB and/or CNB in 25 ablation zones.

Contrast-enhanced US using Sonazoid® was useful modalities for evaluating the efficacy of RF ablation therapy in breast cancer lesions.
Title: $^{18}$F-fluoro-2-deoxy-glucose positron emission tomography/computed tomography evaluation shows the utility for predicting postoperative outcomes on Luminal types breast cancer patients

Kenjiro Aogi¹, Yoshifumi Sugawara¹, Sachiko Kiyoto¹, Takayuki Kadoya², Hideo Shigematsu², Norio Masumoto² and Morihito Okada³. ¹NHO Shikoku Cancer Center, Matsuyama, Ehime, Japan and ²Hiroshima University, Hiroshima, Japan.

Body: Background: Hormonal receptor-positive, i.e. Luminal types, breast cancer has better prognosis compared with other phenotypes, but predictive factors for postoperative outcomes have been in research besides hormonal receptor expressions. $^{18}$F-fluoro-2-deoxy-glucose positron emission tomography/computed tomography ($^{18}$F-FDG PET/CT) using maximum standardized uptake value (maxSUV) has been reported its utility as one of predictive markers for relapse-free survival (RFS) on breast cancer patients. In this retrospective study, the utility of $^{18}$F-FDG PET/CT was evaluated on predicting postoperative outcomes especially on Luminal types breast cancer patients. Methods: Three hundred forty four breast cancer patients with clinical Stage I-III who underwent preoperative $^{18}$F-FDG PET/CT were enrolled between January 2006 and December 2011 in two institutes. Patients classified into Luminal types, Luminal A (166 patients): Estrogen receptor (ER)(+), Progesterone receptor (PR)(+), Nuclear grade (NG) 1-2 or Luminal B (96 patients): ER(+) or PR(+), HER2(-), NG3, were evaluated in this study. Moreover, patients were also divided in two groups according to cut-off maxSUV on receiver operating characteristic (ROC) analysis (≤4.0 group vs >4.0 group, AUC=0.830). Statistical analyses were performed using Student t test and log-rank test, and p values of less than 0.05 were considered to be statistically significant. Results: Significant correlations were shown between maxSUV and clinical T-factor, nuclear grade, vascular invasion and presence or absence of recurrence (p<0.0001, p<0.0001, p=0.0181, and p=0.0056, respectively). In uni- and multivariate analyses using Cox model for recurrence, only maxSUV was shown to be significant (p=0.021 and p=0.043, respectively). In RFS, the maxSUV≤4.0 group showed significantly better prognosis compared to that of the maxSUV >4.0 group (p=0.003). Conclusion: $^{18}$F-FDG PET/CT using maxSUV was concluded to be useful for predicting postoperative prognosis on luminal types breast cancer patients.
Title: Clinical studies of palpation imaging of the breast on over 1000 patients

Cary S Kaufman¹, Jae S Son², Eli Yered² and Armen Sarvazyan³. ¹Bellingham Regional Breast Center, Bellingham, WA; ²Medical Tactile, Inc, Los Angeles, CA and ³ARTANN Laboratories, Inc, West Trenton, NJ.

Body: INTRODUCTION
Palpation Imaging (PI) of the breast is based on the principles of tactile imaging, a method for measuring the elasticity lesions using a tactile sensor array. PI provides objective data beyond subjective manual palpation by quantifying the shape and hardness of a lesion as well as creating a permanent digital image. PI is also known as "Tactile Imaging", "Mechanical Imaging", "Stress Imaging" or "Computerized Palpation". It quantitatively evaluates mechanical and structural properties of a breast lesion that is altered by tumor growth, and provides a quantitative characterization of the detected lesion. PI is a radiation-free and an inexpensive in comparison to other imaging devices. The purpose of this paper is to evaluate the clinical efficacy of PI by summarizing all clinical studies conducted to date with this technology.

METHODS
The two step process to identify the clinical presence of a breast cancer include identifying the presence of an abnormality and secondarily to determine if that abnormality is malignant. Not all studies examined each portion of this two-step process. There have been 9 clinical studies involving 1,155 symptomatic patients using PI in the U.S., China, and UK. To examine the ability of PI to detect an abnormal breast mass, we reviewed the sensitivity comparisons with CBE. To determine whether a breast lesion was malignant, we reviewed the analysis of the sensitivity, specificity and accuracy for the standard imaging modalities; mammography, breast ultrasound, clinical breast examinations (CBE) and MRI using pathology as the gold standard. Calcifications were not included because they represent DCIS which does not present clinically and is the subject of over diagnosis.

RESULTS
All studies included in this initial meta-analysis were on symptomatic patients.
To identify the presence of an abnormality, the sensitivity of PI to detect breast masses was 89% in comparison with CBE which was 83%.
To identify the presence of breast cancer,
- The sensitivity of PI in detecting breast cancer was 85%, in comparison to CBE (59%), mammography (76%) and ultrasound (90%).
- The specificity of PI in detecting breast cancer was 79% in comparison to CBE (78%), mammography (71%) and ultrasound (79%).
- The Accuracy of PI in detecting breast cancer was 82% comparison with CBE (67%), mammography (74%) and ultrasound (84%).

Breast Cancer Detection Comparison

<table>
<thead>
<tr>
<th></th>
<th>Palpation Imaging</th>
<th>CBE</th>
<th>Mammogram</th>
<th>Ultrasound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity for Cancer Detection</td>
<td>85</td>
<td>59</td>
<td>76</td>
<td>90</td>
</tr>
<tr>
<td>Specificity for Cancer Detection</td>
<td>79</td>
<td>78</td>
<td>71</td>
<td>79</td>
</tr>
<tr>
<td>Accuracy for Cancer Detection</td>
<td>82</td>
<td>67</td>
<td>74</td>
<td>84</td>
</tr>
</tbody>
</table>

CONCLUSIONS
A summary of clinical studies conducted on symptomatic patients to date suggest that PI is (a) sensitive in the detection of breast masses and b) identifies breast cancer with similar sensitivity and specificity compared with other standard imaging modalities while being cost effective. PI may fill a gap in developing countries where little breast screening infrastructure exists. Ongoing
clinical research with PI is warranted to evaluate its potential in the early diagnosis and screening for breast cancer in the U.S.
Contrast enhanced spectral mammography (CESM) is a new breast imaging contrast study: A review of indications, protocol, interpretation and pitfalls

Lydia Liao¹, Luna Li¹, Pauline Germaine¹, Elizabeth Tinney¹, Kristin Brill² and Karen Hendershott². ¹Cooper University Hospital Women's Imaging, Voorhees, NJ.

Purpose: This presentation is to discuss the indications, protocol, interpretation and pitfalls of CESM using illustrations of breast malignant and benign cases performed in our institution, after a brief literature review. CESM characteristics for both benign and malignant lesions are defined after comparison to other imaging studies and correlation to final tissue diagnosis.

Methods: 1000 CESM cases including 100 breast cancer and 200 benign lesions were retrospectively analyzed. These cases were performed in our institution from October 2012 to March 2014. GE Senographe Essential Full Field Digital System (SenoBright) was utilized for CESM studies. Both low and high energy images were obtained after intravenous 75-100cc Isovue 370 administration at flow rate of 1.5-2cc per second. Both low energy and subtracted images were reviewed on GE Centricity Imagecast on 5 megapixel monitor. Abnormal CESM lesions underwent further evaluation and tissue sampling for a final diagnosis to determine the presence of breast cancer.

Results: CESM is one of the contrast studies. It provides both morphologic and functional information of breast lesions. The sensitivity of CESM for cancer detection was 93-100% per patient in contrast to 95-100% of breast MRI. There are morphological and enhancement features for both malignant and benign lesions on CESM. CESM could pick up lesions when regular digital mammography and ultrasound are negative. CESM is a part of diagnostic study on selected patients. It has a short duration exam time and results are easily accessible. Some CESM artifacts are unique and commonly occurred.

Conclusion: CESM is a new breast imaging contrast study. The sensitivity for breast cancer detection is comparable to breast MRI. With its easy accessibility and cost effectiveness, CESM may play an important role in breast cancer detection in conjunction with diagnostic mammography and breast ultrasound. Recognition of CESM artifacts helps to increase diagnostic accuracy.
Role of FDG-PET/CT in prediction of underestimation of invasive breast cancer in cases of ductal carcinoma in situ diagnosed at needle biopsy

Hideo Shigematsu\(^1\), Takayuki Kadoya\(^1\), Noriko Yoshimura\(^1\), Keiko Kajitani\(^1\), Akiko Emi\(^1\), Noriko Masumoto\(^1\), Rumi Haruta\(^2\), Tsuyoshi Kataoka\(^2\) and Morihito Okada\(^1\). \(^1\)Research Institute for Radiation Biology and Medicine, Hiroshima University, Hiroshima, Japan and \(^2\)Hiroshima University, Hiroshima, Japan.

**Body:** Background: The aim of this study was to evaluate the significance of 18F-fluorodeoxyglucose positron emission tomography/computed tomography (FDG-PET/CT) for predicting the underestimation of invasive breast cancer in cases of ductal carcinoma in situ (DCIS) at needle biopsy.

Patients and Methods: Of 83 consecutive cases with diagnoses of DCIS at primary needle biopsy who underwent curative surgery between 2010 and 2013, the association between the maximum standardized uptake value (SUVmax) on FDG-PET/CT before excision and the underestimation of invasive breast cancer was examined.

Results: There were 29 (34.9%) cases diagnosed to have invasive breast cancer at excision. Receiver operating characteristics (ROC) curve analysis showed the cutoff value of SUVmax to predict underestimation of invasive breast cancer was 1.6. The rates of underestimation were 61.5% for patients with a tumor of SUVmax > 1.6 and 11.4% for patients with a tumor SUV max \(\leq\) 1.6 (p < 0.001). High value of SUVmax was significantly associated with symptomatic presentation (p < 0.001), palpability (p < 0.001), mass formation (p = 0.013), high Breast Imaging Reporting and Data System category (p = 0.01) and core needle biopsy (p = 0.007). In multivariate analysis, high SUVmax was only significant predictive factor of underestimation of invasive breast cancer (HR 11.7, 95% CI 3.70-37.0, p < 0.001).

Association between each preoperative variable and underestimation of invasive component in multivariate analysis in cases of DCIS diagnosed at needle biopsy

<table>
<thead>
<tr>
<th>Variables</th>
<th>HR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presentation</td>
<td>symptomatic vs. screening detected</td>
<td>1.64 (0.43-6.22)</td>
</tr>
<tr>
<td>Palpable</td>
<td>yes vs. no</td>
<td>1.54 (0.36-6.69)</td>
</tr>
<tr>
<td>Lesion size</td>
<td>&lt; 2cm vs. (\geq) 2cm</td>
<td>1.23 (0.34-4.40)</td>
</tr>
<tr>
<td>Mass formation</td>
<td>yes vs. no</td>
<td>1.28 (0.36-4.44)</td>
</tr>
<tr>
<td>BI RADS category</td>
<td>5 vs. 3, 4</td>
<td>2.92 (0.80-10.7)</td>
</tr>
<tr>
<td>Biopsy device</td>
<td>mammoatome biopsy vs. CNB</td>
<td>2.16 (0.64-7.30)</td>
</tr>
<tr>
<td>Nuclear grade</td>
<td>high vs. not high</td>
<td>high vs. not high 2.24 (0.51-9.78)</td>
</tr>
<tr>
<td>SUVmax</td>
<td>higher vs. lower</td>
<td>11.7 (3.70-37.0)</td>
</tr>
</tbody>
</table>

**Conclusion:** SUVmax on FDG-PET/CT is useful for predicting the underestimation of invasive breast cancer in cases of DCIS at needle biopsy.
**Title:** Cardiac function in BRCA1/2 mutation carriers with a history of breast cancer (BC) treated with anthracyclines (anthra)

Filipa Lynce¹, Ana Barac², Karen L Smith⁴, Mihriye Mete⁵, Lynette Wray², Madeline Nardacci², Pia Herbolsheimer¹, Raquel Nunes¹, Sandra M Swain¹, Robert Warren³ and Claudine Isaacs¹. ¹Washington Cancer Institute, MedStar Washington Hospital Center, Washington, DC; ²MedStar Heart Institute, MedStar Washington Hospital Center, Washington, DC; ³Lombardi Comprehensive Cancer Center, MedStar Georgetown University Hospital, Washington, DC; ⁴Johns Hopkins Sidney Kimmel Cancer Center, Washington, DC and ⁵MedStar Health Research Institute.

**Body:** Introduction: Animal data suggest that cardiac-specific loss of BRCA1/2 genes leads to increased cardiac dysfunction in the setting of stressors such as ischemia or anthra chemotherapy (CT). Women with BRCA1/2 mutations who develop BC may receive anthra-based CT. It is not known whether BRCA1/2 mutation carriers treated for early stage BC with anthra-based CT are at higher risk for anthra-induced cardiac toxicity than similarly treated women with sporadic BC. This study investigates left ventricular ejection fraction (LVEF) and myocardial global longitudinal strain (GLS), a novel echocardiographic measure of cardiac contractility, in women with and without BRCA1/2 mutations who were treated with anthra-based CT.

Methods: We identified 41 women with a history of BRCA1/2 mutation-associated BC and 52 women with a history of sporadic BC, all of whom received anthra-based adjuvant or neoadjuvant CT. We excluded women who received HER2 therapy or who had stage IV disease. BC CT was completed at least 6 months prior to study enrollment. All participants completed a cardiac questionnaire and underwent a comprehensive 2D and 3D echocardiographic exam with assessment of myocardial GLS using speckle-tracking software (QLAB10, Philips, Andover, MA). Abnormal LVEF was defined as less than 55% (50-54.9% borderline reduced and 40-49% mildly reduced) and abnormal GLS was defined as absolute value of less than 18.9%. Patients with history of hypertension (HTN) were excluded from this analysis to avoid confounding effects of HTN on GLS.

Results: We present data on 57 normotensive participants, 34 BRCA1/2 mutation-carriers and 23 non-mutation carriers. Mean age was 49.5 +/- 8.7 years in the BRCA mutation-carrier group and 51.1 +/- 8.7 years in the non-mutation carrier group (p=0.496). Women with BRCA1/2 mutations were more likely to be Caucasian (p=0.026) and have undergone oophorectomy (p<0.001). The average time from anthra treatment to study enrollment was 5 +/- 4.4 years. Groups were well balanced with regards to age, diabetes, hyperlipidemia, history of coronary disease, smoking and cumulative dose of anthra. Four BRCA1/2 mutation-carriers (12%) and 4 non-mutation carriers (17%) had reduced GLS (p= 0.450). Mean LVEF was similar between the groups: LVEF was borderline reduced in one BRCA1/2 mutation-carrier (3%) and borderline or mildly reduced in 4 non-mutation carriers (18%)(p= 0.091).

Conclusions: In this population, reduced LVEF and myocardial GLS were present in a small percentage of women treated with anthra-based CT for early stage BC. However, reduction in LVEF and myocardial GLS did not differ between the BRCA1/2 mutation carriers and the women with a history of sporadic BC. Larger studies are needed to definitively rule out a difference in cardiac toxicity by BRCA mutation status in BC survivors treated with anthra. This project is supported by Fisher Center for Familial Cancer Research Award at Lombardi Comprehensive Cancer Center. AB is supported by Georgetown-Howard Universities Center for Clinical & Translational Science (GHUCCTS) post-doctoral KL2 Award (5KL2TR000102-04).
Title: CYP2D6 intermediate metabolizers includes patient groups with distinct metabolic activity

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Background: Tamoxifen is a selective estrogen receptor modulator that is the most commonly used and cost effective hormonal agent for pre-menopausal hormone-receptor positive breast cancer patients. CYP2D6 activity phenotype, which is classified by genotype, predicts the extent of metabolic activation of tamoxifen to endoxifen. We previously reported that increasing the daily dose to 40 mg/day in intermediate metabolizers (IMs), but not poor metabolizers (PMs), achieves target endoxifen concentrations, defined as that of extensive metabolizers (EMs) on 20 mg/day. There was substantial endoxifen variability in the IM phenotype group, which is composed of several discrete diplophenotypes (EM/IM, EM/PM, IM/IM, IM/PM). We enrolled a second, larger cohort of patients in order to determine whether these diplophenotypes should be combined into a single IM phenotype or segregated.

Methods: 380 patients on tamoxifen ≥ 4 months and not on potent CYP2D6 inhibiting medications enrolled in Lineberger Comprehensive Cancer Center (LCCC) trial 0801. Genotyping was performed using the Amplichip® CYP450 test (Roche Diagnostics) for CYP2D6, followed by systematic assignment of phenotype based on diplophenotype. Tamoxifen was increased from 20 to 40 mg/day in PMs and IMs. Endoxifen concentrations in IM diplophenotypes were compared with EM/EMs and PM/PMs at baseline and at 4 months (after dose increase in patients with IM and PM phenotypes).

Results: After exclusion of UM patients and patients missing endoxifen data at baseline and/or 4 months, 295 patients were included in this analysis. At baseline the EM/IM patients had similar endoxifen level to the EM/EM patients while the IM/IM and IM/PM patients had similar levels to the PM/PMs. After 4 months on 40 mg/day the endoxifen concentrations in EM/IM patients were significantly greater than EM/EMs; EM/PM and IM/IM patients were similar to EM/EMs; but IM/PM patients remained significantly lower than EM/EMs and similar to PM/PMs (See Table 1 for results).

Conclusions: The large group of patients currently defined as CYP2D6 intermediate metabolizers is comprised of four distinct CYP2D6 diplophenotypes. The most metabolically active diplophenotype (EM/IM) are very similar to EM/EMs while the least active diplophenotype (IM/PM) are similar to PM/PMs. A more accurate CYP2D6 activity classification system may be necessary if genetic association testing and genotype-guided therapy are pursued.

<table>
<thead>
<tr>
<th>Diplophenotype</th>
<th>n</th>
<th>Baseline Endoxifen</th>
<th>4-Month Endoxifen</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Median (SD)</td>
<td>P-val vs. EM/EM</td>
</tr>
<tr>
<td>EM/EM¹</td>
<td>103</td>
<td>8.67 (6.01) NA</td>
<td>p=0.0001</td>
</tr>
<tr>
<td>EM/IM²</td>
<td>56</td>
<td>8.02 (4.75) p=0.09</td>
<td>p=0.002</td>
</tr>
<tr>
<td>EM/PM²</td>
<td>74</td>
<td>5.72 (4.45) p=0.0001</td>
<td>p=0.02</td>
</tr>
<tr>
<td>IM/IM²</td>
<td>17</td>
<td>4.29 (4.10) p=0.001</td>
<td>p=0.26</td>
</tr>
<tr>
<td>IM/PM²</td>
<td>32</td>
<td>3.90 (3.17) p&lt;0.0001</td>
<td>p=0.48</td>
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<td>---</td>
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</tr>
<tr>
<td>PM/PM³</td>
<td>13</td>
<td>3.33 (2.89)</td>
<td>p=0.0001</td>
</tr>
</tbody>
</table>

¹Diplophenotype classified as extensive metabolizer phenotype, continued on 20 mg/day. ²Diplophenotypes classified as intermediate metabolizer phenotype, changed to 40 mg/day. ³Diplophenotype classified as poor metabolizer phenotype, changed to 40 mg/day.
2014 San Antonio Breast Cancer Symposium

Publication Number: P1-03-03
Average Grade: 5.00

Title: Experience in the community oncology practice with a 25-gene hereditary cancer panel

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Body: Introduction:
The identification of patients with an inherited cancer syndrome is becoming increasingly relevant in the treatment and prevention of cancer. Testing with a panel of genes provides the opportunity to rapidly identify or rule out deleterious mutations in several genes simultaneously and, thus, has the potential to streamline the testing process and more efficiently and accurately provide results. We report on the experience of panel-based testing in the community oncology setting.

Objectives:
To evaluate the performance of a 25-gene hereditary cancer test in the community oncology setting and describe the patient characteristics and test findings.

Methods:
We retrospectively evaluated the 25-gene panel testing data obtained between September 2013 and February 2014 in 6 large community oncology practices. The gene panel was based on next generation sequencing and rearrangement analysis of 25 genes with cancer risk data: BRCA1, BRCA2, MLH1, MSH2, MSH6, PMS2, EPCAM, APC, MUTYH, CDKN2A, CDK4, PALB2, CHEK2, SMAD4, BMPR1A, STK11, TP53, CDH1, PTEN, ATM, NBN, BARD1, BRIP1, RAD51C, and RAD51D. Personal and family history was obtained by health care provider report on test requisition forms.

Results:
The panel test was performed on 359 individuals during the time period. 69.9% of these patients had a phx of at least one of the eight panel cancers: breast, ovarian, colorectal, endometrium, pancreas, melanoma, prostate, and/or stomach. cancer diagnosis. 97.8% of the patients tested met the 2013 NCCN guideline criteria for HBOC, 2012 NCCN guideline criteria for Lynch Syndrome, or both. 37 pathogenic mutations were found in 34 distinct patients. The variant rate in this population was 35.4%. Mutations were identified in 16 different genes, and only 20 of the 37 mutations (54.1%) were found to be in the 6 genes included in HBOC and Lynch Syndrome testing (BRCA1, BRCA2, MSH6, MLH1, MSH2, PMS2).

Conclusion:
The 25-gene hereditary cancer panel increased the identification of deleterious mutations which would otherwise not have been detected. The variant rate in this setting is similar to the rate reported previously in both community and academic centers where panel testing is used. Use of a hereditary cancer panel in the community oncology practice may improve detection rates and provide an opportunity for enhanced cancer management.
2014 San Antonio Breast Cancer Symposium

Publication Number: P1-03-04
Average Grade: 4.33

Title: The impact of Angelina Jolie (AJ)'s story on genetic referral and testing at an academic cancer centre

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Body: Background
In May 2013, AJ revealed to the media that she had undergone preventive double mastectomy. The actress had a family history of breast and ovarian cancer and tested positive for the BRCA1 gene mutation. Media coverage has been extensive, but it's not clear what messages the public and professional medical staff took from this personal story.

Methods
We conducted a retrospective review using data from the clinical database of the Familial Cancer Program in a tertiary care cancer centre. The impact of AJ's story on genetic counseling referrals was assessed by comparing the number of referrals made before and after the story. In addition, the appropriateness of referrals was reported by comparing the number of patients who qualified for genetic testing as defined by the Ontario Ministry of Health and Long Term Care and the ones who were found to carry a BRCA1/2 mutation before and after the media release. We previously reported the impact of AJ's story, 6 months before and after, and showed that all the numbers practically doubled. This is an update of this assessment 1 year before and after the story.

Results
The increase in the number of women referred for genetic counseling was persistent after one year with an increase by 88% compared to 90% originally: 1042 referrals before versus 1962 referrals after. There was still an increase in the number of women who qualified for a genetic testing, but it was was less than previously reported. Originally there was an increase of 105% after 6 months. After 12 months, the increase is 68%: 493 women qualified for the testing before versus 827 women after. Furthermore the preliminary increase in the number of BRCA1/2 carriers identified is also less pronounced. The preliminary increase was by 110%. After 12 months, it is 42%: 80 BRCA1/2 carriers identified (45 BRCA1, 35 BRCA2) before versus 114 after (63 BRCA1, 51 BRCA2).

Conclusion
In our preliminary report we showed that after AJ's story, the number of referrals doubled and the quality of referrals remained the same. In this updated analysis, the number of referrals after the story remained as high as previously, however the quality of referrals did not remain the same. With the longer period of observation, fewer referred women qualified for genetic testing and fewer BRCA1/2 carriers were identified. If the positive "celebrity effect" reported previously will begin to subside, our next challenge will not only be to meet the increased demand for cancer genetic services but also to ensure that referrals to our clinic are appropriate by working to reduce the referrals of low risk women triggered by media attention.
Title: Genetic variant in the OPG gene is associated with aromatase inhibitor-related musculoskeletal toxicity in breast cancer patients

Anneleen Lintermans¹, Kathleen Van Asten¹, Annouschka Laenen¹, Thomas Van Brussel¹, Johan Verhaeghe¹, Dirk Vanderschueren¹, Diether Lambrechts¹ and Patrick Neven¹. ¹KU Leuven, Leuven, Belgium; ²VIB and ³University Hospitals, Leuven, Belgium.

Body: Background
Aromatase inhibitor (AI) therapy is associated with musculoskeletal toxicity, which adversely affects quality of life and therapy adherence. Our objective was to evaluate genetic predictors for endocrine therapy-related musculoskeletal pain in a prospective cohort study, which was previously published.

Patients & methods
254 early breast cancer patients starting AI (n=159) or tamoxifen therapy (n=95) were included in this genetic biomarker study. Musculoskeletal symptoms were assessed at baseline and at 3, 6 and 12 months thereafter. AI-induced musculoskeletal pain was defined as an increase in arthralgia or myalgia relative to baseline. Single nucleotide polymorphisms (SNP) in candidate genes involved in estrogen signaling or reported to have associations with AI-related musculoskeletal pain or estrogen levels were selected. Of the 40 selected SNPs, 7 failed genotyping and were excluded from further analysis.

Results
Twenty SNPs exhibited a minor allele frequency <0.05 and were omitted from the final analysis. Overall, 13 SNPs in CYP19, CYP17, osteoprotegerin (OPG) and estrogen receptor 1 were included.

74% of AI-users reported new or worsened musculoskeletal pain compared to 33% of tamoxifen-treated patients. The OPG SNP rs2073618 was significantly associated with AI-induced musculoskeletal toxicity after correction for multiple testing (p=0.039). None of the other SNPs showed significant associations, both in AI and tamoxifen-treated patients.

Conclusion
The SNP rs2073618 in OPG predicts the risk of musculoskeletal symptoms with AI therapy, which has not been reported so far. Validation of this finding and further functional studies are needed.
Title: BRCA1 and BRCA2 mutations in ethnic Lebanese Arab high risk women for hereditary breast cancer

Nagi El Saghir¹, Nancy Uhrhammer², Hussein Assi¹, Katia Khoury¹, Stephanie Decousous², Yannick Bidet², Sara Jaber¹, Raghid Charara¹, Rania Farhat¹, Ziad Salem¹, Ali Shamseddine¹, Arafat Tfayli¹, Jaber Abbas¹, Faek Jamal¹, Muhieddine Seoud¹, Deborah Armstrong³, Yves-Jean Bignon² and Nathalie Zgheib¹. ¹American University of Beirut Medical Center; ²Centre Jean Perrin, Clermont-Ferrand, France and ³Johns Hopkins Kimmel Cancer Center.

Body: Background: Breast cancer is the most common malignancy in women in Lebanon and Arab countries with 50% of cases below age 50. The incidence of hereditary breast cancer in Lebanese and Arab women is unknown.

Methods: 250 Lebanese women with breast cancer, of young age with or without family history, were recruited at the American University of Beirut Medical Center (AUBMC) between 2009 and 2012. Study was approved by IRB. All signed an informed consent. Risk assessment questionnaire, medical chart review, and whole blood were collected. Coding exons and intron-exon boundaries of BRCA1 and BRCA2 were sequenced. Full BRCA gene sequencing was performed at Institut Jean Perrin, France. Study was funded in part by an Ethnic Research Initiative (ERI) grant awarded by GSK.

Results:
14 out of the 250 patients (5.6%) had a deleterious BRCA mutation (7 BRCA1, 7 BRCA2) and 31 (12.4%) had a variant of uncertain significance (VUS). Table 1 shows deleterious BRCA mutations based on age group and FH.

Deleterious BRCA mutations based on age group and family history

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>Age ≤ 40 (with no FH)</th>
<th>Age ≤ 40 (with positive FH)</th>
<th>Age 41-50 (with positive FH)</th>
<th>Age ≥ 51 (with positive FH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients</td>
<td>250</td>
<td>74</td>
<td>74</td>
<td>75</td>
<td>27</td>
</tr>
<tr>
<td>Patients with deleterious mutations (%)</td>
<td>14 (5.6%)</td>
<td>1 (1.4%)</td>
<td>8 (10.8%)</td>
<td>4 (5.3%)</td>
<td>1 (3.7%)</td>
</tr>
<tr>
<td>BRCA 1</td>
<td>7</td>
<td>0</td>
<td>6</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>BRCA 2</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
</tbody>
</table>

All 7 BRCA1 mutation carriers had a positive family history, were between 32 and 48 years of age, and had Grade 3 IDC with negative ER, PR, HER2 receptors (TNBC). Six BRCA2 mutation carriers had IDC with positive hormone receptors (HR) and 2 had HER2-positive disease. We found 31 VUS. One VUS (BRCA2) was seen in two sisters with breast cancer. One VUS (BRCA2) was seen in 4 patients and another in 2 patients, while 2 VUS (BRCA1) mutations were seen in 2 sets of 2 patients. The significance of these VUS cannot be ascertained at this time. Haplotype analysis is ongoing.

Conclusions:
This is the first large study of ethnic Lebanese Arab women with breast cancer. The prevalence of BRCA deleterious mutations in women with breast cancer who are considered high risk of carrying a BRCA mutation is 5.6% in our total cohort, while in patients ≤40 with positive FH it is 10.6%. Those numbers are lower than expected from US and European populations. Tumor grade and pathology characteristics in this patient population correlated with that previously documented for BRCA1 (TNBC) and BRCA2 (positive HR) associated breast cancers. Our data supports use of young age together with positive FH should be used to select patients for counseling and BRCA testing in Lebanon and Arab countries with resource-sensitive guidelines. Several VUS were found in patients and sisters with breast cancer. The finding that 94.4% of high risk patients had no deleterious BRCA mutations suggests the need to look for alternate gene mutations and other factors that may contribute to the development of breast cancer in these high risk patients. Conclusions regarding haplotypes and diversity will be reported at the meeting.
2014 San Antonio Breast Cancer Symposium

Publication Number: P1-03-08
Average Grade: 6.00

Title: Different genomic rearrangements account for 17% of BRCA1/2 mutations in Greece

Angela Apessos¹, Eirini Papadopoulou¹, Vassiliki Metaxa-Mariatou¹, Konstantinos Agiannitopoulos¹, Christos Markopoulou², Vasileios Venizelos³, Grigoris Xepapadakis⁴, Maria Vasilaki-Antonatou⁵, Antonios Keramopoulos⁶, Nikolaos Bredakis⁶, Aristidis Tsiftsoglou⁷, Georgios Kesisis⁷, Stylianos Kakolyris⁸, Nikolaos Touroutoglou⁹, Ioannis Natsiopoulos⁹, Konstantinos Papazisis¹⁰ and Georgios Nasioulas¹.

¹Genekor MSA, Athens, Greece; ²Medical School, University of Athens, Athens, Greece; ³Metropolitan Hospital, Athens, Greece; ⁴Rea Maternity Hospital, Athens, Greece; ⁵IASO General Hospital, Athens, Greece; ⁶IASO Maternity Hospital, Athens, Greece; ⁷St Luke's Hospital, Thessaloniki, Greece; ⁸University General Hospital of Alexandroupoli, Alexandroupoli, Greece; ⁹Interbalkan Medical Center of Thessaloniki, Thessaloniki, Greece and ¹⁰Euromedica General Clinic of Thessaloniki, Thessaloniki, Greece.

Body: AIM: The aim of this study was to further delineate the extent and nature of mutations in the BRCA1 and BRCA2 genes, responsible for hereditary breast and ovarian cancer in Greek families.

MATERIALS & METHODS: Genomic DNA was isolated from whole peripheral blood of patients referred to our center for mutation analysis of the BRCA1 and BRCA2 genes. Patients were included on the basis of affected family members, types of cancer present in the family and age at diagnosis of breast cancer in the proband. Families were subdivided into high, medium and low risk depending on the number of affected family members, types of cancer diagnosed in the family and age at diagnosis of affected family members. In total, 675 families have been analyzed by our group in the past 4 years. Mutation analysis in all cases included sequencing of the coding region and the splice sites of the two genes. In addition, MLPA analysis was carried in 585 of the patients.

RESULTS: In total, a pathogenic mutation has been identified in 12% of the 675 patients analyzed. Of the 78 mutations identified in total, 13 (17%) were large genomic rearrangements. These were deletions of exons 8, 20, 23, 23-24 and the entire BRCA1 gene, in addition to a duplication of exons 3-8 of the BRCA1 gene. As far as BRCA2 is involved deletions of exons 3, 15 and the entire BRCA2 gene were detected. All deletions were confirmed by use of other MLPA probe sets and/or relative quantitation by Real Time PCR. Of the rearrangements identified, two, namely deletions of exon 20 and exons 23-24 of the BRCA1 gene were identified in more than one unrelated families. In addition, the recurrent mutations 5382insC and G1738R, which have been previously identified as founder mutations in the Greek population, were identified in multiple unrelated analyzed families.

CONCLUSIONS: Our results indicate that different large genomic rearrangements account for an important proportion (17%) of the mutations in the BRCA1 and BRCA2 genes, in Greek families at risk of carrying a germline mutation as judged by family / personal history. The use of the available technologies for the identification of such mutational events is therefore necessary when carrying out complete analysis of the genes in high risk families of Greek background.
Title: Identification of BRCA1 and BRCA2 founder mutations in population isolates from Colombia

Luis G Carvajal-Carmona, Anna Marie D Tuazon, Carolina Ramirez, Mabel Bohorquez, Rodrigo Prieto, Jorge Castro, Gilbert Mateus, Magdalena Echeverry, COLOMBUS Consortium, Justo Olaya and Alejandro Velez. 1University of California, Davis, CA; 2University of Tolima, Colombia and 3Hospital Pablo Tobon Uribe, Colombia.

Body: Breast cancer (BC) is the most common malignancy in women. Germline mutations in breast cancer risk genes, such as BRCA1 and BRCA2, account for a large portion of hereditary breast cancer families. The contribution of mutations in breast cancer susceptibility genes has been poorly investigated in minority populations, such as in Hispanics from South America. While mutations in BRCA1 and BRCA2 display a heterogeneous mutation spectrum, specific mutations often occur in population isolates. Using the KASP genotyping technology, we screened unselected Colombian breast cancer patients for three common BRCA1 and one BRCA2 founder mutations in the Hispanic population. The BRCA2 c.2806_2809delAAAC founder mutation was identified in 3 of the 476 BC cases (0.6%). Strikingly, 28 out of 580 cases (5.0%) harbored the BRCA1 founder mutation c.3331_3334delCAAG, which has also been reported in Southern European populations, including Spain and Portugal. The clinical data and family history of these patients have highlighted a distinct geographic region, Neiva, from where these cases originate. 10% of the BC cases recruited from Neiva harbored the same BRCA1 mutation, potentially representing one of the most remarkable founder effects reported in human populations. To date the arrival of the BRCA1 c.3331_3334delCAAG mutation to Colombia, we are completing haplotype analysis of the patients and their families, along with BC cases from Spain and Portugal that harbor the same mutation. Interestingly, haplotype analyses of our BC cases have revealed a haplotype larger than 3.5 cM. In the future, we aim to perform haplotype analysis of family members of the mutation carriers and BC cases from Portugal and Spain to further study the haplotype around this BRCA1 mutation, which will allow us to date the origin of the mutation. By understanding the prevalence of mutations in known breast cancer risk genes in minority populations, cancer disparities can be better addressed and breast cancer screening can be improved. Furthermore, the ability to identify such strong founder effects highlights an advantage of using this Colombian population isolate in facilitating the identification of novel BC risk genes.
Exome sequencing identified emergence of HER2 kinase domain mutations in trastuzumab-resistant breast cancer

Yizhou Jiang¹, Ke-Da Yu¹, Wen-Jia Zuo¹, Yu-Jie Wang¹ and Zhi-Ming Shao¹. ¹Fudan University Shanghai Cancer Center, Shanghai, China.

Purpose
Human epidermal growth factor receptor 2 (HER2)-positive breast cancer is primarily treated with trastuzumab and other HER2-targeted therapies. However, approximately 30% of HER2-positive tumors exhibit de novo resistance, whereas 40% acquire resistance to these therapies. The mechanisms underlying drug resistance are unclear, and a better understanding of acquired resistance to HER2-targeted therapy is essential for the development of more effective therapeutics.

Methods
We enrolled 11 patients with HER2-positive breast cancer, all of whom had been treated with trastuzumab for one year and developed lung metastasis in the follow-up. We conducted exome sequencing of the primary and paired metastatic tumors from four patients. We identified somatic mutations in HER2 (K753E and L755S) affecting the kinase domain only in the metastatic lesions of two cases. Sanger sequencing was performed to analyze all exons of the HER2 gene in the paired primary and metastatic tumors of the remaining seven cases. Furthermore, we enrolled 48 patients with HER2-positive disease who underwent trastuzumab-based neoadjuvant therapy. We performed Sanger sequencing of the HER2 gene in paired tumor DNA before and after neoadjuvant therapy. To determine the phenotype of these mutations, we functionally characterized the HER2 mutations using protein structure analysis, in vitro kinase assays, cell culture, and xenograft experiments.

Results
We identified somatic mutations in HER2 affecting the kinase domain in four of 11 metastatic tumors (one case with K753E and three cases with L755S). None of the primary tumors harbored HER2 mutations. Furthermore, Sanger sequencing in the neoadjuvant setting revealed two of 48 cases harboring HER2 mutations (K753E and L755S) in the post-treatment samples, none of whom had any HER2 mutation in the paired pre-treatment tumor. Protein structure visualization hinted that the HER2 L753 and L755 side chains were in close proximity to the binding site for small-molecule inhibitors, indicating mutations at this residue might produce drug resistance. In vitro kinase assays revealed that HER2 K753E had greater tyrosine kinase-specific activity than wild-type. HER2 autophosphorylation, phosphorylation of HER2’s dimerization partners (EGFR or HER3) and downstream signaling proteins were significantly upregulated in MCF10A and MCF7 cells overexpressing HER2 K753E or L755S. Trastuzumab or lapatinib treatment did not decrease the number of colonies formed in MCF10A cells transduced with K753E or L755S. MCF7 cells bearing K753E or L755S mutant could reverse the inhibition of lapatinib in xenografts. The MCF10A-HER2 K753E and L755S cells were resistant to trastuzumab (IC₅₀>1000μg/mL) and lapatinib (IC₅₀>10μmol/L) but could be readily inhibited by neratinib (IC₅₀ of 15 nmol/L), an irreversible kinase inhibitor of HER2.

Conclusion
These data implicate that mutations in HER2 kinase domain are a key mechanism in resistance to HER2-targeted therapy. The selection of functional HER2 mutations might act as potential drivers of trastuzumab resistance during the progression of HER2-positive disease. Furthermore, more potent HER2 inhibitors such as neratinib might be of therapeutic benefit for trastuzumab-resistant breast cancer.
Title: Prognostic and predictive implications of monosomy 17 in curable HER2-amplified breast cancer

David Page¹, Yong H Wen¹, Dana Dure¹, Clifford Hudis¹, Edi Brogi¹ and Heather McArthur¹. ¹Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Background: Monosomy of chromosome 17 is identified in approximately 6% of HER2-amplified breast cancers. Monosomy 17 has been retrospectively associated with inferior response to trastuzumab in the metastatic setting. The adverse impact of monosomy 17 could be related to the loss of putative genes encoding DNA-binding regulatory proteins, such as ROX or TOP2A. To date, the prognostic or predictive significance of monosomy 17 in women with potentially curable HER2-positive breast cancer has not been fully evaluated.

Methods: Through a search of institutional databases, we identified women with HER2+ breast carcinoma with monosomy 17 by fluorescence in situ hybridization (FISH) and treated at our center between 1/1/00 and 6/1/11. HER2-amplification was defined as a HER2/CEP17 copy ratio ≥2.0, and monosomy 17 was defined as the presence of ≤1.4 CEP17 copies/cell. Clinical outcomes were determined by chart review, defined by Standardized Definitions for Efficacy (STEEP). Differences in treatment and other disease characteristics were compared by the log-rank test.

Results: We identified 99 women with stage I-III HER2-positive breast cancer showing monosomy 17 by FISH: 51% (n=50) were treated with trastuzumab plus chemotherapy (tras/chemo), 31% (n=31) with chemotherapy (chemo alone), and 18% (n=18) with neither (no tras/chemo). An adjuvant anthracycline was administered in 82% of women in the tras/chemo group and 97% of women in the chemo group. The 3 treatment groups were balanced for age (median: tras/chemo 49y; chemo 53y; neither 56y), but were not balanced for hormone receptor (HR)-status (HR+: tras/chemo 96%; chemo 81%; neither 78%, p=0.04) or TNM stage (stage III: tras/chemo 16%; chemo 27%; neither 0%, p=0.01). With a median follow-up of 7y (0.16-13.4y), 4y distant relapse free survival (DRFS) was 100% for the tras/chemo group, 87% for the chemo alone group, and 80% for the no tras/chemo group (p=0.018); and 4y overall survival (OS) was 100% for the tras/chemo group, 93% for the chemo alone group, and 81% for the no tras/chemo group (p=0.005).

Conclusions: Here we report the largest retrospective series of patients with curable HER2-amplified breast cancer and monosomy 17. HER2-amplified monosomy 17 does not appear to adversely affect survival in the curative setting, with outcomes comparable to those reported in large phase III adjuvant trastuzumab trials. Despite receiving less anthracycline-based chemotherapy, better OS and DRFS were observed in women treated with tras/chemo compared with women treated with chemo alone, thereby supporting the critical role of trastuzumab in this patient population, despite data to the contrary from the metastatic setting.
Title: Activating HER2 mutations promote oncogenesis and resistance to HER2-targeted therapies in breast cancer

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Body: Purpose: Somatic mutations in the tyrosine kinase domain of human epidermal growth factor receptor2 (HER2) have been reported to lead to resistance to HER2-targeted therapies in HER2-positive breast cancer, while activating mutations of HER2 have been described in HER2-negative breast cancer. The prevalence, clinicopathological characteristics, and phenotypes of HER2 mutations are not well established, thus we sought to describe the HER2 mutation profile of Chinese breast cancer patients.

Methods: DNA samples were gathered from breast cancer patients undergoing neoadjuvant (N=102) or adjuvant therapy (N=498) at Fudan University Shanghai Cancer Center between January 1, 2006 and December 31, 2012. Sanger sequencing was performed to analyze all exons of HER2 to identify somatic mutations. To determine the phenotypes of novel HER2 mutations, in vitro kinase assays, protein structure analysis, cell culture, and xenograft experiments were conducted.

Results: 10 HER2 somatic mutations were observed in 17 patients (17/600, 2.83%). 7 novel HER2 mutations were uncovered, 4 in the transmembrane domain and 3 in the kinase domain. Kinase domain mutations L768S and V773L were detected in HER2-negative tumors, while K753E was found in HER2-positive disease. In vitro kinase assays found that L768S and V773L exhibited a significant increase of tyrosine kinase-specific activity, while Western blots showed that L768S and V773L strongly increased phosphorylation of all signaling proteins in both MCF10A and MCF7 cell lines, indicating that they were activating mutations. In Matrigel cultures, L768S and V773L formed acini when seeded in vehicle, but maintained spherical morphology when seeded in culture containing trastuzumab. The addition of lapatinib in Matrigel culture inhibited the growth of all except K753E, which was successfully inhibited by neratinib. Similarly, L768S, V773L and K753E increased the number of cell colonies formed in soft agar, trastuzumab and lapatinib treatment decreased the number of colonies formed by L768S and V773L, but only neratinib could inhibit the colony growth of K753E. Xenograft showed L768S and V773L displayed a more rapid growth, while K753E showed resistance to lapatinib in vivo. MCF10A cells bearing K753E mutation were found to be resistant to lapatinib (IC50>10,000 nmol/L), but could be inhibited by neratinib, though requiring a relatively higher dosage (IC50 of 32 nmol/L) than HER2 WT (IC50 of 480 nmol/L for lapatinib, <2 nmol/L for neratinib) and other HER2 mutations. Meanwhile, clinical follow-up showed that the 2 patients with K753E mutation who received adjuvant trastuzumab treatment presented with either brain or bone metastasis, in their 3rd and 5th year after initial cancer diagnosis, suggesting K753E mutation may have a role in trastuzumab resistance as well.

Conclusions: HER2 somatic mutations were found in 2.83% of patients in this study. HER2-positive tumors harboring certain HER2 kinase domain resistance mutations may not benefit from trastuzumab or lapatinib treatment, and neratinib may offer an alternative treatment option for these patients. HER2-negative disease with activating mutations may benefit from HER2-targeted therapies, and may be of interest in prospective clinical trials.
Title: Heterogeneity and clonal evolution revealed by exome sequencing of primary breast cancers with paired metastatic lesions

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Body: Genomic heterogeneity in primary solid tumors has been extensively studied using deep sequencing technologies during the last decade. However, little has been described about the genomic profiles of the metastatic lesions and their relation to its original malignant cell population. Here, we report the exome sequences of paired primary-metastasis tumors from ten breast cancer patients. The heterogeneity of cancer tumors is today a well-established concept with a low number of genes being recurrently mutated in over 10% of the tumors, for example TP53 and PIK3CA. However, most available data relates to the primary breast cancer tumors and little has been described about the mutational profiles of the metastatic lesions and their relation to its original malignant cell population. Prospective and numerous retrospective studies have demonstrated lack of stability of the standard breast cancer prognostic and therapy predictive factor Er, Pr and Her-2. Altered receptor status in the metastatic lesion occurs at high rates ranging from during cancer progression and is additionally affected by adjuvant therapy with major implications for management of the metastatic disease. Recently, a number of studies have described how genetic diversity develops sequentially from primary tumor, to lymph node and distant metastasis.

We compared the exome sequences from ten pairs of primary breast carcinomas and their metastatic lesion. We found a marked heterogeneity of somatic mutations as well as chromosomal aberrations in the metastatic lesions, which had sometimes greatly diverged from the primary tumors. A number of mutated genes were enriched in the metastases including, significantly, members of the A-kinase anchoring protein (AKAPs), a family of adaptor proteins that anchor Protein kinase A (PKA) to diverse subcellular locations. In addition to somatic mutations and deletions the gene expression of the AKAP family followed a pattern that relates tumor intrinsic subtype with PKA subcellular localization.

Our findings indicate that in metastatic lesions, the primary tumor genome is extensively transformed, with enrichment of mutations in a distinct set of genes. Together, these findings suggest the involvement of AKAPs in the metastatic process and provide a potential avenue for targeted therapy directed at metastatic breast cancer. Molecular and genetic characterization of the metastatic lesions is not only important in the clinical setting but should also provide the means to reveal genetic patterns specific for the disseminated malignancy.
Title: Prevalence of PIK3CA mutations in tumor tissue of a consecutive cohort of breast cancer patients (n=700)

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Body: Introduction:
The AKT / mTOR pathway is activated by phosphorylation of the lipid kinase phosphatidylinositol 3-kinase (PI3K) and regulates proliferation, apoptosis, survival and adhesion of tumor cells. In up to 30% of breast cancers, dysregulation of PI3K is reported, resulting from mutations in the PIK3CA gene that encodes the catalytic subunit (p110a). Hotspots of mutations were described in exon 9 (helical domain, E542K, G > A) and exon 20 (kinase domain, H1047R, A > G). Prevalence and the prognostic impact of the PIK3CA mutations as well as the predictive value with regard to endocrine therapy are controversially discussed. In this study we describe the prevalence of PIK3CA mutations in a consecutive cohort of breast cancer patients and its association to tumor characteristics and known prognostic factors.

Material & Methods:
Fresh frozen tumor samples were obtained from a consecutive cohort of 700 breast cancer patients (inclusion criteria: pTx pNx M0 Gx HRx HER2x) who were primarily operated in 6 centres between 2009 and 2011. Tumor DNA was extracted and analyzed by conventional and quantitative PCR (exon 9: cosmid 763 and exon 20: cosmid 775). Patient data, tumor characteristics and prognostic factors were obtained from patients charts. ER, PgR and HER2 results were based on local pathology, uPA and PAI-1 values were determined centrally.

Results:
All isolated DNA (n=700) were analyzable by PCR and showed a mutation rate of 22% in the entire cohort; including two tumors with both mutations. Among the tumors with a positive steroid hormone receptor (HR) status (n=606; 87%), we found a mutation rate of 24% for PIK3CA, whereas only 9 (10%) of 94 HR-negative tumors were mutated. Tumors with a PIK3CA mutation were significantly associated with HR positivity / HER2 negativity (p=0.001). 14% of the cohort was HER2 positive. In 98 cases with a HER2 positive tumor 17 DNAs were mutated (17%) and 139 DNAs of the 602 HER2 negative tumors had a positive PIK3Ca mutation status (23%). Of the mutated tumors, 94 % were HR positive, and 11% HER2 positive. Only three samples were HER2 positive and HR-negative. 6% of the mutated tumors were triple negative.

No significant association was found to age, menopausal status, tumor stage, nodal status, grading and uPA/PAI-1 status. Most mutant tumors were histological grade 2 (p=0.001). A quarter of the intermediate risk group according to St. Gallen risk estimation had a somatic PIK3CA mutation.

Conclusion:
In our cohort, nearly all mutated tumors are hormone receptor positive and a minority of all mutated samples is HER2 positive. This data adds important information to the heterogeneous results of other previously published patient cohorts. It suggests a possible role of PI3K-dysregulation with regard to resistance against endocrine therapy and anti-HER2-treatment.
Title: Optimized mixed micellar nanoparticle as carrier improves therapeutic effect of PLK1 siRNA for breast cancer

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Body: Small interfering RNA (siRNA) brings a new dawn for the treatment of breast cancer. However, the biggest challenge in systemic administration of siRNA drugs is the siRNA clearance in vivo. Nano drug delivery systems for siRNA delivery have made significant progresses, but the size effect of nanoparticles affecting its efficacy for cancer therapy is controversial. We successfully established a new method for preparing mixed-micelle nanoparticles (MMP) with controllable sizes (40, 90, 130 and 180 nm). All MMPs could load, release and protect siRNA from the RNase degradation. The MMPs were proved to be stable in the serum and with low toxicity. In addition, all MMPs carrying FAM-siRNA were taken up by breast cancer cells, while larger MNPs were more taken up by the cells due to the extra endocytosis pathways. MMPs loaded with Polo-like kinase 1 siRNA (siPLK1) significantly inhibited the proliferation of breast cancer cells and induced cell apoptosis through inhibiting PLK1 gene expression. In addition, such effect was strengthened along with the increased size. Moreover, MMPs loaded with siPLK1 inhibited tumor growth in tumor-bearing mice, but the nanoparticles with average diameter of 90 nm showed strongest effect. Although nanoparticles with smaller diameters (40 and 90 nm) had longer blood circulation time and showed more accumulation in tumor tissue following systemic administration, MNPs with larger diameters (130 and 180 nm) were more efficiently taken up by cancer cells after they arrived the tumor tissue in vivo. Therefore, therapeutic effect of MNPs carry siPLK1 to breast cancer showed an equilibrium effect and reached highest inhibitory effect to tumor growth when nanoparticles with a diameter of 90 nm was used as the carrier. In conclusion, we demonstrate that optimized mixed micellar nanoparticle as carrier of siPLK1 significantly improves the therapeutic effect to breast cancer treatment, which provides a powerful technic to improve the efficacy of siRNA delivery for breast cancer therapy.
Title: Histone deacetylase HDAC7 is a putative therapeutic target in invasive lobular carcinoma

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Body: Invasive lobular carcinoma (ILC) is the second most common histological subtype of breast cancer, accounting for 10-15% of cases. Though ILC represents a minority of breast cancer patients, ILC affects over 30,000 women annually in the US, making ILC the 6th most common women’s cancer. Despite this incidence, no ILC-specific therapeutic options exist. The majority of ILC are estrogen receptor (ER)-positive (>90%); this and other biomarkers suggest that ILC patients are ideal candidates for endocrine therapy. However, the efficacy of endocrine therapy in ILC is poorly understood since no ILC-specific prospective clinical trial data exist. Recent retrospective analyses suggest that a subset of ILC patients may not benefit from endocrine therapy. Understanding the unique mechanisms of endocrine response and resistance in ILC is critical to improving ILC patient outcomes.

We recently identified estrogen-mediated gene expression specific to the ILC cell lines MDA MB 134VI and SUM44PE. ILC-specific ER target genes were enriched for repression by estrogen. Further, tamoxifen regulated estrogen-repressed genes as an agonist in MDA MB 134VI cells, in parallel with tamoxifen-induced growth. Thus, ER-mediated repression of target genes may be critical in maintaining cell growth and survival. Our laboratory previously identified that ER-mediated gene repression in breast cancer cells is mediated by histone deacetylase 7 (HDAC7). E2-induced repression requires specifically HDAC7, not HDAC1-6/8-10. In a panel of breast cancer cell lines, HDAC7 was most strongly expressed in MDA-MB-134VI cells, the only cell line among the panel derived from an ILC. Increased HDAC7 expression in ILC versus invasive ductal carcinoma (IDC) can also be observed in the Cancer Genome Atlas; mean HDAC7 expression in ER-positive tumors is 2-fold higher in ILC (n=148) versus IDC (n=508) (p<0.0001). We hypothesize that HDAC7 mediates the unique ER-mediated gene repression in ILC cells, and may serve as a novel therapeutic target in conjunction with endocrine therapy for ILC.

To model HDAC7 inhibition, we generated stable cell lines carrying inducible HDAC7 shRNA constructs from MCF-7 (IDC) and MDA MB 134VI (ILC). These lines are being used to examine the effects of HDAC7 depletion on estrogen- and tamoxifen-mediated gene expression, cell proliferation, anoikis resistance, and endocrine resistance. Parallel studies using the class Ila HDAC inhibitor MC1568 are being used as proof-of-principle for using HDAC7-specific inhibitors. Additionally, we identified novel splice variants of HDAC7 in breast cancer cells. Based on the mouse homolog Hdac7, these splice variants may alter the ability of HDAC7 to interact with transcription factors, or change its activity as a transcriptional repressor.

Our observations suggest that HDAC7 may mediate ER-mediate gene repression in ILC cells, which may be necessary for cell proliferation and endocrine resistance in this breast cancer subtype. Preclinical studies using ILC model systems will identify the role of HDAC7 and specific splice variants in controlling ER-driven gene expression. Further studies using HDAC7-specific inhibitors will determine whether targeting HDAC7 in ILC patients can improve endocrine response and inhibit endocrine resistance.
Identifying hypermethylated tumor suppressor genes in breast cancer with an in vivo total genome knockdown screen

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Changes in gene expression are required for the progression of breast cancer; and DNA methylation, which silences tumor suppressor genes, is often responsible for these changes. Several de-methylating/DNA synthesis inhibiting drugs (such as decitabine) are potential breast cancer therapies; however, for their application to be successful, we need to identify which patients would most benefit from these treatments. To accomplish this, the critical genes, which must be silenced by hypermethylation in order for breast cancer to progress, need to be identified. For this purpose, we have performed an in vivo total genome knockdown screen.

A genome-wide lentiviral-based shRNA screen was performed with breast cancer cell line MDA-MB-231 tumors implanted in the mammary fat pads of female NOD/SCID mice, with or without decitabine treatment (50mg/kg). The resulting tumors were harvested and shRNA sequences retrieved and hybridized to a Decode microarray, allowing for the identification of shRNA sequences that were enriched or depleted under decitabine treatment. The enriched shRNA sequences likely target genes of two categories: 1) they are methylated in the tumor and when expressed have tumor suppressive function, or 2) they are required for decitabine-mediated DNA synthesis inhibition and apoptosis. To determine if the shRNA-targeted genes identified in the screen fall within category 1 or 2, further analysis was done using GEO datasets for methylation in breast cancer cell lines, primary tumors, and normal breast tissues.

In replicates of 6 mice, 111 shRNA sequences were enriched more than 2-fold in 5/6 mice. Of the 111 genes, 29 genes showed significant enrichment (p<0.05) in the decitabine-treated tumors. Using the GEO datasets, 19 of those significantly enriched genes fit a tumor suppressor methylation profile and merit further investigation of their methylation status in the MDA-MB-231 cell line. The remaining enriched genes may play a role in DNA synthesis inhibition and apoptosis- this will be confirmed with in vitro apoptosis assays. DAVID analyses did not reveal any functional clustering of the genes of interest, but did implicate several pathways that involved a single potential hypermethylated tumor suppressor gene.

We have identified several genes that are hypermethylated in breast cancer and are potentially involved in tumor progression. Confirmation experiments will reveal a list of genes that when found hypermethylated in patient tumors would identify candidate breast cancer patients who would benefit from decitabine treatment.
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Title: Maintenance of a lean phenotype is associated with increased ERβ expression and ERβ gene intron methylation in murine MMTVneu luminal mammary cancer

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Body: Background: Obesity is associated with increased breast cancer mortality in premenopausal women, who predominately develop estrogen receptor (ER)-α negative breast tumors. New mechanism-based targets and intervention strategies for offsetting the procancer effects of obesity are urgently needed given the rising rates of obesity in women throughout the world and the lack of effective targeted therapies for ER-negative breast cancer. Unfortunately, the mechanisms by which obesity impacts ER-negative breast cancer prognosis remain unclear. Methods: In this study, we utilized the MMTV-neu transgenic mouse model of Her2+ breast cancer to test the hypothesis that dietary energy balance modulation alters mammary tumor development and progression through epigenetic regulation of ER in the mammary epithelium of these mice. MMTV-neu transgenic mice form spontaneous mammary tumors that progress from an ERα-positive hyperplasia to aggressive ERα-negative ductal adenocarcinomas. Female MMTV-neu transgenic mice were randomized to 3 diet regimens (30/regimen), resulting in either an obese, overweight or lean phenotype. A subset of mice were killed at baseline, 1, 3, and 5 months following diet initiation, and tissues were collected for analysis; remaining animals were followed for up to a 22 month survival study. Results: Gene and protein expression analysis revealed that mammary ERα expression, known to be lost by 8 weeks of age in normoweight MMTV-neu transgenic mice, was maintained in lean mice but lost in the overweight and obese mice (which did not statistically differ in any parameter studied). Additionally, we found lean mice had significantly increased mammary ER gene expression and delayed onset of hyperplasia relative to the overweight/obese mice. ERα and ERβ gene intron methylation increased from 5 months to 12 months on diet in lean mice and conversely decreased in overweight/obese mice, which parallels the ER gene expression data. After 22 months of feeding, lean mice had significantly increased tumor-free survival in relative to the overweight/obese mice. Interestingly, human and mouse HER2+ (but not but not HER2-) breast cancer cell lines, when treated with serum from obese (BMI>30), women resulted in a significant down-regulation of ERβ gene expression. Conclusions: In MMTV-neu transgenic mice, dietary energy balance modulation impacts spontaneous mammary tumor development and ERα and ERβ levels in normal and tumor tissue, possibly through epigenetic mechanisms. Moreover, increased mammary ERβ expression may represent a novel target for interventions to offset the enhancing effects of obesity on breast cancer.
**Title:** Super-enhancer analysis defines breast cancer subtype and identifies tumor dependencies

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**Body:** Epigenomic modifications define gene regulatory features that control transcription and disease cell state. Recent studies of these regulatory features have identified large clusters of enhancers, termed super-enhancers, which define key cell identity and disease genes. Using ChIP-seq and RNA-seq analysis, we have discovered Super-enhancers in breast cancer cell line models and in primary tissue and have characterized their roles in establishing tumor cell state. We find that Super-enhancers recapitulate clinical subgroups in both breast cancer cell line models and in invasive ductal carcinoma. Super-enhancer-associated genes encode known and novel therapeutic targets including kinases, phosphatases, chromatin regulators and transmembrane proteins. Such genes include key drivers such as ERRB2 in HER2+ patient samples, ESR1 in estrogen receptor positive samples, and CCND1 in samples of luminal subtype. We describe the biological and disease relevance of Super-enhancer-associated genes in the context of tumor cell state and drug target discovery.
Title: DNA methylation-based classification are mostly concordant with intrinsic subtypes of breast cancer

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Body: Backgrounds:
Breast cancer (BC) is a heterogeneous disease and usually divided into 5 subtypes according to clinicopathologic features. These subtypes have concordance with mRNA-based subtypes called as "intrinsic subtypes". Further gene expression analysis categorized triple negative BCs into 7 sub-subtypes which relate to clinical behaviors. DNA methylation is one of the epigenetic systems to regulate gene transcription. DNA methylation in CpG islands (CGIs) associated with the gene promoter region affects gene expression levels. Recently genome-wide analyses suggested that methylation status not only in CGIs, but also in CpG shores (within 2000 bps from CGIs) and CpG shelves (2000-4000 bps from CGIs) contribute to gene silencing. Thus, we hypothesized that intrinsic subtypes may possess specific methylation profiles especially in regions surrounding CGIs (islands, shores and shelves). To date, several reports demonstrated each intrinsic subtype has specific methylation patterns in CGIs. They indicate that DNA methylation is promising markers for cancer detection, prediction of therapeutic response and clinical outcomes. However, there were no report that conducted a genome-wide high-resolution epigenetic analysis for BC.

Objectives:
This study aims to determine whether specific DNA methylation patterns exist in CGIs, shores and shelves in BC cells, and whether the DNA methylation-based classification correlates with intrinsic subtypes, furthermore, to identify the subtype specific epigenetic markers using high-resolution methylation array.

Materials and Methods:
DNA was extracted from 31 samples of 28 BC cell lines (8 luminal, 18 basal, 2 unclassified) and 3 samples of 3 non-BC cell lines. We performed genome-wide methylation analysis using Illumina Infinium HumanMethylation450 BeadChip. We did unsupervised hierarchical clustering analysis of cell lines using top 3000 variably methylated (VM) loci among cancer cell lines. We also analyzed top 3000 VM CGI-related loci. Gene functional annotation clustering was performed using DAVID.

Results:
In top 3000 VM loci, CGIs were most frequently observed (43.0%). According to clustering analysis using top 3000 VM loci, cell lines were roughly classified into 4 clusters. There were trends that cell lines of same subtype fell into the same cluster, but not definitely. In case of clustering using top 3000 VM CGI-related loci, cell lines were clearly divided into 2 clusters, high and low methylated clusters. High methylated cluster contained almost all luminal cell lines and low methylated cluster contained most of basal cell lines and all non-BC cell lines. In low methylated cluster, cell lines of the same sub-subtype, such as Basal-like 1 and 2, were clustered closely. Gene functional annotation clustering suggested that genes related to “transcription” and “differentiation” were more frequently regulated by epigenetics.

Conclusions:
High-resolution methylation analysis revealed methylation-based clusters were concordant with intrinsic subtypes in BC cells. Transcriptional factors and differentiation-related genes could be differently regulated by epigenetics among different subtypes of BC.
Title: Meta-analysis reveal novel mechanism of bisphenol A effect on mammary gland

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Body: Pubertal exposure to bisphenol A (BPA) has been shown to cause abnormal mammary gland development and neoplastic transformation in adults. However, the mechanism of this BPA effect still remains largely unknown. We exposed 21-day-old Balb/c mice to BPA by gavage (25 µg/kg/day) during puberty for 3 weeks. Primary mammary cells were isolated at 6 weeks, and 2 and 4 months of age and subsequently sorted into Lin-CD49f-CD24-stromal cells, Lin-CD49fhighCD24med MaSCs-enriched basal cells and Lin-CD49flowCD24high luminal progenitor-enriched cells with FACS for gene expression analysis. The Gene Ontology (GO) Enrichment Analysis shows that upon BPA treatment, the biological processes (BPs) significantly enriched in luminal compartment were related to response to organic substance and hormone stimuli at early stage (2 month old) whereas to cell cycle regulation at late stage (4 month old), consistent with published study in mammary gland morphology where BPA promoted the expansion of luminal population. However, the BPs enriched in spheres derived from basal cells (highly enriched with MaSCs) collected at late stage (4 month old) were mainly related to cell adhesion, epithelial differentiation and in stromal cells were related with vascular development for tissues collected either immediately after BPA treatment or 6 weeks later at 4-month of age. More interestingly, the Downstream Function Effects Analysis performed by Ingenuity Pathway Analysis reveal the changes in any compartments will all lead to significant activation of cancer-related downstream effects including hyperplasia, neoplasm of mammary tumor, proliferation of tumor cells and invasion of breast cancer, although there are few overlapping common genes in these compartments and conditions upon BPA treatment, indicating the different mechanism in response to BPA treatment and tumorigenesis. Network analysis revealed that the potential upstream regulators for these enriched BPs are TGFβ1, VEGF, PPARG, Estrogen/Estrogen Receptor, glucocorticoid, ERK and NOTCH signaling. Together with our recently published data, this study reveals the difference in immediate and delayed response to pubertal BPA treatment and indicates that cross-talk between several growth factor and hormonal receptor signaling pathways may contribute to the altered cellular processes by BPA, consistent with the role of BPA as an endocrine disruptor.
Title: Modeling breast cancer dormancy and re-emergence

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Body: Most breast cancer (BrCa) mortality results from distant metastases. Current evidence strongly suggests that in some instances these disseminated cells remain dormant for long periods of time. Both the non-proliferative state and protective microenvironment of the metastatic niche likely contribute to the observed resistance of metastases to chemotherapies that are otherwise effective against the primary tumor. Although significant interventional progress has been made on primary tumors, the lack of relevant accessible model systems for metastases has hindered the development of therapies against this stage. To address this gap, we developed an innovative all-human 3D ex vivo hepatic microphysiological system (MPS) to faithfully reproduce human physiology and thereby facilitate the investigation of BrCa behavior in a micrometastatic niche. The liver is a major site of metastasis for carcinomas and is also the primary site of drug metabolism (activation and/or detoxification), which is a significant factor in determining efficacy and limiting toxicities of cancer therapies.

The MPS incorporates hepatocytes and nonparenchymal cells (NPC) isolated from fresh human liver resections. BrCa cells (RFP+) are seeded on day 3 and afforded time to intercalate into the hepatic tissue until treatment with chemotherapy on day 7 for 72h. Surviving BrCa cells are stimulated on day 13 with LPS/EGF and cultured through day 15. Proliferation is monitored by RFP quantification, Ki67 staining and EdU incorporation. Physiological function of the hepatic tissue is monitored throughout the experiment by protein catabolism (urea), active metabolism (glucose, CYP P450) and injury markers (AST, ALT, A1AT, fibrinogen). Luminex assays (55 analytes) were used to provide insights into the communication networks in the hepatic metastatic milieu during different stages of dormancy and progression, and identify potential metastatic biomarkers via computational approaches.

The MPS maintains the physiologic function of the hepatic niche through 15 days and BrCa cells effectively integrate into the established niche. Spontaneous dormancy is observed amongst a subpopulation of BrCa cells, indicated by the absence of Ki67 staining and EdU incorporation after 12 days of culture. Further, we demonstrate that the BrCa cells surviving chemotherapy (doxorubicin) are non-proliferating (Ki67-/EdU-). Notably, ‘re-awakening’ of the surviving non-proliferating cancer cells is observed in the presence of physiological inflammatory stressors (LPS/EGF). Luminex analyses of the milieu effluent identified signaling molecules from NPC influenced the metastatic cell fraction entering dormancy.

This MPS provides unprecedented insitters into the tumor biology of dormant micrometastases. We demonstrate the recreation of spontaneous, rather than engineered, BrCa dormancy in an all-human ex vivo hepatic MPS. Mimicking the dormancy and outgrowth observed in patients, we found that dormant breast cancer cells that are resistant to chemotherapy can be stimulated to re-emerge following an inflammatory insult. Ultimately, this MPS provides an accessible tool to identify new therapeutic strategies for metastasis during initial seeding, dormancy and re-emergence, while concurrently evaluating agent efficacy for metastasis, metabolism and dose-limiting toxicity.
Title: Mesenchymal stem cell regulated microRNAs converge on the speech gene FOXP2 and regulate breast cancer metastasis

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Body: About 90% of breast cancer mortalities are due to the spread of breast cancer cells (BCCs) from a primary tumor to distant organs, a process known as metastasis. However, the molecular mechanisms underlying metastasis remain poorly understood. Substantial evidence now supports a major role for the tumor microenvironment (TME) in catalyzing breast cancer metastasis. Indeed, observations indicate that proximal interactions between BCCs and cells of the TME induce altered gene expression programs in BCCs, allowing for the navigation of the various steps of the metastatic cascade. Our group and others observed that breast tumors recruit mesenchymal stem cells (MSCs): multipotent fibroblasts that normally exert tissue maintenance functions. We and others have observed that physical interactions of MSCs with BCCs are sufficient to drive their metastatic dissemination in murine xenograft models, via the induction of epithelial-mesenchymal transition (EMT) and dedifferentiation into stem cell-like states (cancer stem cells, or CSCs), states tightly associated with the capacity to seed new tumors (for example in foreign tissues) and with chemotherapeutic resistance. However, the TME-induced molecular pathways regulating such mechanisms remain poorly understood.

MicroRNAs (miRNAs, miRs) are small noncoding RNAs that regulate gene expression via base-pair interactions with messenger RNAs (mRNAs), resulting in mRNA degradation or translational inhibition. Due to their ability to interact with large numbers of target mRNAs simultaneously, miRNAs are major regulators of cell identity, and thereby serve critical roles in metastasis. We performed miRnome-wide screening of MSC-stimulated BCCs to determine if TME interactions might contribute to BCC metastasis via the deregulation of miRNAs. We observed that proximal MSCs induce aberrant expression of a specific set of miRNAs in BCCs, which had not been previously implicated in breast cancer pathogenesis. These miRNAs, led by the transcriptionally co-regulated miR-199a-3p and miR-214, were sufficient to actuate the metastasis of weakly metastatic human BCCs in xenograft models. We observed that exogenous expression of the miRNAs provided BCCs with phenotypes and gene markers characteristic of CSCs, including enhanced tumor initiation capacities. Interestingly, we found that the MSC-induced miRNAs function as an interrelated network, and converge upon a common novel target: the speech associated gene FOXP2. Knockdown of FOXP2 phenocopied the metastatic phenotypes observed in MSC-induced miRNA expressing BCCs. Importantly, elevated levels of the MSC-induced miRNAs or depressed levels of FOXP2 could predict patient prognosis in the clinic. Altogether, our results incriminate FOXP2 and it’s MSC-induced miRNA regulatory network as novel determinants of breast cancer metastasis.
Title: Dual inhibition of IGF1R and insulin receptor in estrogen receptor positive and triple negative breast cancer and monitoring blockade of metastasis using novel MRI

Body: Insulin-like growth factors (IGFs) and insulin acting via the type I IGF receptor (IGF1R) and insulin receptor (IR) respectively regulate biology of estrogen positive (ER+) and triple negative (TN) breast cancer cells. In animal models, inhibition of IGF1R alone with antibodies has demonstrated significant inhibition of tumor growth and/or metastasis of several types of cancer cells. Unfortunately, the results of initial clinical trials with anti-IGF1R antibodies have been disappointing. One reason for this is that inhibition of the related IR through which insulin and IGF-II signal, may be necessary for optimal efficacy of this targeted approach. Second, there is a need to develop markers that can be used to stratify patients who may benefit from these drugs and/or monitor response to these drugs.

To examine the effectiveness of dual inhibition of IGF1R and IR, we evaluated the effects of BMS-754807, a small molecule dual tyrosine kinase inhibitor of IGF1R/IR on ER+ and TN breast cancer cells. In ER+ cells (MCF-7, T47D and ZR-75-1), BMS-754807 inhibited IGF-I, IGF-II and insulin stimulated activation of downstream PI3K and MAPK pathways, proliferation and anchorage-independent growth in vitro. BMS-754807 also blocked signaling in MCF-7 tumors and inhibited xenograft growth of MCF-7 tumors (n=10 per treatment) compared to vehicle. Interestingly, while tumor growth was suppressed over a period of five weeks, eventually the tumors displayed resistance to BMS-754807. In TN cell lines (MDA-MB-231; MDA-MB-231-LM2 and MDA-231-BoM, lung seeking and bone specific metastatic variants respectively of MDA-MB-231; and MDA-MB435A/LCC6) BMS-754807 also inhibited activation of the PI3K pathway and motility in vitro. In contrast to ER+ cells, BMS-754807 did not inhibit primary tumor growth of TN breast cancers cells injected into the mammary fat pad of mice. But BMS-754807 (50 mg/kg daily by oral gavage) inhibited metastasis of TN cells, MDA-231-LM2 and MDA-MB435A/LCC6 cells, in the orthotopic and tail vein models of metastasis compared to vehicle (n=10/group). Our data indicate that regulation of metastases and tumor growth by IGF1R can be discrete events and functional imaging to identify biological properties of metastatic breast cancer regulated by IGF1R/IR are needed to better define treatments. Therefore, we used a novel MR sequence called sweep imaging with Fourier transformation (SWIFT), where the data is acquired quasi-simultaneously with the radiofrequency pulse, to monitor response to BMS-754807. We used MDA-231-LM2 cells with the tail vein injection model of metastasis. Mice injected with breast cancer cells were treated with either vehicle or BMS-754807 and metastasis was monitored by BLI and SWIFT MRI weekly. SWIFT was sensitive in detecting inhibition of metastasis by BMS-754807.

Our results suggest that dual inhibition of IGF1R and IR is effective in blocking growth of ER+ and metastasis of TN breast cancers. However, combination of this therapeutic strategy with other agents may be necessary to prevent or delay onset of resistance. Further, noninvasive biomarkers of response to IGF1R/IR targeted drugs can be developed with SWIFT imaging.
Title: Triple negative breast cancer metastases: Protein signal transduction networks within the tumor-stromal microenvironment complement genomic analysis and stratify local versus distant metastasis

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Body: Background: Genomic profiling of primary TNBC may not reflect the lethal metastatic (mTNBC) lesions that constitute a selected subclone of the primary tumor. Moreover, while the stroma microenvironment of primary tumors has been the subject of study, almost nothing is known about the stromal microenvironment of metastasis. We conducted proteomic signal pathway network analysis of microdissected tumor cells and stroma from mTNBC samples for which whole genome sequencing was known. We addressed the following questions: 1) Can potentially actionable driver mutations in the metastasis be inferred from the activated/ suppressed protein signaling pathways in the tumor cells? 2) Are the tumor cell signaling pathways different between local and distant metastasis? 3) Does the proteomic signaling profile of the stroma provide strategies for stromal therapy of mTNBC?

Methods: Enriched populations of mTNBC cells and adjacent stromal cells were collected by Laser Capture Microdissection from 13 fresh frozen mTNBC samples. Signal pathway profiling of 130 protein and/or phosphoprotein endpoints were quantified by reverse phase protein arrays (RPPA). The samples represented local (regional lymph node and chest wall) (n=7) and distant (n=6) metastasis. Spearman rho correlation analysis identified pair-wise protein linakges (rho=0.8, p<0.01). Whole genome sequencing was also performed on the mTNBC samples (Craig DW. Mol Cancer Ther, 2012).

Results: We compared the protein signaling network of the tumor and matched stroma to investigate tumor-stroma crosstalk, and also to the genomic alterations (GAs) in the same tumor. GAs in mTNBC fell into two major categories: DNA repair or cell cycle/growth factor signaling. Proteomic analysis confirmed the down-regulation of endocrine (ER, AR, PR) and HER2 pathways. PhosphoJAK1/2 and phosphoHer3 were markedly down-regulated in mTNBCs, while EGFR and Her4 signaling were not. Spearman rho pairwise correlations revealed differences between local and distant metastases as well as between local and distant stroma. Cyclin D1 in locally metastatic tumor cells was linked with Autotaxin, PLCgamma and RUNX1, whereas in distant metastatic tumor cells, Cyclin D1 was linked with HIF-1alpha, phosphoJAK1, and HSP70. Growth factor signaling via phosphoEFGR was more prevalent in distant mTNBC tumor samples (322 linkages) compared to local mTNBC tumor samples (146 linkages). Distant stromal cell linkages were dominated by growth factor (phospho mTOR) and immune cell crosstalk, e.g. 21 linkages between CD63 in distant stromal samples compared to 1 CD63 linkage in local stromal cells.

Discussion: Proteomic signal pathway data can stratify metastatic lesions into functionally important groups based on a) clinical phenotype (distant versus local metastasis) and b) GA subtype (DNA repair vs growth factor signaling), thereby providing new actionable strategies for novel therapies, e.g. anti-JAK1 and anti-HSP70, and providing a means to prioritize potential driver mutations. Profiling the signal pathway network of mTNBC stroma, for the first time, provides novel strategies for targeting immune cell and mTOR pathways, for new classes of stromal therapy.
Title: Activation of oncogenic pathways by mitochondrial reprogramming in triple negative breast cancer

Jun Hyoung Park¹, Santhosh Kumar¹, Sajna Vithayathil¹, Kavisha Arora¹, Nagireddy Putluri², Efrosini Tsouko³, Taraka R Donti¹, Daniel E Frigo³, Chad J Creighton², Michael T Lewis², Arun Sreekumar², Lee-Jun Wong¹² and Benny Abraham Kaipparettu¹².¹ Baylor College of Medicine, Houston, TX and ²Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX.

Body: Triple negative breast cancer (TN BCa) Driver pathway is still poorly understood. Thus, it is important to identify the underlying mechanisms of triple negative breast cancer progression. Mitochondria, a semiautonomous organelle in cells, play an important role in cellular energy metabolism, free radical generation, and apoptosis. Mitochondria-nuclear crosstalk is a bidirectional pathway of communication between mitochondria and nucleus that influences many cellular and organismal activities. This crosstalk can regulate several oncogenic pathways involved in tumorigenesis. Using transmitochondrial cybrid (cybrid) technology, we generated different cybrid models under common nuclear background of TN BCa. Mitochondria from cells of different cancer potential including benign breast epithelium, moderately and highly metastatic breast cancer cell lines were used to understand cancer mitochondria regulated tumor pathways. Tumor and gene expression analysis suggested that among different cancer pathways, c-Src signaling pathway is one of the most consistently activated pathways in cybrids with TN BCa cancer mitochondria. Further analysis in parental cells and other tumor models suggested that autophosphorylation of c-Src is regulated by mitochondrial tumor characteristics. Our preliminary analysis also suggest that mitochondria targeted drugs are promising combination therapy for the management of Src-driven TN BCa. This finding is particularly important while considering the poor response rate observed after single drug therapy with Src family tyrosine kinase inhibitor Dasatinib in unselected TN BCa patients.
Title: Recurrent ESR1-CCDC170 rearrangements in an aggressive subset of estrogen-receptor positive breast cancers

Jamunarani Veeraraghavan¹, Ying Tan¹, Xi-Xi Cao¹, Jin-Ah Kim¹, Xian Wang¹, Dean P Edwards¹, Alejandro Contreras¹, Susan G Hilsenbeck¹, Eric C Chang¹, Rachel Schiff¹ and Xiao-Song Wang¹. ¹Baylor College of Medicine, Houston, TX.

Body: The crucial role of gene fusions in epithelial tumorigenesis has been recently appreciated by several milestone discoveries, but no significant recurrent translocations have yet been found in the vast majority of breast cancers that express the estrogen receptor (ER). While a majority of ER+ tumors can be effectively treated by endocrine therapy, tumors of the luminal B subtype are more aggressive and endocrine therapy-resistant. Further, the molecular blueprint of these aggressive tumors is poorly understood. Thus, characterizing the genetic alterations underlying the more aggressive forms of ER+ breast cancer is of critical significance in breast cancer management. In this study, by large-scale analyses of breast cancer transcriptome and copy number alterations, we identified recurrent rearrangements between estrogen receptor gene, ESR1 and its neighbor gene, CCDC170, which are enriched in the more aggressive and endocrine-resistant luminal-B tumors. Further screening of 200 ER+ breast tumor tissues by RT-PCR identified eight ESR1-CCDC170 positive tumors. The genomic rearrangements underlying these fusions were verified by genomic PCR and capillary sequencing. CCDC170 encodes a protein with unknown function. The observed fusion joins the 5’-untranslated region of ESR1 upstream to the coding region of CCDC170, enabling the expression of N-terminally truncated CCDC170 (∆CCDC170) under the promoter of the ESR1 gene. Consistent with the behavior of luminal B tumors, forced expression of ∆CCDC170 in ER+ breast cancer cells leads to markedly increased cell motility, invasiveness and anchorage-independent growth, and reduced endocrine sensitivity in vitro, as well as enhanced xenograft tumor formation and reduced endocrine sensitivity of the tumors in vivo. When introduced into benign breast epithelial cells, ∆CCDC170 impairs acini morphogenesis and enhances cell motility and invasiveness. Further, Knockdown of the endogenous ESR1-CCDC170 fusion variant expressed in HCC1428 cells potently inhibited cancer cell proliferation and migration, which can be rescued by forced expression of this fusion. Mechanistic studies suggest that ∆CCDC170 engages Gab1 signalosome to potentiate growth factor signaling and enhance cell motility. The augmented growth factor signaling driven by ∆CCDC170 appears to be sustained even after withdrawal of serum, and is not affected by endocrine treatment. Together, this study identified neoplastic ESR1-CCDC170 fusions in a more aggressive subset of ER+ breast cancer, which also suggests a new role of ER in breast tumorigenesis by contributing its promoter to an oncogene.
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Publication Number: P1-07-08
Average Grade: 0

Title: TP53 alteration in morphologically benign breast tissue in patients with ER/PR negative high grade breast carcinomas: Marker for early detection of high risk patients for high grade carcinomas?

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Body: Background: TP53 alterations have been identified in approximately 20% of breast carcinomas, especially in ER/PR negative high grade carcinomas. It is believed that TP53 alteration is an early event in the carcinogenesis of high grade breast carcinomas. TP53 alteration could even be identified in morphologically "benign" appearing breast tissues. Nevertheless, the precursor lesion of high grade ductal carcinoma in situ and/or invasive carcinoma is not well recognized morphologically.

Design: We retrospectively analyzed the TP53 status of 108 cases with high grade (modified Bloom-Richardson grade 3) invasive ductal carcinoma, 51 cases with non-high grade invasive ductal carcinoma, and 50 cases with benign breast from mammoplasty. The high grade carcinomas were all estrogen receptor (ER) and progesterone receptor (PR) negative and 96.3% of them were HER-2 negative. The non-high grade carcinomas were all ER and/or PR positive, 92.2% Her2 negative. Blocks with tumor and/or benign tissue were selected from each case. Immunohistochemical stain for p53 was performed. TP53 gene sequencing was performed on selected tumors with inconclusive p53 staining. Further ER and Ki67 immunohistochemical stains were performed on the blocks with p53 positive benign tissue.

Results: Of the 108 high grade carcinomas, 48 (44%) were positive for TP53 alteration. Seventeen of the 48 (35.4%) cases also showed focal p53 staining in the adjacent benign appearing tissue. Only one case was negative for TP53 alteration in tumor but with focal p53 staining in benign tissue. Of the non-high grade carcinomas, 5 (9.8%) were positive for TP53 alteration, one of which had focal p53 staining in adjacent benign breast tissue. No p53 staining positivity was identified in the mammoplasty specimens. The p53 staining positive benign glands were ER negative, and did not show an increase in Ki67 labeling index. The morphology of these cells appeared to be slightly enlarged nuclei, with a mildly increased nuclear/cytoplasm ratio, slightly irregular nuclear contours, open chromatin, and prominent nucleoli, but still within the limits of "benign" glands.

Conclusion: TP53 is already altered in "benign" appearing breast glands in patients with high grade breast carcinomas. These glands show the same ER status as their counterpart carcinomas, but remain inactive with low Ki67 labeling index, indicating further genetic changes are needed to trigger the unlimited tumor growth and fully malignant transformation. The special morphology and p53 positivity of the "benign" appearing cells could serve as an indicator for future potential risk of TP53 positive high grade breast carcinoma.
SMAD nuclear staining patterns in inflammatory breast cancer suggest non-canonical TGFβ-signalling establishing collective, sheet-like invasion

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Introduction: Inflammatory Breast Cancer (IBC) is an aggressive and highly metastatic form of breast cancer. Recent expression profiling studies revealed IBC-specific modulation of TGFβ-signalling. In the present study, we aim to validate these findings by evaluating nuclear SMAD staining patterns.

Materials and Methods: Immunohistochemistry (IHC) was performed for SMAD2, -3, -4 and -6 on tissue sections from patients with (N=79) and without (N=133) IBC. Protocols for IHC were established on cell blocks from breast cancer cell lines with and without SMAD staining identified by western blotting on nuclear protein extracts. A staining score was assigned by multiplying the percentage of stained cancer cell nuclei by the staining intensity evaluated on a three-scale basis (1=weak, 2=intermediate, 3=strong). Expression data were subjected to unsupervised hierarchical cluster analysis (UHCA) and statistical assessment of staining differences was done using uni- and multivariate models. Additionally, we looked at the correlation between nuclear SMAD expression and gene expression in 11 patients. For each gene, we generated a spearman correlation coefficient, and selected only those genes that were positively correlated with nuclear SMAD expression in 11 patients (p<0.05).

Results: UHCA for SMAD2, -3 and -4 nuclear protein expression data identified two sample clusters downstream of the first bifurcation. IBC samples were significantly (OR=60.34, P<0.001) enriched in the cluster characterized by increased nuclear SMAD2 protein expression and absence of both nuclear SMAD3 and -4 protein expression. Univariate analysis revealed that these staining patterns are significant (all Ps<0.01). Multivariate analysis demonstrated that gain of SMAD2 nuclear expression and loss of SMAD3 nuclear expression in IBC are unrelated observations and independent of histological grade, hormone receptor expression, ErbB2 amplification and tumour stage. SMAD correlation analysis showed us that genes positively correlated with nuclear SMAD2 expression included CD44 and REL, both markers that represent crucial pathways in IBC. Interestingly, nuclear SMAD3 expression was positively correlated with ZEB1.

Discussion: In line with the expectations, our data show that TGFβ-signalling indeed differs between samples from patients with and without IBC. In addition, this study does offer novel insights on IBC biology. First, SMAD2 nuclear staining is gained in IBC in the absence of its canonical binding partners SMAD3 and -4, suggesting non-canonical TGFβ-signalling in IBC. Second, it has been shown that SMAD2 induces an invasive response program in cancer cells without affecting their epithelial morphology, while SMAD3 accounts for invasion by induction of Epithelial-to-Mesenchymal transition (EMT). Thus our results indicate that IBC cell invasion occurs in a collective, sheet-like fashion instead of EMT, which is probably responsible for the invasion of non-IBC cells.
Title: Immunohistochemical (IHC) marker discordance between primary breast cancer biopsy and recurrent cancer: Would IHC testing of the surgical breast or lymph node have altered treatment?

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Body: Objective: Based on emerging data on tumor heterogeneity and the evolutionary branching of tumor cells, tumor cells in the lymph node may represent more virulent clones with the inherent capability of metastasis. IHC discordance from original cancer diagnosis to recurrence is documented to occur in up to 20% of cases, raising the question if characterization of these likely more virulent cells would more accurately guide treatment and predict prognosis. Our pilot study sought to determine if crucial clinical information is gained by IHC testing of the surgical breast or lymph node specimens at the time of initial surgery.

Methods: Using the cancer registry and oncology records, all invasive breast cancers diagnosed after 2001 with subsequent recurrence were identified. Cases missing all IHC data were disqualified. We then evaluated ER and HER2 of the primary cancer biopsy and recurrence biopsy to identify discordances. Those with discordances who had surgical breast and lymph node specimens available were accessed, tested, and evaluated by our breast cancer pathologist.

Results: A total of 128 recurrence cases with partial or complete primary and recurrence IHC data were identified. Of the 95 initially ER positive cases with recurrence IHC available, 13/95 had discordant, or ER negative, recurrence. Additionally, 5/27 initially ER negative tumors, 3/14 initially HER2 positive tumors, and 6/69 initially HER2 negative tumors had discordant recurrence results. In 128 cases, 27 cases were identified to have ER or HER2 discordance from primary biopsy diagnosis to recurrence. Of all cases with original surgical breast or positive lymph node specimen available, 9 markers on 7 patients were performed for our pilot study. One of seven surgical breast specimens and one of two lymph node specimens were concordant with the recurrence, but not the initial biopsy. The tested surgical breast was ER positive, while the surgical lymph node was HER2 positive, concordant to their recurrences, but discordant with initial biopsy.

<table>
<thead>
<tr>
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<th>Breast Biopsy</th>
<th>Recurrence Concordant</th>
<th>Recurrence Discordant</th>
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<tbody>
<tr>
<td>ER Positive</td>
<td>98/127</td>
<td>82/95</td>
<td>13/95 (14%)</td>
</tr>
<tr>
<td>ER Negative</td>
<td>29/127</td>
<td>24/27</td>
<td>5/27 (19%)</td>
</tr>
<tr>
<td><strong>Total ER Discordance</strong></td>
<td></td>
<td></td>
<td>18/122 (15%)</td>
</tr>
<tr>
<td>HER2 Positive</td>
<td>19/119</td>
<td>11/14</td>
<td>3/14 (22%)</td>
</tr>
<tr>
<td>HER2 Negative</td>
<td>100/119</td>
<td>63/69</td>
<td>6/69 (9%)</td>
</tr>
<tr>
<td><strong>Total HER2 Discordance</strong></td>
<td></td>
<td></td>
<td>9/83 (11%)</td>
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Conclusion: Tumor discordance of the original cancer biopsy and recurrence is not uncommon. Our pilot study demonstrated that ER and HER2 discordance occurred in 15% and 11% of cases, respectively. Though our pilot study was limited by small sample size, we found that IHC testing of the surgical breast and lymph node specimen may provide additional clinical information and affect management. Of the two cases that had a positive lymph node available, one was HER2 positive and concordant with the recurrence. Of the seven breast specimens tested, one was ER positive and concordant with the recurrence. Had IHC testing been performed at that time of surgery, adjuvant treatment management would have been altered. Further testing of our IHC discordant recurrence patient population will be pursued to investigate the potential benefits of surgical breast and lymph node IHC testing.
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Title: Promotion of lymphangiogenesis by postpartum breast tumor cells

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Body: Multiple studies report poor survival rates in postpartum women with breast cancer[1, 2]. A recent study from our group has specifically shown that incidence of recurrence and death from breast cancer is increased approximately 3-fold in postpartum patients compared to nulliparous patients[3]. We define postpartum breast cancers as those diagnosed within 5 years of birth and as a highly metastatic subset of young women’s breast cancer[4]. We have recently shown increased incidence of lymph node involvement in patients diagnosed less than 2 years postpartum and have utilized tissues from our clinical cohort to show increased lymphatic vessel density in stage II postpartum patient samples compared to nulliparous. Using xenograft and isograft murine models we have modeled postpartum breast cancer to show that postpartum tumors are larger[5], exhibit increased lymphatic vessel density, and more frequently spread to mouse lymph node and lung tissues[5] compared to tumors in nulliparous mice. In order to determine whether postpartum tumor cells specifically promote lymphangiogenesis, we utilized postpartum tumor cells ex vivo to show that lymphatic structure formation is promoted via increased postpartum tumor cell secretion of PGE2, which acts on the EP2 receptor on lymphatic endothelial cells. We have also performed expression analyses on postpartum tumor cells isolated from our xenograft model to reveal increased expression of COX-2, VEGF-C, and Semaphorin 7a; all three of these molecules have reported, and possibly interrelated, roles in promotion of lymphangiogenesis[6, 7]. Importantly, both in vivo inhibition and knockdown of COX-2 decrease postpartum tumor associated lymphangiogenesis in pre-clinical models. These results, along with our results indicating that COX-2 inhibition during the postpartum period decreases metastasis in our pre-clinical models, have led us to propose that COX-2 dependent promotion of lymphangiogenesis may facilitate, in part, metastasis of postpartum tumors.

Title: Defining the role of the kynurenine pathway in mediating anoikis resistance in triple negative breast cancer

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Body: Background: Anoikis resistance is thought to be a critical trait of metastatic cancer cells, enabling them to leave the primary tumor and travel through extracellular matrix, intravasate, and survive in the vasculature or lymphatics in transit to a metastatic site. This is particularly important for the triple-negative breast cancer (TNBC) subtype, which has a peak risk of recurrence within the first three years post-diagnosis and an increased mortality rate in the first five years as compared to other subtypes. We performed global profiling of TNBC cells in attached versus forced suspension culture conditions (using poly-HEMA coated plates) for 24 hours. These data revealed that TNBC cells surviving in suspension upregulate multiple genes involved in tryptophan catabolism, also known as the kynurenine pathway (KP), including the rate limiting enzyme tryptophan 2,3,-dioxygenase (TDO) and kynureninase (KYNU). A key metabolite of this pathway has been found to activate the aryl hydrocarbon receptor (AhR), which was also up-regulated in suspended cells.

Hypothesis: We hypothesize that the ability to upregulate the kynurenine pathway (KP) facilitates TNBC cell survival in suspension and mediates the migratory/invasive potential of TNBC.

Methods: We assessed mRNA and protein levels of TDO2, KYNU and AhR by RT-qPCR and western blot. AhR luciferase reporter activity, as well as known AhR regulated genes, were measured in suspension compared to adherent conditions. TNBC cells were treated with small molecule inhibitors of AhR and TDO2. Additionally, secretion of endogenous kynurenine was measured by high performance liquid chromatography (HPLC). Purified kynurenine was added to rescue AhR activity following TDO2 inhibition. Finally, anoikis sensitivity and migratory potential were measured following pharmacological inhibition of TDO2 and AhR.

Results: Relative mRNA levels of TDO2, KYNU, and AhR increase 9, 7, and 2 fold respectively in suspended TNBC cells compared to adherent conditions (P<0.0001). Estrogen receptor positive breast cancer cells lines do not significantly upregulate these genes. AhR reporter activity (P<0.0001) and nuclear localization increase in suspended TNBC cells. Additionally, AhR reporter activity (P<0.0001) and AhR target gene expression (CYP1A1, CYP1B1: P<0.0001) decreased in TNBC cells treated either AhR antagonist (CH223191) or selective TDO2 inhibitor (680C91). Conversely, when purified kynurenine was added to TNBC cells in vitro, AhR activity increased (P<0.05). Furthermore, kynurenine secretion, as measured by HPLC, increased 2.5 fold in suspended TNBC cells and this increase in kynurenine was reduced by addition of 680C91 (P<0.0001). Finally, targeting TDO2 or AhR increased anoikis sensitivity and decreased migration of attached and suspended TNBC cells.

Conclusions: Collectively, these results suggest that the kynurenine pathway may play a critical role in metastatic TNBC. Further mechanistic studies will focus on how the kynurenine pathway is mediating these tumorigenic properties either through the de novo synthesis of NAD+ and/or activation of AhR by kynurenine. Targeting the kynurenine pathway in the clinic may provide a therapeutic strategy to reduce TNBC mortality rates.
Title: Extramedullary hematopoiesis aids initiation of cancer metastasis

Kenji Yokoi¹, Tomonori Tanei¹, Megumi Kai¹, Yuki Saito¹, Yan Ting Liu¹ and Mauro Ferrari¹. ¹Houston Methodist Research Institute, Houston, TX.

Body: Despite the recent advances in treatment of primary tumors, metastatic disease is resistant to current therapies and remains the primary cause of cancer-related death. Therefore, prevention and improved therapy of cancer metastasis are the ultimate goals of cancer research. Although tumor cells are the driving force of metastasis, novel findings suggest that the tumor microenvironment also plays a key role. Microenvironments distant from the primary tumor are primed for future metastatic growth by the recruitment of bone marrow-derived myeloid cells, creating "pre-metastatic niches", and ready for arrival of circulating cancer cells. The number of platelets is often increased in the late stage of cancer patients and experimental evidences show that platelets facilitate tumor metastasis. Within the circulatory system, platelets surround and guard tumor cells from immune elimination and produce various kinds of growth factors and cytokines to aid establishment of metastasis. But the role of platelets within the pre-metastatic niches has not yet been explored, particularly with respect to their ability to aid circulating cancer cells to arrest, survive and grow.

In our study, 4T1 murine breast cancer cells, implanted into mammary fat pad (mfp) of mice were spontaneously metastasized to the lung and liver around 6 weeks after the implantation. Interestingly, splenomegaly was found in these mice bearing 4T1 tumor in the mfp. Pathological and immunofluorescence analysis of the spleen revealed extramedullary hematopoiesis including megakaryopoiesis. Consequently, platelets accumulated into the other organs of the tumor bearing mice as early as 2 weeks after the implantation of 4T1 cells to the mfp. Interestingly, platelets accumulated into lung and liver, but not to the other major organs. To validate the effect of extramedullary hematopoiesis, 4T1 cells expressing luciferase (4T1-luc) were implanted into the mfp of normal mice or mice resected with spleen in advance. Then, development of spontaneous metastasis was monitored by luminescence imaging of the live mice. There was a significant reduction in the number of metastasis in the mice without spleen as compared to that in the normal mice, indicating crucial role of extramedullary hematopoiesis in the spleen in spontaneous metastases. Next, to evaluate biological effect of accumulated platelets into the lungs, normal mice or mice bearing 4T1 in the mfp (2 weeks after the implantation) were injected intravenously with 4T1-luc cells through tail vein. The amount of the arrested, survived and growing tumor cells in the lung was imaged and quantified using luminescence imaging of the live mice. There was a significant increase in these parameters in the mice bearing 4T1 tumor in the mfp as compared to those in the normal mice. These data strongly indicates that accumulated platelets aid circulating tumor cells to arrest, survive and grow at the lungs. Therefore platelets accumulated into the secondary sites can serve as the pre-metastatic niches. To validate these findings, mice bearing 4T1 cells in the mfp will be pre-treated with CD42 antibodies to delete circulating platelets or glycoprotein GP IIb/IIIa inhibitors which can reduce binding of activated platelets to endothelial cells.
Title: P40 suppresses breast cancer metastasis by inducing epigenetic silence of FZD10

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Body: P40 is a component of the SIN3A-HDAC corepressor complex that suppresses target genes transcription. It shares homology with BRMS, which suppresses metastasis of multiple types of malignancies. Although it was reported that p40 inhibits the growth of a human lung cancer cell line, the contribution of p40 in breast cancer development is not well characterized. In the present study, we found that reduced p40 in breast cancer tissues is associated with larger tumor size, higher clinical stage, aggressive distance metastasis, as well as poor patient clinical outcome. Functionally, we demonstrated that p40 suppresses migration and invasion of breast cancer cells by inhibiting epithelial-mesenchymal transition (EMT). These biological effects are mediated by epigenetic silencing of FZD10, a seven-transmembrane receptor for Wnt signaling, by facilitating the recruitment of HDAC1 to its promoter and enhancing histone H3K9 deacetylation. Consequently, p40-induced FZD10 silencing inhibits aberrant activation of Wnt3-FZD10-β-catenin signaling. Furthermore, p40 is a target of miR-106b and miR-106b upregulation leads to p40 reduction in breast cancer cells. In vivo, RNAi-mediated silencing of p40 expression promotes metastasis breast cancer xenografts in immunocompromised mice, while ectopic p40 expression inhibits metastasis. In summary, p40 provides epigenetic regulation of Wnt signaling in breast cancer cells and acts as a breast cancer metastasis suppressor. P40 down-regulation may not only serve as a prognostic factor to predict clinical outcome for breast cancer patients, but also a therapeutic target as ectopic p40 expression efficiently inhibits breast cancer metastasis by suppressing the activities of Wnt signaling.
Title: Galectin-7 increases resistance of breast cancer cells to drug-induced apoptosis and promotes tumor escape by killing T cells

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Body: Resistance to apoptosis induced by anti-cancer drugs is a major obstacle for the treatment of aggressive forms of breast cancer. Galectin-7 was recently shown to be specifically expressed in basal-like and HER-2 positive but not in luminal subtypes of human breast cancer. Galectin-7 also increases the metastatic behavior of breast cancer cells since overexpression of this protein in poorly metastatic breast cancer cells increases their ability to metastasize to the bone and to the lung. This pro-tumoral activity of galectin-7 in breast cancer is surprising since galectin-7 had been previously associated with activation of p53 and breast cancer cells often harbor a transcriptionally inactive form of p53. We have recently reconcile this apparent contradiction by showing that elevated levels of galectin-7 in breast cancer cells occurs via a gain-of-function mechanism of mutant p53. In p53-null breast cancer cells, we have shown that C/EBPβ-2 (also known as LAP2), the most transcriptionally active of the C/EBPβ isoforms, is responsible for the upregulation of galectin-7. How galectin-7 contributes to the progression of breast cancer remains unclear. Up to now, regulation of apoptosis by intracellular galectins has been largely attributed to their ability to translocate to mitochondria, possibly following their interaction with bcl-2. Here, we report that a mutant of galectin-7 that is unable to translocate to mitochondria induces resistance of human breast cancer cells to apoptosis induced by etoposide or by hypoxia-mimicking conditions. Surprisingly, this mutant and the wild-type form of galectin-7 bind equally well to bcl-2 in vitro and in vivo. Interestingly, both forms decreases the translocation of p53 to the nucleus and reduce the expression of p21 following treatment with doxorubicin. We also found that galectin-7 was released by breast cancer cells and that recombinant galectin-7 increased apoptosis of monocytes, T CD4+ and T CD8+ cells. This suggests that galectin-7 contributes to the establishment of an immunosuppressive tumor microenvironment by killing helper and effector T cells. Taken together, these results challenges the current paradigm that mitochondrial galectins are important for resistance to apoptosis and call for a greater focus on the role of galectin-7 in breast cancer. They also indicate that targeting both cytosolic and extracellular galectin-7 could improve the efficacy of anti-cancer drugs for the treatment of aggressive forms of breast cancer.
Title: Transcription factor Fra-1 modulates the adhesive properties of breast cancer cells contributing to a metastatic phenotype

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Body: Fra-1, a component of the transcription factor AP-1 family, has been found to be overexpressed in carcinomas with high metastatic potential such as in triple-negative breast cancer. The effect of Fra-1 on the morphology, motility and invasive potential of breast cancer cells has been previously described. Since tumor cell adhesion plays an essential role in the metastatic process, especially for extravasation from blood vessels, we investigated the influence of Fra-1 on breast cancer cell interactions with the endothelium.

Using the human breast cancer cell lines MCF7 (weakly invasive, estrogen receptor (ER)-positive) and MDA MB 231 (strongly invasive, ER-negative) with stable Fra-1 overexpression, we performed dynamic cell-flow adhesion assays on surfaces coated with E-selectin or with human pulmonary microvascular endothelial cells (HPMEC). Our analyses revealed increased adhesion of Fra-1 overexpressing MCF7 cells to E-selectin but also to activated endothelial cells, whereas the per se highly invasive and Fra-1-positive MDA MB 231 cell line showed enhanced cell rolling and tethering on E-selectin and endothelium-coated surfaces, but no increased firm adhesion after Fra-1 overexpression. These different behaviors correspond to an up-regulation of various adhesion-related proteins such as CD44, Integrin α5 and CEACAM6 in Fra-1 overexpressing MCF7 cells measured by microarray analysis and flow cytometry in comparison to weaker differences in the expression of adhesion molecules found in the Fra-1 overexpressing MDA MB 231 cell line. In line with these results and based on cDNA microarray data of breast cancer patients (n=175) high Fra-1 expression significantly correlates with shorter overall survival and higher rate of lung metastasis in ER-positive breast cancer patients (n=130), but has no impact on the prognosis of patients with ER-negative tumors.

Thus, in addition to its pro-invasive and pro-migratory effect, Fra-1 might influence the metastatic potential of breast cancer cells by changing the expression of adhesion molecules resulting in increased adherence to endothelial cells under flow conditions.
Body: Background: Triple-negative breast cancer (TNBC) is a highly aggressive tumor subtype associated with poor prognosis. The onset of metastasis in organs such as the lung, bone and brain is a major cause of mortality in TNBC patients. Thus, identification of novel targets for the treatment of triple-negative breast cancer is urgent for improved outcomes in patients with this disease.

Methods and Results: Here we conducted quantitative proteomic analyses in breast cancer cell lines, which include a normal, primary tumor and a metastatic tumor that were isolated from a single patient. Stable isotope labeling of amino acid in cell culture followed by LC-MS/MS analysis was performed and deregulated proteins were identified using statistical analysis. We identified for the first time that Rab1B, a member of RAS oncogene family, was down-regulated in high metastatic human breast cancer cells. In a panel of breast tumor tissues, Immunohistochemical analysis demonstrated an inverse correlation between metastatic propensity and the expression of Rab1B. Compared with primary tumors, Rab1B expression was notably decreased in lymph node metastases. Low Rab1B expression also correlated with poorer survival in triple-negative breast cancer patients. Through in vitro assays, we confirmed that silencing of Rab1B expression by RNAi in MDA-MB-231 and MDA-MB-468 breast cancer cells, can potentiate the proclivity to metastasize in Transwell assay and enhance lung colonization by tail vein injection in mice. In a reverse-complimentary approach, we determined that elevated Rab1B expression in highly metastatic breast cancer cell lines (MDA-MB-231HM and MDA-MB-231Bo) can suppress the ability of invasion and metastasis in vitro and in vivo. Mechanistically, we establish that the metastatic behavior strongly correlates with increased phosphorylated Smad3 levels and overexpression of TGF-β type I receptor (TβRI). Conversely, restoration of Rab1B can inhibit the activation of this pathway in MDA-MB-231HM and MDA-MB-231Bo cells.

Conclusion: By utilizing a series of mammary tumor cell lines, animal models, and clinically sourced tissues, we demonstrate that Rab1B acts as a metastasis suppressor in triple-negative breast cancer through regulating TGF-β/Smad signaling pathway.
Title: Effects of JAK pathway inhibition in pre-clinical models of breast cancer bone marrow metastases

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Body: Bone marrow (BM) metastases are an important problem in metastatic breast cancer. Metastatic-relapse occurs even decades after the first diagnosis and can show different clinical features compared to the primary disease. The cross-talk between cancer cells and the microenvironment influences tumor behaviour and impinges on therapeutic efficiency.

To uncover new targets for the inhibition of BM metastases, we performed a transcriptional analysis of BM stromal cells using in-vivo models of breast cancer bone metastases. We identified IL6, and its downstream JAK kinases pathway, as major up-regulated signals in the bone stroma of tumor-bearing mice. Moreover, we found that a significant proportion of BM metastases from breast cancer patient biopsies are positive for pSTAT3 staining, attesting to activation of JAK-STAT signaling. These results provide a rational to investigate the therapeutic potential of a JAK inhibition in bone metastases. We evaluated the effect of two JAK inhibitors: the BSK805, a JAK2 inhibitor, and ruxolitinib a JAK1-JAK2 inhibitor currently in clinical trials for metastatic breast cancer. In pre-clinical models of breast cancer metastases (the triple negative MDA-MB231 SCP1833 and the ER+ EO771), treatment with the JAK inhibitors decreased pSTAT3 signaling in the bone, but surprisingly increased metastatic tumor burden.

Considering the importance of the JAK-STAT pathway as a therapeutic target, we are investigating the possible bystander effects of blocking JAK kinases. Analysis of the BM stroma showed that JAK inhibition decreased TRAP+ osteoclasts and affected the mesenchymal compartment, with a significant increase in the BM fat compartment in tumor-bearing animals. The reasons for and the consequences of these changes in the bone stroma are under investigation. As the JAK-STAT signaling pathway is also a key regulator of the hematopoietic compartment, we are studying how JAK inhibitors influence the immune response in tumor bearing mice. Further dissection of the immunomodulatory effect of JAK inhibition and how this influences tumor dissemination and expansion is warranted.
Title: Melatonin's inhibitory effect on breast cancer metastasis mediated by ROCK-1

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Body: The metastasis occurrence, an important prognostic factor, depends on peculiarities such as cellular invasiveness and cell migration, mechanisms controlled by regulatory and effector molecules such as Rho-associated kinase protein (ROCK-1). An increased expression of this protein promotes tumor growth and metastasis, a mechanism which can be restricted by the use of the effector’s inhibitors. Melatonin, a hormone secreted by the pineal gland, has shown oncostatic action and anti-metastatic effects and can modulate the ROCK-1 expression. The objective of this study was to investigate the anti-metastatic response mediated by ROCK-1 and through the action of melatonin and its specific inhibitor (Y27632) in vitro and in vivo breast cancer models. Cells from metastatic (MDA-MB-231) and non-metastatic (MCF-7) breast cancer lines were treated with melatonin and Y27632. Cell viability was verified by MTT assay, cell migration/invasion assays in Boyden chamber, ROCK-1 protein and gene expression by western blot and quantitative real time PCR, respectively. In addition, the in vivo metastasis study was performed using female athymic nude mice induced by injection of 2x10⁵ MDA-MB-231 viable cells by tail vein for lung metastasis and by intracardiac for bone metastasis, during 3 weeks. The animals were treated with melatonin and Y27632 for 2 weeks. The metastasis developments were evaluated by single photon emission computed tomography (SPECT). Treatment with melatonin reduced cell viability and migration of both cell lines (p<0.05). The use of melatonin and Y27632, in association or not, decreased the ROCK-1 protein expression in metastatic cells, not significantly altering its expression in the non-metastatic line (p>0.05). An statistically significant reduction of ROCK-1 gene expression was observed in all treatment groups (p<0.05). ROCK-1 downexpression was more efficient in the group with associated treatments for both lines (p < 0.05). In vivo SPECT images showed multiple foci in the lungs (on Tc-99-tetrofomin images) and in the vertebrae (on Tc-99-MDP images). The numbers of “hot” spots were significantly higher in lung metastasis of control animals compared to treated groups. Semi quantitative analysis showed significantly lower activity in animal lungs that received treatment (p<0.05). The bone metastasis did not show difference between control and treatment groups. Melatonin and Y27632 are effective drugs of metastatic breast cancer in vitro treatment, confirming their effects in decreasing cell viability, invasion, migration and protein expression of ROCK-1 in these cells. In vivo, melatonin and Y27632 treatments seem to be effective reducing lung metastasis but not bone metastasis. Melatonin, in particular, appears to be more effective when combined to ROCK-1 inhibitor.

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Title: N-glycosylation changes in tumour cells are associated with vascular invasion and metastasis in breast cancer patients

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Background: In a recent study based on cDNA microarray data of breast cancer patients, we could show that the mRNA expression of some glycosylation enzymes involved in synthesis and trimming of N-glycans is significantly associated with metastasis and recurrence in breast cancer patients suggesting an important role of N-glycosylation in tumour progression (Milde-Langosch et al., Breast Cancer Res Treat 2014). The aim of the present study was to further investigate this issue on a protein and glycoprotein level (1.) by western blot analysis of selected glycosylation enzymes and (2.) by lectin histochemistry using lectins which bind to O- and/or N-glycans.

Methods: In order to confirm the role of two selected glycosylation enzymes, ribophorin (RPN1) and mannosidase (MAN1A1) on protein level, we analyzed their expression in a cohort of 196 breast cancer samples by western blot analysis. In addition, we studied tumour cell binding of various lectins, which recognize diverse O- or N-glycans (UEA1, HPA, PNA, GNA, PHA-L), using a tissue microarray with up to 293 evaluable breast cancer samples. These results were correlated with recurrence-free survival (RFS) and overall survival (OS) and data on vascular and lymphatic invasion.

Results: By western blot analysis, the negative prognostic significance of ribophorin (RPN1), a key enzyme of N-glycosylation, was corroborated on a protein level. For MAN1A1 which is involved in trimming of N-glycan structures, two isoforms with different molecular weight could be detected on western blots, showing contradictory associations with OS. Protein levels of RPN1 and total MAN1A1 showed significant correlations to the respective mRNA expression data. By lectin histochemistry, we found significant correlations of lectin binding to lymph node metastasis, vascular invasion and lymphangiosis in breast cancer samples. Binding of PHA-L and GNA (binding to N-glycans) and PNA (binding to O- and N-glycans) was significantly associated with vascular invasion, whereas PNA and PHA-L binding correlated with lymph node involvement. In addition, PNA and HPA reactivity was associated with microscopically detected lymphangiosis. PNA binding also correlated with shorter RFS and OS.

Conclusion: While the role of O-glycans in breast cancer metastasis has already been described, our data point to an additional strong impact of N-glycosylation on metastasis and progression of mammary carcinomas.
Title: Investigating the role of Duffy antigen receptor for chemokines (DARC) isoforms in breast cancer metastasis

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Body: Duffy Antigen Receptor for chemokines (DARC) is known to regulate the biological activities of chemokines. It has been implicated in the progression of various diseases including different types of cancer, affecting survival, proliferation and metastasis. Genetic variants in the DARC genes are strongly associated with African ancestry and are linked to malaria resistance. There are at least two isoforms of DARC, which have not been appreciably investigated in these contexts, that may have differing chemokine activity functions. To fill this knowledge gap, we have been characterizing the impact of DARC gene variants on DARC isoform expression in specific tissue types in order to define relationships between the polymorphic DARC and its effect as an immune regulator in breast cancers. We hypothesize that DARC variants, which are known to impact expression of the gene on erythrocytes, also are associated with functional activity related to DARC’s control of metastatic potential in breast cancer. Specifically, we have determined that one DARC polymorphism which is well defined in ancestral groups (i.e. Duffy null - rs2814778; -541T>C) is associated with differential expression of DARC isoforms in epithelial cell types, including lymphoblast and breast tissues.

We investigated the erythroid silencing Duffy Null allele of DARC using DNA sequencing and quantitated DARC isoform expression by real time PCR in breast cancer cell lines, ancestral cell lines and breast cancer tumor tissues. We will show that DARC isoforms have significantly variant expression among ancestral groups that is associated with the Duffy Null allele. We will also show that certain types of breast tumors have variable DARC expression that is also associated with this genotype. Additionally, we visualized the subcellular localization of DARC and known interacting chemokines breast cancer cells with IHC and IF confocal microscopy.

Specifically, our results indicate breast tumors differentially express DARC in which may be affecting the chemokine pool and immune response within the tumor and its microenvironment. In our Hapmap ancestral populations, all DARC genotypes expressed the gene in lymphoblast, with significantly different levels of isoforms among African, African American and European individuals. Breast cancer cells also show different levels of specific DARC isoform mRNA levels and distinct levels and patterns of binding chemokines, related to tumor subtype origins (i.e. ER status).

Based on these findings we conclude that DARC isoforms have differential expression levels in breast cancer cells within tumor and non-tumor regions that may drive both pro and anti tumorigenic events with varying chemokine binding or localizations during tumorigenesis of specific breast cancer subtypes. Different levels of isoforms in epithelial cells may contribute to its essential function as atypical chemokine receptor. We hypothesize that immune responses in breast cancer patients with Duffy Null genotypes will differ and may express more severe metastasis due to altered epithelial regulation of alternate DARC isoforms.
Title: Pathway activation mapping of metastatic breast cancer identifies potential organ-specific signatures: Implications for patient stratification to targeted treatment

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Body: Background: The development of distant metastases is the strongest prognostic factor associated with cancer mortality. Customization of treatment based on molecular profiling of a patient’s primary tumor has yielded promising results and has opened a new paradigm for treating patients with advanced disease. Nevertheless profiling the primary tumor may not reflect the actionable molecular drivers of the metastatic lesions. In the last few years a large number of genomic and proteomic studies have demonstrated that at the molecular level metastatic lesions significantly differ from matched primary tumors. The impact of metastasis site organ microenvironments on breast cancer primary tumors has been only partially explored. Since most targeted therapies work by modulating aberrantly-activated protein kinase signaling, the aim of this study was to utilize reverse phase protein arrays (RPPA) to explore whether metastatic lesions derived from different patients, but invading the same host target organ showed similarities in their signaling architecture and presented with organ-specific targetable signatures. Methods: Snap frozen material collected from 14 metastatic breast cancer patients enrolled in a prospective phase II trial (“Side Out II”) were used for this analysis. Sites of metastasis were: liver (n=7), skin/chest wall (n=4), and lung (n=3). All samples were subjected to Laser Capture Microdissection (LCM) and RPPA to measure the activation/phosphorylation levels of 12 FDA approved drug targets and linked substrates. Results: Among the 7 patients with liver metastases, p70S6K, c-Abl, ERK 1/2 were highly phosphorylated in 5 cases (71.4%), ErbB2/HER2 in 4 cases (57.1%), and AKT in 3 cases (42.8%). On the contrary, of the 4 patients with skin/chest wall lesions 3 showed high activation of ErbB2/HER2, ErbB3/HER3, and ERK 1/2 (75.0%) and 2 of EGFR (50.0%). Of note, none of the skin/chest wall lesions showed activation of AKT and only 1 case presented with activated p70S6K (25.0%). Lung metastasis showed an overall low activation of all 12 analytes measured. Discussion: Although this data is based on a relatively small number of samples and needs further validation, our initial analysis revealed potentially important trends that could have an impact in the prioritization and selection of targeted agents for metastatic breast cancer trials. We found that the distribution of the 12 FDA approved drug targets and downstream substrates varies between different metastatic sites. Thus, the emergent hypothesis from this work is that breast cancer patients with liver metastases may be more susceptible to AKT-mTOR directed targeted therapeutics and patients with skin/chest wall metastasis may benefit from therapies that target EGFR and/or downstream ERK signaling.
Title: Modeling breast cancer metastasis in the mouse via measurement of circulating tumor cells

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Body: Objectives: Advances in the detection of circulating tumor cells (CTC) in the blood of metastatic breast cancer patients have indicated that patients with greater than 5 CTC/7.5mL of blood have poorer prognosis; however, little is known about the biology of these cells and what characteristics are critical for breast tumor cell dissemination. We are developing and evaluating several mouse models of breast cancer to characterize important markers that distinguish CTCs from other cell types in the blood as well as to assess whether silencing of various metastasis supporting genes leads to reductions in CTC counts and formation of metastatic tumors.

Methods: This study involves two separate approaches to characterize CTCs. The first involves xenograft models using human breast cancer cells injected into the fat pad of immunocompromised mice. These cells are tagged with both mCherry, for detection in blood, and luciferase, to track metastatic spread of the primary tumor cells to other sites via intravital imaging. Blood was collected from tumor bearing mice at end point, processed and stained for characterized CTC markers. The second approach involves endogenous mouse models to characterize CTC counts and relevant markers in a variety of metastatic and non-metastatic murine breast cancer models using negative depletion to remove contaminating normal and hematopoietic cells. In both approaches CTCs have been assessed using the Imagestream by Amnis.

Results: Initial xenograft experiments with MDA-MB-231 cells injected into the mouse mammary gland fat pad revealed a low concentration of mCherry+/cytokeratin+/Hoechst+/CD45- cells in the blood of mice without confirmed metastatic dissemination. Pilot studies on endogenous tumor models have demonstrated the utility of our negative depletion approach to remove contaminating hematopoietic cells as well as the detection of a population of EpCAM+/Hoechst+/CD45- cells.

Conclusions: Initial experiments have provided feasibility for detailed characterization of mouse CTCs, which will be presented. This work complements our study being carried out on characterizing CTCs in metastatic breast cancer patients. Increasing understanding of the biology of circulating tumor cells and relating their characteristics to those of both the primary and metastatic tumors may reveal mediators common and functionally important to all 3 tumor cell populations and may therefore serve as better targets for treatments that are effective against both the primary and metastatic tumors.
Title: SSA, a novel sulindac sulfide derivative, inhibits tumor cell growth and invasion in breast cancer

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Body: Nonsteroidal anti-inflammatory drugs (NSAIDs), such as sulindac sulfide (SS), have been reported for striking chemopreventive activities in various types of human malignances, including breast cancer. However, the toxicities related to cyclooxygenase (COX) inhibition resulting in suppression of physiologically important prostaglandins limit their clinical use for chemoprevention in human cancer. We recently developed a novel amine derivative of SS named as SSA, which lacks inhibitory effect on COX-1 or -2, yet shows 10 times greater suppressive effect than SS on growth of a panel of breast cells. Moreover, SSA treatment at sub-cytotoxic concentration (4µM) for 36 hr can significantly inhibit both migration and invasion of highly aggressive breast tumor MDA-MB-231 cells. To understand the molecular mechanism accounting for this activity, we examined 4 oncogenic miRNAs, including mir-17-92, mir-9, mir-10b, and mir-21 that were previously reported by our group to be associated with SS anti-invasive activity in breast and colorectal cancer. We found SSA could significantly down-regulate these miRNAs; whereas their forced expression was able to counteract the anti-invasive activity of SSA in MDA-MB-231 cells. These results imply that significance of SSA with non-COX inhibitory properties in suppression of tumor cell invasion could provide novel insights into development of safer and more effective strategies for prevention of breast cancer progression and metastasis. In addition, after inducing mobility of non-invasive breast MCF-7 cells by using TGF-β1, we treated these invading cells with SSA and found that their mobility was significantly decreased. These results support anti-invasive activity of SSA in human breast cancer cells and inhibition of TGF-β1 as well as oncogenic miRNAs may be responsible for the mechanistic basis by which SSA prevents breast tumor cell invasion.

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Title: Global analysis of the transcriptome in matched primary and metastatic tumours defines ER specific gene alterations

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Body: The incidence of brain metastasis (BM) among women with metastatic breast cancer (MBC) is currently on the rise, and it is estimated that between 10-30% of patients diagnosed with breast cancer may also develop BM (Weil 2005). BM is considered a late complication in the clinical setting and is an infrequent occurrence even in mice studies that readily metastasise to other organs (Valiente 2014). While pre-menopausal status and negative estrogen receptor (ER) status are seen as main risk factors (Gil-Gil 2013), it is now recognised that BM is emerging as an increasing clinical problem across the various MBC subtypes. BM patients have very poor overall survival and as a result it is crucial to identify the factors that promote the survival and adaptation of breast cancer cells to allow them to colonise distant sites such as the brain. The ability to predict the BM potential could be of great clinical importance and in this study we decided to investigate the mechanisms underlying BM across the various MBC subtypes.

To our surprise, in a cohort of breast cancer patients we found a comparable number of ER positive and ER negative BM. We established that ER positive patients (n=10) had a substantially longer disease latency period (median disease free survival (DFS) 36 months, range 8-85 months) in comparison to ER negative patients (n=8, HER2 positive, n=2, triple negative, n=6), (median DFS 24 months, range 9-60 months). To understand and elucidate the gene alterations required for successful colonisation of the brain we undertook RNAseq of sequential primary and brain metastatic tumours from ER positive (n=4) and ER negative (n=3) breast cancer patients. Significant elevations in gene expression were observed in the brain metastatic tumours in comparison to the matched primary. Though a large degree of patient heterogeneity was observed, common ER-specific metastatic pathways were identified.

This study highlights the requirement of unique gene sets for the colonisation to the brain and that functional characterisation of the differentially expressed genes will enable the identification of novel molecular targets for prevention and treatment of breast cancer BM.
Title: Neutrophil elastase modulates breast cancer progression by fostering collective cell detachment and tumor emboli dissemination

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Body: Proteases constitute a large family of enzymes involved in several processes, including degradation and remodeling of the extracellular matrix to drive dissemination of cancer cells into adjacent tissue. Within this large family, the serine protease neutrophil elastase (NE) has been proven clinically meaningful in breast cancer. Indeed, high levels of NE correlate with poor outcome and endocrine resistance in breast cancer patients.

In an experimental model it has been demonstrated that estrogen receptor positive (ER+) MCF7 breast cancer cells grow in suspension as 3D-spheroids in NE-addicted medium, thus resembling the micropapillae of a human breast cancer characterized by high propensity to metastasize, i.e. the micropapillary carcinoma. We hypothesized that NE may produce disarrangement of tumor cell adhesion to the substrate fostering neoplastic lymphovascular invasion (LVI) and metastasis. ER+/E-Cadherin (E-CAD+)/HER2- (MCF7, T-47D, ZR-75-1), ER+/E-CAD+/HER2+ (BT-474), ER-/E-CAD+/HER2+ (SK-BR-3) and ER-/E-CAD-/HER2- (MDA-MB-231) cells were grown with serine proteases (NE, cathepsin-G), hyaluronidase and collagenase. NE and cathepsin-G led to 3D-spheroid formation of ER+/E-CAD+ cells only. In 3D-spheroids from MCF7 cells grown with NE the luminal Epithelial Membrane Antigen (EMA) lined the external border of cell clusters, which faced cancer associated fibroblasts in co-cultures experiments, thus recapitulating the inverted polarity of MPCs. MCF7 3D-spheroids were tamoxifen resistant. Injection of NE in tumors of MCF7 cells in SCID mice triggered neoplastic cluster detachment, micropapillae, vascular emboli similar to 3D-spheroids and metastases. In a cohort of human breast carcinomas with LVI the MCF7-3D-spheroid-alike pattern was the most prevalent in tumor emboli. Immunohistochemical NE expression was mainly detected in polymorphous neutrophilic granulocytes (PMNs) within vessels and in the stroma and PMNs were significantly higher in breast carcinomas with LVI. In fully developed metastases within lymph-nodes, which reverted the EMA expression as in the primary tumor, no NE+ PMNs were observed. Our results may explain why high levels of NE negatively act on patient prognosis by creating a favorable environment for breast cancer invasion and metastasis.
Title: Expression of S100A14 promotes cancer cell invasion and metastasis and is associated with poor prognosis in breast cancer

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Body: Introduction: Identifying key molecules involved in cancer metastasis is important in anticancer drug discovery. To identify such molecules, we used a dual approach involving: 1) gene screening and in vivo confirmation of metastasis-promoting molecules using a mouse mammary tumor model; and 2) functional and clinicopathological analyses of the candidate molecules in a human cancer. The products of several candidate genes promoted metastasis of mouse mammary tumors. A protein designated S100A14 promoted metastasis in the mouse model and was found to be associated with poor prognosis in human breast cancer patients.

Methods and Results: 1) Identification of metastasis-related molecules: We established two highly metastatic cell lines, 66Lu10 and 66HM, which formed multiple metastatic colonies in general organs, from MCH66 mouse mammary tumor cells. Two low-metastasis cell lines, 66C8 and 66LM1, were also established. DNA microarray analysis was used to identify candidate metastasis-related genes based on differential expression in 66Lu10 and 66HM cells compared with 66C8 and 66LM1. An in vivo metastasis assay in which the highly metastatic cells were transfected with gene-specific siRNAs and orthotopically inoculated into mice was performed to confirm the metastasis-promoting activity of the candidate genes. Several novel molecules that promoted metastasis in the mouse model were identified, including S100A14. 2) Significance of S100A14 in human cancer: Analysis of S100A14 protein expression in various human cancer cell lines and tissues revealed that S100A14 expression is upregulated in breast cancer. Functional analysis using MCF7 and SK-BR-3 human breast cancer cells showed that S100A14 colocalizes with F-actin on the cell membrane at cell-cell attachment sites and on focal adhesions at the leading edge. Immunoprecipitation analysis also demonstrated an interaction between S100A14 and F-actin. Boyden chamber and wound-healing assays showed that S100A14 knockdown substantially suppresses the invasive activity of both cell lines. Immunohistochemical analysis of archival specimens of primary tumors from 167 breast cancer patients showed strong membranous staining of S100A14 (53%). Elevated expression of this protein was significantly associated with younger age (<60 years), ER-negative status, HER2-positive status, and poorer prognosis (P=0.0002).

Conclusions: Using our mouse mammary tumor model for identifying metastasis-related genes, we identified S100A14 as a strong candidate metastasis-promoting molecule in vivo. Functional analyses using human breast cancer cell lines indicated that S100A14 promotes invasive activity via interaction with cytoskeletal components. Upregulated expression of S100A14 is associated with poor prognosis in breast cancer patients. Therefore, S100A14 is a potential prognostic biomarker and breast cancer therapeutic target.
Title: The role of cytoplasmic polyadenylation element binding protein-2 in breast cancer

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Body: **Background:** We had shown that over-expression of the inflammation-associated enzyme cyclo-oxygenase (COX)-2 promotes breast cancer progression and metastasis and sustains stem-like cells (SLC), believed to evade traditional therapy and promote tumor reoccurrence. Stable transfection of the COX-2 gene into COX-2–, HER-2–, non-metastatic human MCF-7 breast cancer cell line (named MCF-7-COX-2) led to a highly aggressive phenotype *in vitro*, where we observed epithelial-mesenchymal transition, increased migration, invasion, clonogenic tumorsphere forming ability (surrogate of SLC) and expression of SLC marker ALDH. Furthermore, we observed an increase in the tumorigenic and metastatic capacity of the cells in immuno-compromised mice *in vivo*. Combined gene expression and microRNA (miRNA) micro-array analysis of MCF-7 and MCF-7-COX-2 cells revealed COX-2 induced up-regulation of two miRNAs, miR-526b and miR-655, and down-regulation of their common gene target, cytoplasmic polyadenylation element binding protein (CPEB)-2. While both miRNAs were found to be oncogenic in functional assays, the function of CPEB-2 in breast cancer remains untested. **Hypothesis:** miR-526b and miR-655 down-regulate CPEB-2 to promote breast cancer cell aggressiveness and the SLC phenotype. **Approaches & Results:** (1) To test if expression levels of COX-2 and CPEB-2 are inversely related, we measured both gene and protein expression in multiple COX-2 disparate breast cancer cell lines. (2) We also tested if there is an inverse relationship between miRNA expression and the expression levels of their target gene CPEB-2. We found that high COX-2/miRNA expressing cell lines such as MDA-MB-231 and MCF-7-COX-2 had significantly lower expression of CPEB-2 than MCF-7 cells (low COX-2/low miRNA). (3) To test the functional effects of CPEB-2 gene manipulation *in vitro* and *in vivo*, we knocked down CPEB-2 in CPEB-2-high MCF-7 cell line. MCF-7-CPEB-2-KD cells were found to be more migratory and invasive than control cells in transwell assays. Ongoing studies are to test (a) whether there is a cause and effect relationship between the expression of the miRNAs and CPEB-2 expression, that is, whether knocking down or knocking in the miRNAs respectively up-regulates and down-regulates CPEB-2 expression; and (b) whether CPEB-2 expression is lower in breast cancer tissues than in adjacent non-tumor tissues, and inversely correlated with the miRNAs in breast cancer tissues. **Conclusion and Significance:** If CPEB-2 is proven to be tumor suppressor, these oncogenic miRNAs and putatively anti-oncogenic CPEB-2 may serve as potential biomarkers in personalizing breast cancer therapy with COX-2 inhibitors as adjuvant. (Supported by funds of the OICR to PKL. AH and MM are CaRTT/TBCRU scholars).
Title: FOXC1/FOXA1 transcriptional balance in breast cancer: From acquisition of mesenchymal and stem cell traits to occult lymph node independent breast cancer metastasis

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Body: Background: Distant metastatic spread of cancer cells to other organs from the primary site of origin currently constitutes the most significant contributor to cancer-related morbidity and mortality. Epithelial-to-mesenchymal transition (EMT) is a biologic transformation of cancer cells from a non-migratory phenotype to a migratory one, and is thought to initiate the metastatic cascade in cancer. EMT has also been reported to trigger acquisition of stem cell traits in breast cancer. Transcription factor (TF) Forkhead box C1 (FOXC1), strongly associated with the basal-like and claudin-low breast cancer molecular subtypes, is a powerful EMT inducer and is also a marker of stem/progenitor cells. In contrast, TF forkhead box A1 (FOXA1), strongly associated with luminal subtypes, is an EMT repressor and a luminal differentiation marker, thus seemingly exerting reciprocally opposite transcriptional effects to that of FOXC1. We hypothesized that effective EMT program activation status in breast cancer might be better predicted by examining the expression ratio of an EMT inducer and EMT repressor, such as FOXC1/FOXA1, theoretically being more reflective of net transcriptional effect than either component alone. Methods: Herein we utilize RNA-Seq profiling of the HRAS-transformed MCF10A cell series, a well characterized and widely accepted in vitro model of breast cancer progression and metastasis, to correlate measured FOXC1/FOXA1 ratios to dynamic shifts in EMT marker expression in 3D matrigel cultures and to stem cell traits observed in primary and secondary mammosphere suspension cultures. We further test the ability of the FOXC1/FOXA1 expression ratio to predict lymph node independent breast cancer metastasis and death in independent human breast cancer gene expression datasets. Results: RNA-Seq and qRT-PCR profiling confirmed progressive increase in FOXC1/FOXA1 ratio to correlate with a progressive loss of E-cadherin expression and synchronous gain of EMT markers N-cadherin, Fibronectin, and Vimentin. FOXC1/FOXA1 ratio was found to be directly proportional to mammosphere formation efficiency, a surrogate indicator of stem cell enrichment. In patients without any evidence of nodal metastasis, elevated FOXC1/FOXA1 ratio was associated with significantly decreased 10 year Overall Survival (HR 2.58;95%CI 1.39 to 4.80, p = 0.003, 295 patient Van de Vijver dataset), 10 year Disease-specific Survival (HR 1.74;95%CI 1.16 to 2.61, p = 0.008,1992 patient Curtis dataset) and predicted the development of lung metastasis. Conclusion: Elevated FOXC1/FOXA1 expression ratio indicates EMT program activation in breast cancer, and predicts the associated occurrence of lymph-node-independent distant metastasis and death in human patients. These findings may allow for the early (pre-symptomatic) diagnosis of clinically occult (node negative) metastasis by using the FOXC1/FOXA1 ratio as a biomarker of metastasis and permit institution of appropriate therapy earlier than currently possible. The current study improves our understanding of EMT and highlights the importance of future studies geared towards unraveling mechanisms involved in regulating FOXC1 and FOXA1 expression in breast cancer.
Title: Role of astrocyte in the triple-negative breast cancer brain metastatic growth

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Body: Patients with triple-negative breast cancer (TNBC) have the worst outcome among breast cancer patients due to high propensity for brain metastatic spread. So far, no targeted therapies have been developed to help prevent brain metastasis in patients with TNBC. Therefore, it is critical to address the gap in knowledge because it will allow for targeting of triple-negative cells, thereby preventing brain metastasis formation in these patients. The outcome of metastatic process depends on interactions of metastatic cells with the specific organ microenvironment. In the brain microenvironment, astrocytes are one of the first cell types in the blood-brain barrier that brain-invading cancer cells encounter post extravasation. Reactive astrocytes have been found in close proximity to individual cancer cells in BM, which suggests the importance of astrocytes in tumor BM formation. To investigate the role of astrocyte in TNBC brain metastasis formation, we sequentially passaged TNBC MDA-MB-231 cells in primary rat neonatal or adult astrocyte culture media, and then the astrocyte media conditioned tumor cells were used for further experiments. Our results showed that cultured astrocyte-secreted factor(s) induced a correlated morphological and functional alteration of MDA-MB-231 cells, which endowed the tumor cell with a more invasive phenotype and enhanced their ability of metastatic growth in the brain. Furthermore, analysis of the gene-expression profile of the conditioned MDA-MB-231 cells showed dramatic alteration of some tumor related genes, and the tumor genetic alteration happened at transcriptional level in several critical tumor genes, such as SPP1 and Angptl4. Although reactive astrocytes surrounding tumor brain metastases has been observed, our data provides direct evidence that astrocyte secreted factors support TNBC cell brain metastatic growth. The underlying mechanisms for how astrocyte secreted factors induce tumor genetic alterations that contribute to TNBC to grow in the brain are actively being investigated by us currently with the purpose to target the astrocyte secreted factor(s) and the key corresponding tumor cell genetic alterations as clinical targets for therapy of TNBC brain metastasis.
Activated thrombin activatable fibrinolysis inhibitor is a novel anti-metastatic factor in breast cancer

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**Body:** Thrombin activatable fibrinolysis inhibitor (TAFI) is a plasma zymogen initially known for its role in attenuating fibrinolysis. Activated TAFI (TAFIa) is formed through proteolytic cleavage by thrombin, plasmin or, its most effective activator, thrombin in complex with the endothelial cofactor thrombomodulin (TM). TAFIa is a carboxypeptidase, which acts by cleaving carboxyl terminal lysine and arginine residues from protein and peptide substrates, including plasminogen binding sites on cell surface receptors. Carboxyl terminal lysine residues play a vital role in accelerating plasminogen activation to plasmin on the cell surface. Plasmin has many critical functions including cleaving components of the extracellular matrix (ECM), which enhances invasion and migration of cancer cells. In addition, plasmin can activate matrix metalloproteinases (MMPs), which also play a role in degrading the ECM. Furthermore, the expression of TM in tumours is inversely correlated to metastasis. Studies have shown that the anti-metastatic effects of TM result from its ability to bind thrombin. Given that the thrombin/TM complex is responsible for the activation of TAFI, the anti-metastatic effects of TM may be modulated by TAFIa. We therefore hypothesize that the activation of TAFI on the cell surface inhibits plasminogen activation and decreases breast cancer cell invasion and migration. Expression of TAFI and TM were assessed in breast cancer cells with varying degrees of metastatic potential. Although TAFI mRNA levels did not correlate with malignancy, TM mRNA levels were found to be inversely correlated with breast cancer cell malignancy. Moreover, cell invasion and migration of MDA-MB-231 and SUM149 cells were assessed upon treatment with potato tuber carboxypeptidase inhibitor (PTCI), which is a specific inhibitor of TAFIa. Inhibition of TAFIa resulted in a significant increase in cell invasion and migration of both cell lines. Cell invasion and migration of MDA-MB-231 and SUM149 cells were also assessed upon treatment with TM. Treatment with TM significantly decreased cell invasion and migration of both MDA-MB-231 and SUM149 cells. Additionally, experiments using a fluorogenic collagen substrate showed an increase in extracellular collagen cleavage after PTCI treatment, using both MDA-MB-231 and SUM149 cells. Furthermore, the ability of TAFIa to inhibit pericellular plasminogen activation was evaluated. Plasminogen activation was significantly decreased on the surface of MDA-MB-231 and SUM149 cells following treatment with various concentrations of TAFIa. Taken together, these results indicate a vital role for TAFIa in regulating pericellular plasminogen activation and ultimately ECM proteolysis. Enhancement of TAFI activation in the breast cancer tumour microenvironment may be a therapeutic strategy to inhibit invasion and prevent metastasis of breast cancer cells.
Title: CIP4 promotes triple-negative breast cancer metastasis and is associated with poor prognosis

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Body: Epidermal growth factor receptor (EGFR) and Src kinase pathways are key drivers of triple-negative breast cancer (TNBC) cell invasion and tumor metastasis. Cdc42-interacting protein-4 (CIP4) is an adaptor protein targeted by these pathways, and has been identified as a key regulator of cancer cell motility. Here, we address the role of CIP4 in TNBC metastasis in vivo, and CIP4 expression profiling in human breast cancer patients. Using an inducible CIP4 knock-down (KD) system in human TNBC cell lines, we observed effects of CIP4 silencing on EGFR signaling to Akt and MAPK pathways. Although CIP4 KD cells were capable of growth in spheroid assays, we observed a significant reduction in invasion of the surrounding extracellular matrix compared to control cells. Profiling of conditioned media from these cell lines revealed a role for CIP4 in promoting expression of matrix metalloproteinase-2 (MMP-2). CIP4 silencing in mammary orthotopic xenograft assays using both human and rat breast cancer models, led to defects in lung metastases. Profiling CIP4 expression in primary human breast tumors by immunohistochemistry revealed high CIP4 levels in myoepithelial cells and within subtypes with high risk of metastasis (HER2, TNBC). In a cohort of 245 patients with invasive ductal carcinoma, patients with high CIP4 levels were more at risk of developing metastases. Together, these findings implicate CIP4 in promoting breast cancer metastasis in mice, and identifies a link between CIP4 expression levels and risk of metastasis in breast cancer patients.
Title: A breast angiosarcoma treated by bilateral mastectomy in a patient who had undergone to arm amputation for Stewart Treves syndrome following breast conserving surgery: Case report

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Body: INTRODUCTION: Stewart Treves Syndrome is a rare disease who is undergone breast conserving surgery or total mastectomy accompanied by curettage of axillary lymph nodes. There is a risk for chronic subclinical edema and angiosarcoma secondary to radiotherapy. We represent a case that we have performed bilateral mastectomy and right arm amputation to a 61 year old woman who had undergone to breast conserving surgery for invasive ductal carcinoma ten years a go.

CASE: A 61 year old woman who had undergone to breast conserving surgery and axillary curettage for invasive ductal carcinoma 10 years ago, had also received radiotherapy, chemotherapy and hormonotherapy. The patient who was given a couple of different treatments with the complaints of swelling and erythema in her arm, was diagnosed as CD34+ lymphangiosarcoma by punch biopsy performed because of the unresponsiveness of other treatments. In her PET-CT scan, there was not seen any involvement except primary tumor at right elbow. Disarticulation to the right upper extremity was performed. One month after the operation, there was appeared erythema, swelling and bruise also on the skin of her right breast and biopsies were obtained from the lesions. Mastectomy was considered for the patient who was histopathologically diagnosed as CD31+, Fli 1 + angiosarcoma. Because of the fact that there was also macromastia in her left breast, not to cause a postural distortion and changes in balance bilateral mastectomy was planned as the patient agreed.

After the operation as the tumor was reported as high grade angiosarcoma (Ki67>20) histopathologically, Paclitaxel 80mg/m2 D1,D8, D15 was administered every 28 days. In her imaging studies after four cycles of chemotherapy, there was no signs demonstrate relapse or metastasis. There was not seen adverse effectss except grade 1 fatigue and grade 1 neurotoxicity.

RESULTS: While the risk for angiosarcoma following the breast conserving surgery is %0.14, recent studies shows an increase in this precentage. After radiotherapy, along with chronic subclinical edema cytokines such as endothelial growth factor can induce proliferation of lymphatic vessels and fibrosis which can be resulted in angiosarcoma. On the other hand, it is suggested that the lymph dissection creates a predisposition to angiosarcoma. Breast conversing surgery is equal to mastectomy for early breast cancers and the numbers of this operation increase at the present. Therefore, there is a possibility of an increase in the risk of angiosarcoma development. Because of the fact that early detection of Stewart Treves Syndrome is very important, the skin changes must be well observed in the patients who had undergone axillary dissection and receive radiotherapy.
Fatty acid binding protein 5 promotes metastatic potential of triple negative breast cancer

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Body: Triple negative breast cancer (TNBC) is an aggressive subtype of breast cancer that has a poor prognosis for patients, not only due to its aggressive nature, but also due to the lack of effective therapeutic strategies. High mortality is associated with metastasis of the primary TNBC. We analyzed the role of fatty acid binding protein 5 (FABP5), an intracellular fatty acid transport protein, in aggressive breast cancers, especially TNBC. FABP5 expression was analyzed in a tumor microarray (TMA) containing 423 breast cancer patient samples and found that FABP5 expression is associated with tumor grade, triple negative status, and disease-free survival. Next, a mechanistic approach was taken and found that FABP5 knockdown TNBC cell lines express lesser EGFR protein compared to control, and upon EGFR activation express less phospho-FAK and phospho-Pyk2. We found that EGFR was not down regulated at the transcriptional level in FABP5 knockout cells. Further analysis indicated the role for Cbl, an E3 ubiquitin-protein ligase, as a potential mechanistic target for the down-regulation of EGFR protein in FABP5 knockdown cell lines. EGFR is over-expressed in aggressive breast cancers, especially TNBC. Though EGFR is highly expressed in aggressive subtypes of breast cancer, targeted therapeutic strategies have not been successful in clinic. EGFR has been shown to be involved in distant metastasis in aggressive breast cancers. We found a correlation between FABP5 and EGFR expression and metastasis-free survival using publically available datasets from The Cancer Genome Atlas. We next studied the functional consequence of FABP5 knockdown in TNBC cell lines. We showed that EGF-induced FABP5 knockdown cells migrated less compared to control. Additionally, FABP5 knockdown cells were significantly less able to migrate into a wound in response to EGF stimulation. EGF-induced cell attachment of FABP5 knockdown cells is significantly decreased compared to control. Our findings suggest FABP5 modulates metastatic potential of TNBC through alterations in EGFR expression and downstream migratory signaling molecules.
Title: Signaling consequences and rational therapeutic combinations with glutaminase inhibitor, CB-839, in basal breast cancer

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Body: Purpose: Proliferation rates of basal breast cancers are dependent on glutamine metabolism as previously demonstrated by glutamine withdrawal or glutaminase inhibition by CB-839 in cell line models. Early and late adaptive signaling and metabolic responses to glutamine stress may enable cells to proliferate or evade cell death. We hypothesized that inhibition of these pathways would be synergistic in combination with glutaminase inhibition by CB-839.

Methods: Protein signaling responses in HCC1806 cells (basal A breast cancer) were quantified using reverse phase protein arrays (RPPA) at early (4 h) and late (24 h) time points in response to low glucose or low glutamine concentrations as well as treatment with CB-839, a selective glutaminase inhibitor. Signaling pathways evaluated included mTORC1/2, PI3K/AKT, MAPK, and AMPK. ADP and ATP were quantified by ion exchange chromatography. Drug combinations predicted by RPPA were evaluated at therapeutically relevant concentrations using proliferation or apoptosis assays after 72 h of continuous treatment.

Results: In agreement with previous reports of glutamine withdrawal, low glutamine and glutaminase inhibition resulted in inhibition of proliferation and early inhibition of mTORC1 signaling including pmTOR, pP70S6K, p4EBP1, and pS6. In contrast, for cells incubated in low glucose, proliferation and mTOR signaling were maintained at early time points despite robust pAMPK/pACC signaling and increased ADP/ATP ratios (2-orders of magnitude higher than baseline); mTOR signaling was only inhibited after 24 h when ATP concentrations were reduced. Bioenergetic stress as detected by pAMPK and pACC was not observed with glutaminase inhibition – ATP concentrations were increased by CB-839 treatment. Importantly, 2-deoxyglucose, a compound used to reduce ATP levels and activate AMPK signaling, was antagonistic in combination with CB-839. In contrast, hydroxychloroquine, an inhibitor of autophagy activated by mTOR inhibition, was synergistic with CB-839. Consistent with mTORC1-mediated negative feedback loops, CB-839 treatment also increased AKT and ERK signaling. Combinations of CB-839 with AKT or MEK inhibitors (AZD5363 and GSK1120212B) were at synergistic and additive, respectively, for cell proliferation. The MEK inhibitor in combination with CB-839 resulted in supra-additive effects using apoptosis as an endpoint.

Conclusion: Glutaminase inhibition by CB-839 acutely inhibited mTORC1 signaling independently of AMPK signaling in HCC1806 cells. MEK and AKT signaling increased, likely in response to negative feedback loops with mTORC1. Combinatorial approaches with CB-839 targeting these compensation pathways resulted in increased responses supporting further preclinical and clinical studies.
Title: Metformin blocks de novo synthesis of cholesterol in triple negative breast cancer cells

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Body: Background: Metabolic shifts in cancer and stem cells are well recognized (the so called Warburg Effect). Tumor cells frequently demonstrate alterations in lipid metabolism. The anti-diabetic drug metformin has been shown to have broad anti-cancer activity, reducing both the incidence and mortality of several types of cancer. Metformin inhibits pro-oncogenic cell signaling and metabolic pathways, and has demonstrated unique anti-stem cell activity. We have reported that metformin is particularly active against triple negative (TN) breast cancer cells, inducing S phase arrest and apoptosis. Metformin down regulates de novo fatty acid synthesis, significantly reducing fatty acid synthase (FASN) and FASN protein expression. Mechanistically, these shifts appear secondary to a metformin associated up regulation of miR-193a, which directly targets the FASN 3'UTR (In press, Wahdan-Alaswad et al, Hormones and Cancer).

Cancer cells require high cholesterol for membrane synthesis, rigidity, the formation of lipid rafts (membrane regions which organize critical receptor/signaling molecules including the epidermal growth factor receptor (EGFR)), and to provide precursor molecules for hormones and sterols. While de novo cholesterol biosynthesis and high systemic cholesterol levels have been associated with an increase in breast cancer risk and a worse prognosis for women with the disease, clinical studies of statins (drugs to inhibit the mevalonate synthesis pathway and thus block cholesterol synthesis) have shown mixed results. Silvente-Poirot S et al have suggested that the contradictory effects of anti-cholesterol strategies may be due to the complexity of the genes and enzymes controlling the "sterolome" (Science, 2014).

Results: The effects of metformin on de novo cholesterol biosynthesis in the TN breast cancer cells, MDA-MB-231 and MDA-MB-468, were studied using expression profiling and miRNA microarrays. Metformin treatment for 24 h down regulated the expression of 21 genes encoding enzymes in the cholesterol biosynthesis pathway at high levels (up to 9 fold), which has been confirmed by qRT-PCR. Metformin treatment for 6 h also resulted in the upregulation of 17 miRNA, which are predicted to control many of the down regulated genes. These data suggest a post-translational inhibition of cholesterol biosynthesis, similar to what we have reported for fatty acid biosynthesis (see above). The down regulated enzymes include critical steps in complex cholesterol biosynthesis pathway occurring in the cytosol, endoplasmic reticulum and the nuclear envelope. Western blots have confirmed that miRNA 141 and miR192 reversed the metformin induced reduction in p-MEK1/2, p-Akt and pMAPK.

Conclusions: Metformin regulates cancer cells by multiple pathways including reduction in cell growth, increases in apoptosis and alterations in cellular metabolism. The effects on the cholesterol pathway are both extensive and affect multiple downstream pathways including EGFR, Akt, and MAPK.

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Title: Targeting lactate metabolism as a novel therapeutic target in platinum-resistant triple negative breast cancer (TNBC)

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Body: Introduction TNBC is a heterogeneous disease and is often associated with expression of a stem cell signature (CD44⁺, CD24⁻). Recently platinum agents have been shown to improve the pathological response rates in the neo-adjuvant setting; however a significant proportion of patients with TNBC will relapse. The study objective was to evaluate the potential of targeting glycolysis, mitochondrial function and lactate metabolism in platinum resistant TNBC cell lines.

Methods Stem cell signature and glycolytic phenotyping: CD44 and CD24 antibodies were used to measure the percentage of stem cells in each of the cell lines using flow cytometry(FACS). Protein expression of hexokinase, Glut1 transporter, lactate dehydrogenase A/B(LDHA and LDHB), and monocarboxylic transporter 1 and 4 (MCT1 and MCT4) was determined in MCF7(control), MDA-MB231, MDA-MB468, and HCC38 cells. Proliferation and apoptosis assays: IC50s for platinum, metformin, and MCT1 inhibitor AR-C155858, Tocris (MCT1I) were calculated by the sulforhodamine B (SRB) assay. Propidium iodide and AnnexinV staining intensity used to measure apoptosis by FACS. Metabolic analysis: The change in lactate and glucose levels in culture media post-treatment with metformin and MCT1I was quantified by 1H NMR. Intracellular lactate and ATP concentrations were determined by lactate(BioVision) and ATP determination assays(Invitrogen). Mitochondrial membrane potential and Reactive oxygen species (ROS) were measured by MitoTracker Red and MitoSOX Red. 2NBDG was used to assess cell glucose uptake. Combination index SRBs: Cells were treated with increasing concentrations of MCT1I and metformin for 72h. The combination index values were calculated using CalcuSyn® software.

Results MDA-MB231 and HCC38 cells were resistant to cisplatin and associated with a stem cell signature (CD44⁺, CD24⁻) which was not detected in the other cell lines. MCT1 was highly expressed in HCC38 cells but not at all in MDA-MB231. In contrast, MCT4 was abundantly expressed MDA-MB231 but minimally in HCC38 cells. In these two cell lines, only MDA-MB231 was sensitive to metformin which reduced ATP production, induced ROS generation, and increased the percentage of apoptotic cells. HCC38 also produced ROS, but ATP production and apoptosis were unaffected. HCC38 cells were more sensitive to MCT1I than other cells, which was associated with increased intracellular lactate. The NMR data showed significant increases in lactate in culture media following treatment with metformin in MDA-MB231 and HCC38. Only HCC38 had increased 2NBDG uptake and reduced of glucose levels in culture medium by metformin. Metformin significantly enhanced the anticancer efficacy of MCT1I in HCC38 cells, as a synergistic effect of the two agents was observed across the range of different concentrations; whilst an antagonistic effect was shown in MDA-MB231 cells with almost all concentrations.

Conclusions Targeting lactate metabolism selectively inhibits a platinum-resistant stem cell population of TNBC, this effect is enhanced when combined with metformin. The inhibition of glycolysis and lactate metabolism may represent a new therapeutic strategy for this population and warrants further study.
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Title: Metabolic state of reprogrammed breast cancer stem cells

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Body: Purpose: In general, tumor cells display a more glycolytic phenotype compared to the corresponding normal tissue. However, it is becoming increasingly clear that tumors are composed of a heterogeneous population of cells. Breast cancers are organized in a hierarchical manner, with the breast cancer stem cells (BCSCs) at the top of the hierarchy. Here, we demonstrate that breast cancer cells are heterogeneous in their metabolic phenotypes, with the breast cancer stem cells displaying a reliance on oxidative phosphorylation, while the more differentiated progeny display a glycolytic phenotype. The metabolic state of radiation-induced reprogramming of BCSCs was also investigated.

Methods: Luminal, basal, and claudin-low breast cancer cell lines were propagated as mammospheres enriched in BCSCs. Lactate production, glucose consumption and ATP content was compared with differentiated cultures. A metabolic flux analyzer was used to determine the oxygen consumption, extracellular acidification rates, maximal mitochondria capacity and mitochondrial proton leak. The effect of radiation treatment of the metabolic phenotype of each cell population was also determined.

Results: BCSCs consume more glucose, produce less lactate and have higher ATP content compared to their differentiated progeny. BCSCs have higher maximum mitochondrial capacity and mitochondrial proton leak compared to their differentiated progeny. Radiation treatment enhances the higher energetic state of the BCSCs, while decreasing mitochondrial proton leak.

Conclusions: Our study indicated that BCSCs reside in a distinct metabolic state compared to their differentiated progeny, and appear to depend more on oxidative phosphorylation. Interfering with the metabolic requirements of BCSCs may prevent radiation-induced reprogramming of breast cancer cells during radiation therapy, thus improving treatment outcome.
Title: An association between obesity and more aggressive breast cancer subtype

Saranya Chumsri, Paula Rosenblatt, Candace Mainor, Gauri Sabnis, Olga Goloubeva and Angela H Brodie. University of Maryland Greenebaum Cancer Center, Baltimore, MD.

Body: Background: Obesity has become epidemic in the United States with over one third of the adult population being obese. The incidence of obesity is even higher among African American population with slightly more than half of the population being obese. Elevated body mass index (BMI) has been associated with an increased risk of breast cancer in postmenopausal women. This is believed to be due to higher levels of estrogens, inflammation, and insulin. Given that adipose tissue is the major source of estrogen production via the aromatization of androgens into estrogens in postmenopausal women, there is a concern that obese women may have inadequate estrogen suppression with a fixed dose of aromatase inhibitors (AIs). Multiple recent retrospective analyses of large phase III adjuvant endocrine therapy trials demonstrated that obese patients had worse outcome when treated with AIs. However, it remains unclear whether there is also a difference in the tumor subtype among normal weight, overweight, and obese patients.

Method: A retrospective review of breast cancer cases diagnosed from July 2008 and August 2011 was performed. Patient characteristics, BMI, and pathologic findings were collected. An immunohistochemistry (IHC)-based molecular subtypes was assigned based on St. Gallen criteria: Luminal A (ER/PR+, HER2- and Ki67 \leq 14%), Luminal B (ER/PR+, HER2+ or Ki67 > 14% or grade 3), HER2 enriched (ER/PR-, HER2+), and Triple negative breast cancer (TNBC; ER/PR-, HER2-). Using the WHO classification, normal weight was defined as BMI < 25, overweight BMI 25-30, and obese BMI \geq 30.

Result: 143 patients were included in the analysis. The median age was 55 years. The majorities of our patients were African American (63%) and presented with early stage disease (stage I 29%, stage II 45%, stage III 22%, and stage IV 5%). IHC-based molecular subtypes were luminal A 20%, luminal B 48%, HER2 enriched 7%, and TNBC 25%. 21% were normal weight, 30% overweight and 49% obese. As shown in the table below, comparing between normal weight, overweight, and obese patients, there is a possible association between tumor subtype and BMI status (p = 0.03). The majority of TNBC patients (94.4%) were overweight or obese. Specifically, there is significantly more aggressive luminal B subtype compared to luminal A (52.86% vs. 15.71%) among obese patients (p = 0.017). We further evaluated the effect of obesity and tumor growth in xenograft model of MCF7Ca. Comparing between obese mice fed with high fat vs. lean mice fed with chow diet, there is a significant increase in tumor growth among obese mice (p = 0.04) which corresponds to the high proliferation index measured by Ki67 seen in patients with luminal B subtype.

IHC-based molecular subtypes and distribution by weight category

<table>
<thead>
<tr>
<th>Weight Category</th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>HER2 Enriched</th>
<th>TNBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Weight</td>
<td>8 (26.67%)</td>
<td>15 (50%)</td>
<td>5 (16.67%)</td>
<td>2 (6.67%)</td>
</tr>
<tr>
<td>Overweight</td>
<td>9 (20.93%)</td>
<td>17 (39.53%)</td>
<td>2 (4.65%)</td>
<td>15 (34.88%)</td>
</tr>
<tr>
<td>Obese</td>
<td>11 (15.71%)</td>
<td>37 (52.86%)</td>
<td>3 (4.29%)</td>
<td>19 (27.14%)</td>
</tr>
</tbody>
</table>

Conclusion: Our study suggests that obesity may provide a microenvironment that support and accelerate tumor growth, particularly in luminal breast cancer subtypes. Furthermore, the poor outcome seen in obese patients may also be in part due to more aggressive tumor subtype.
The role of nitric oxide synthase uncoupling in breast tumor cell maintenance

Christopher S Rabender¹ and Ross B Mikkelsen¹. ¹Virginia Commonwealth University, Richmond, VA.

Body: Nitric oxide (NO) has been demonstrated to activate both proliferative pathways as well as pro-apoptotic pathways in breast carcinogenesis. One explanation that may account for these apparently conflicting conclusions is the dual activity of nitric oxide synthase (NOS). The unstated assumption in most all studies with tumors is that NOS synthesizes NO from arginine; however, as is well documented in vasculature literature, NOS can have two activities: NO or O2-/ONOO- synthesis. A requirement of NOS for the production of NO is the cofactor tetrahydrobiopterin (BH4) and in inflammatory environments BH4 can undergo direct oxidation to dihydrobiopterin (BH2). It has been demonstrated that the product of NOS catalytic activity is dictated by the ratio of BH4:BH2, as the enzyme has equal affinity for both. When BH4 is bound in the active site, NO is the product; whereas, O2- predominates when BH2 is bound. Given the inflammatory microenvironment of breast tumors, our studies set out to examine NO signaling in breast tumor cells from a new perspective of NOS dysregulation. We examined BH4:BH2 in MCF-7 and MDA231 breast tumor cells both in tissue culture and as flank xenografts as well as in spontaneously derived breast tumors (MMTV-neu) and determined that the BH4:BH2 is significantly reduced over what we measure in normal tissue. This results in NOS uncoupling and leads to decreased NO signaling and increased pro-inflammatory, pro-survival, signaling as a result of the increased generation of ROS/RNS from uncoupled NOS activity. We are able to increase the BH4:BH2 and recouple NOS through the exogenous BH4 precursor, sepiapterin, both in vitro and in vivo, reducing ROS/RNS and reestablishing NO signaling through cGMP protein associated kinase. Reduction of ROS/RNS results in the reduced activity of two major constitutively active transcription factors in breast cancer cells, NFκB and STAT3. In MCF-7 and MDA231 cells we found that increased NO-dependent PKG signaling led to tumor cell toxicity mediated by downregulation of β-catenin expression and TCF-4 promoter activity. Downregulation of β-catenin led to increased protein levels of p21 in MCF-7 and p27 in MDA 231cells, ultimately resulting in cell death in vitro and in vivo as measured by ex vivo clonogenic assay and 18F-deoxyglucose positron emission tomography. These results along with our results demonstrating decreased spontaneously developing mammary carcinogenesis in MMTV-neu mice suggest that there is potential for BH4 as a therapeutic agent.

BH4:BH2 measurements in multiple cell types in vitro and in vivo

<table>
<thead>
<tr>
<th>Normal Tissue</th>
<th>BH4:BH2 +/- SEM</th>
<th>In vitro tumor cell lines</th>
<th>BH4:BH2 +/- SP +/- SEM</th>
<th>In vivo tumors</th>
<th>BH4 +/- SEM</th>
<th>BH4:BH2 + SP +/- SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon</td>
<td>7.1 +/- 0.6</td>
<td>A431</td>
<td>0.3 +/- 0.5</td>
<td>2.1 +/- 0.6</td>
<td>Fadu</td>
<td>0.8 +/- 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.6 +/- 0.3</td>
</tr>
<tr>
<td>Lung</td>
<td>11.1 +/- 2.4</td>
<td>MCF-7</td>
<td>0.3 +/- 0.1</td>
<td>10.1 +/- 0.4</td>
<td>MCF-7</td>
<td>1.1 +/- 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>7.9 +/- 0.2</td>
</tr>
<tr>
<td>Liver</td>
<td>8.3 +/- 0.8</td>
<td>MDA231</td>
<td>1.0 +/- 0.1</td>
<td>5.8 +/- 0.2</td>
<td>MDA231</td>
<td>1.4 +/- 0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>6.4 +/- 0.4</td>
</tr>
<tr>
<td>Kidney</td>
<td>13.4 +/- 1.2</td>
<td>SW1990</td>
<td>1.3 +/- 0.3</td>
<td>3.7 +/- 0.5</td>
<td>HT29</td>
<td>1.1 +/- 0.4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.8 +/- 0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>MCF10a</td>
<td>1.2 +/- 0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.1 +/- 0.3</td>
</tr>
<tr>
<td>MMTVneu mammary fat pad</td>
<td>3.8 +/- 0.5</td>
<td></td>
<td></td>
<td></td>
<td>MMTVnew mammary tumors</td>
<td>1.2 +/- 0.5</td>
</tr>
</tbody>
</table>

Mean +/- SEM based on a minimum of n=3 individual mouse or tissue culture samples.
Title: A novel pharmacodynamic assay to measure glutaminase inhibition following oral administration of CB-839 in triple negative breast cancer biopsies

Andy MacKinnon¹, Mark Bennett¹, Ethan Emberley¹, Mathew Gross¹, Julie Janes¹, Evan Lewis¹, Alison Pan¹, Mirna Rodriguez¹, Peter Shwonek¹, Taotao Wang¹, Jinfu Yang¹, Frances Zhao¹ and Francesco Parlati¹. ¹Calithera Biosciences, South San Francisco, CA.

Body: Triple negative breast cancer (TNBC) cell lines are highly dependent on glutamine (Gln) for growth and survival. A critical step in Gln utilization is its conversion to glutamate (Glu) by the mitochondrial enzyme glutaminase (GLS). CB-839 is a potent inhibitor of GLS that has anti-proliferative activity in TNBC cell lines and antitumor activity in TNBC xenograft models (Gross et al., Mol. Cancer Ther. 13:890). Across a panel of breast cancer cell lines derived from both receptor positive and TNBC tumors, sensitivity to CB-839 was associated with (i) elevated GLS expression, (ii) elevated GLS activity, and (iii) the TNBC subtype. Importantly, many of the determinants of CB-839 sensitivity in cell lines are also present in primary tumor samples, including high mRNA and protein expression of GLS and a high Glu to Gln ratio in TNBC tumors as compared to receptor positive tumors. These observations motivate the Phase 1 clinical study of CB-839 in TNBC patients. To aid in the selection of a recommended Phase 2 dose, we sought to develop a pharmacodynamic (PD) assay to directly measure the GLS activity in breast tumor lysates in order to determine the extent of GLS inhibition in tumor biopsies from CB-839 treated patients.

To develop a robust PD assay, we first identified conditions that maintain the GLS:CB-839 inhibitory complex during preparation of lysates from CB-839 treated samples. High concentrations of KCl (150 mM) and low concentrations of K-phosphate (15 mM) in the lysis buffer, as well as maintaining the lysate at a low temperature stabilized the inhibited complex. Following gel filtration of the lysate to remove unbound CB-839 and exchange the buffer, GLS activity was immediately measured with a coupled enzyme assay. The GLS activity measured at this step reflects the residual activity present in a sample that was exposed to CB-839. To quantify the amount of total GLS present in the sample, we incubated the same lysate for 3 hours at room temperature under conditions of low KCl and high phosphate to allow the the GLS:CB-839 complex to fully dissociate prior to measuring activity. This assay format allows quantitation of the % GLS inhibition from a single tumor lysate sample and eliminates the requirement for multiple biopsies as well as any assay variability due to tumor heterogeneity.

We utilized this tumor PD assay to determine the plasma drug levels required for maximal tumor GLS inhibition in a preclinical TNBC model. Mice bearing HCC1806 TNBC tumors were first treated with a range of CB-839 doses. Four hours after oral administration, a 10 mg/kg dose of CB-839 resulted in >90% inhibition of tumor GLS. CB-839 plasma concentrations of 100 nM corresponded to 50% inhibition of tumor GLS, while maximal inhibition occurred at plasma concentrations ≥300 nM. In xenograft studies, maximal anti-tumor efficacy was achieved with BID dosing at 200 mg/kg, a dose and schedule that resulted in trough plasma levels of CB-839 of ≥300 nM and sustained GLS inhibition in tumors. As part of an ongoing Phase 1 trial, this assay will be utilized to monitor tumor PD responses in TNBC patients undergoing treatment.
**Title:** Reduction of HER2-associated lipogenic phenotype by docosahexaenoic acid (DHA) induces apoptosis in breast tumor cells harboring HER2 overexpression

Graziela R Ravacci¹, Maria M Brentani¹, William Festuccia², Tharcisio Tortelli¹, Angela F Waitzberg³ and Dan L Waitzberg¹.

¹University of São Paulo â–“ FMUSP, Faculty of Medicine, São Paulo, SP, Brazil; ²Institute of BioMedical Science â–“ University of São Paulo â–“ ICB/USP, São Paulo, SP, Brazil and ³Federal University of São Paulo (UNIFESP), São Paulo, SP, Brazil.

**Body:** The lipogenic phenotype is associated with oncogenic transformation and malignancy in the cancer setting. Our hypothesis is that the oncogenic nature of lipogenesis depends on the expression of HER2 oncogene, and its modulation by docosahexaenoic acid-DHA, might induce apoptosis in breast tumor cells with HER2 overexpression. To evaluate if the lipogenic phenotype might be induced by HER2 overexpression, we used a oncogenic transformation cell model in which, cells were engineered to overexpress HER-2 receptor (HB4aC5.2), but are identical to their parental strain (HB4a) in all other aspects, permitting an specific analysis of enhanced HER-2 effects. Toward this end the lipogenesis profiling was characterized, evaluating several molecular features, including synthesis (FASN), uptake(CD36), transport(FABP4) and storage(DGAT) of FA by RT-PCR and lipogenic regulatory pathways (mTOR, DEPTOR, SREBP1 and PPARγ) in both cells. Lipogenic contribution to lipid rafts formation, which is necessary to HER2 receptor location and activation in the cell membrane, was evaluated by gas chromatography and confocal microscopy. The influence of HER2 overexpression and lipogenic phenotype on proteins activated by HER-2 (AKT, ERK1/2 and FASN) was analyzed by western blot. Next, HB4a and HB4aC5.2 cells were treated, alone or in combination, with DHA, Trastuzumab (anti-HER2), and GW9662 (PPARγ inhibitor) for 72h, and the above experiments were repeated. Cell death was analyzed by flow cytometry and confocal microscopy. Results: In HB4aC5.2 cells, the oncogenic transformation by HER2 overexpression was associated with a lipogenic phenotype, which contributed to increase of lipid rafts formation, activation of survival and proliferation signals, as compared to HB4a normal cells (p<0.001). It is believed that PPARγ is the main regulator of cell lipogenesis. However, our data have shown that mTOR/SREBP pathway, exclusively mTORC1, was decreased concomitantly to increase of DEPTOR gene expression and AKT activation, in HB4aC5.2 cells. Also, the treatment with GW9662 did not change the expression of lipogenic genes, suggesting that lipogenesis seems independent of PPARγ activation, and that HER-2 overexpressing cells may use alternative mechanisms to maintain the lipogenic phenotype. DEPTOR is overexpressed in white adipose tissue and is associated with regulation of lipogenesis. Moreover, DEPTOR overexpression suppresses S6K1 but, by relieving feedback inhibition from mTORC1 to PI3K signaling, activates Akt. In HB4aC5.2 cells, only the DHA treatment decreased the lipogenic phenotype, DEPTOR expression, disrupted lipid raft, inhibited HER-2 signaling, and induced apoptosis (p<0.001). In addition the combined treatment using DHA and Trastuzumab increased cell death. Conclusion: We show that lipogenic phenotype was associated with HER-2 in breast cancer, can be induced early in the oncogenic transformation, and it seems important to promote survival and proliferation. Otherwise, its modulation by DHA increased death in tumor cells, suggesting this kind of FA may represent a useful tool for controlling the HER2-positive breast cancer.
Body: Background: Patients (pts) frequently report ‘fatigue’ as a consequence of breast cancer therapy but the true impact of cancer treatments on body composition and energy expenditure has not been measured.

Patients and Methods: Pts with stage 0-III were evaluated prior to any therapeutic intervention to assess baseline health status, at 6 months to assess acute impact of therapy, and at 12 months to assess chronic effects and ‘spontaneous’ recovery. Measurements included body composition, maximum watts produced and power envelope (watts/kg) based on laboratory testing, energy expenditure over 7 days in real world conditions, insulin resistance (HOMA), fatigue (BFI), quality of life scales (FACT-B), and serum for insulin-like growth factor-1 (IGF-1), adiponectin, leptin, markers of bone formation (P1NP, BSAP, osteocalcin), bone resorption (sCTX), and bone mineral metabolism (PTH, 25-OH Vit D).

Results: 45 patients have been enrolled; 25 have completed the 6 month assessment to date. Median age was 52 (30-68). Patients were analyzed separately based on therapies received.

<table>
<thead>
<tr>
<th></th>
<th>Chemotherapy +/- HRT</th>
<th>Hormone Therapy</th>
<th>Local Therapy Only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline (n=23)</td>
<td>Baseline (n=14)</td>
<td>Baseline (n=8)</td>
</tr>
<tr>
<td></td>
<td>6 months (n=12)</td>
<td>6 months (n=11)</td>
<td>6 months (n=2)</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>27.8</td>
<td>26.3</td>
<td>28.7</td>
<td>29.3</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>39.5</td>
<td>36.3</td>
<td>42.0</td>
<td>41.1</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>43.9</td>
<td>47.3</td>
<td>46.5</td>
<td>50.6</td>
</tr>
<tr>
<td>HOMA-IR*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.75</td>
<td>1.77</td>
<td>1.21</td>
<td>1.81</td>
</tr>
<tr>
<td>Peak power (Watts)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>68.2</td>
<td>44.1</td>
<td>70</td>
<td>53</td>
</tr>
<tr>
<td>Watts/kg lean muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.7</td>
<td>1.2</td>
<td>1.7</td>
<td>1.3</td>
</tr>
<tr>
<td>Time to zero power (min.sec)^</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12.3</td>
<td>6.39</td>
<td>9.5</td>
<td>6.9</td>
</tr>
</tbody>
</table>

*HOMA-IR was not assessed in patients known to have diabetes. ^Time to zero power quantifies the time over which patients can sustain generating motive power as a measure of endurance.

Week long bi-axial pedometry studies at baseline and six months revealed a decrease in pt activity between 17 and 23 percent, confirming the decrease in motive power seen in the power envelope and body composition tests. Enrollment continues to a planned total of 75 pts. Additional follow-up with 12 month assessment, as well as QOL and serum data will be presented.

Conclusions: Pts in this study were more deconditioned than expected based on population normative data. Systemic therapy had a profound impact on body composition with a substantial decrease in lean muscle mass with a corresponding increase in % body fat and decrease in the ability to generate and sustain motive power. Our data suggest that most patients would not be able to participate in or comply with commonly proposed exercise interventions and physical activity recommendations. Future interventions need to be restructured, following the model of individualized rehabilitation with careful assessment of the pts baseline status to improve the health of breast cancer survivors.
Title: BMI and metabolic factors in long-term breast cancer survivors: Changes from diagnosis and comparison to non-breast cancer controls

Ana Elisa Lohmann¹,², Marguerite Ennis³ and Pamela J Goodwin¹,². ¹Samuel Lunenfeld Research Institute, Sinai Hospital, Toronto, ON, Canada; ²Division of Medical Oncology and Hematology, University of Toronto, Toronto, ON, Canada and ³Applied Statistician, Toronto, ON, Canada.

Body: Background: The metabolic syndrome is associated with poor breast cancer (BC) outcome. We evaluated changes from diagnosis in metabolic factors and BMI in long-term survivors and compared their status at long-term follow-up (LTFU) to that of age-matched women with no history of BC.

Methods: A total of 535 women with early breast cancer were enrolled between 1989 and 1996 and followed prospectively. From 2005 to 2007, those alive without distant recurrence were re-contacted to participate in a long-term followed-up study and 285 agreed. A control group of 167 age-matched women without cancer history was recruited from women presenting for screening mammograms. Mean changes in metabolic factors from diagnosis to long-term follow-up were assessed with paired t-tests. In spite of matching, controls were younger and had higher income than survivors and the comparison to controls was made using age-adjusted regression models. Variables were transformed to normality before statistical testing.

Results: With a median follow-up of 12.5 years, BC survivors gained on average 2.35 kg and BMI, waist and hip circumference, waist-hip ratio, glucose, insulin, HOMA, total cholesterol and its components (but not triglycerides) increased significantly. After age adjustment, waist circumference, glucose, HOMA and total triglycerides were significantly higher in BC survivors compared to controls

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted mean ± standard deviation</th>
<th>Unadjusted mean ± standard deviation</th>
<th>P-value for age-adjusted difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC patients</td>
<td></td>
<td>controls</td>
<td></td>
</tr>
<tr>
<td>waist circumference (cm)</td>
<td>85 ± 12</td>
<td>81 ± 10</td>
<td>.01</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.5 ± 1.0</td>
<td>5.2 ± 0.9</td>
<td>.01</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>60.9 ± 50.5</td>
<td>47.1 ± 28.3</td>
<td>.06</td>
</tr>
<tr>
<td>HOMA</td>
<td>2.25 ± 2.24</td>
<td>1.64 ± 1.24</td>
<td>.03</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.28 ± 0.64</td>
<td>1.10 ± 0.57</td>
<td>.01</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.3 ± 4.9</td>
<td>25.4 ± 4.5</td>
<td>.26</td>
</tr>
</tbody>
</table>

Despite exclusion of BC patients with diabetes at study entry, 24.9% of BC survivors self reported diabetes or pre-diabetes (1.99%/year) versus 12.6% in controls (OR 2.3, p= .0017).

Conclusion: The metabolic status of long-term BC survivors deteriorated over time and age-adjusted at LTFU were worse with respect to a number of factors compared to the control group.
Title: Prevention of aromatase Inhibitor (AI)-induced joint symptoms with omega-3 fatty acid supplementation: A randomized placebo-controlled pilot study

Maryam B Lustberg1, Tonya Orchard6, Xueliang Pan6, Raquel Reinbolt1, Amanda Logan1, Joanne Lester2, Rachel M Layman1, Erin Macrae1, Ewa Mrozek1, Bhuvaneswari Ramaswamy1, Robert Wesolowski1, Michael Berger1, Michael Knopp2, Charles Loprinzi6, Charles L Shapiro1 and Lisa Yee1. 1Ohio State University Comprehensive Cancer Center & The Stefanie Spielman Comprehensive Breast Center, Columbus, OH; 2Ohio State University Comprehensive Cancer Center, Columbus, OH; 3Ohio State University, Columbus, OH and 4Mayo Clinic, Rochester, MN.

Body: Introduction: AI-induced joint symptoms negatively impact drug adherence and quality of life. Based on observations that n-3 polyunsaturated fatty acids (PUFAs) have anti-inflammatory effects and that the mechanism of AI-induced joint symptoms may be partly due to inflammation, we hypothesized that women taking more n-3 PUFAs are less likely to develop AI-induced joint symptoms.

Methods: We conducted a randomized, double-blind, placebo-controlled study comparing n-3 PUFA vs placebo in postmenopausal breast cancer patients starting adjuvant Al. Participants were randomized to n-3 supplements [2.58 g eicosapentaenoic acid + 1.74 g docosahexaenoic acid/day; Marine Nutriceuticals, Mt. Bethel, PA] vs matched placebo for 24 weeks (wks). Primary endpoints was feasibility; secondary outcomes were self-reported symptoms as assessed by the Brief Pain Inventory short form (BPI-SF), Functional Assessment of Cancer Treatment, Breast & Endocrine Symptoms (FACTB-ES), and Stanford's Health Assessment and Disability Index (HAQ) at baseline prior to AI receipt, 12 and 24 wks. Compliance and toxicity were evaluated monthly. Serial peripheral blood n-3 PUFA levels and inflammatory cytokines (IL-6, TNFR2, IL-17) were drawn. MRI of hands/wrists was performed in selected patients using a 3 Tesla dedicated wrist coil at baseline and treatment end.

Results: Forty-four women were enrolled and randomized to study drug; 42 received ≥1 cycle (4 wks) of treatment; 36 had ≥1 post treatment evaluation at wk 12 or 24. Median age was 59.5 (range 43-76); history of prior taxane (n=15, 34%). The two groups' baseline characteristics were similar. Overall, 93% and 88% of patients took >80% of the placebo and n-3 PUFA doses, respectively. Baseline erythrocyte n-3 PUFA was similar for both groups (6.6% ± 1.6%, 7.2% ±1.9%, p=0.20), but higher in the n-3 PUFA arm by wk 24 (6.5%±1.0% vs 15.0%±3.3%, p<0.001). Most toxicities were grade 1; the n-3 PUFA arm had only 1 (2.5%) grade 3 toxicity (diarrhea). The n-3 PUFA arm reported lower mean BPI-SF scores after treatment [(-.0.28/-.25 at week 12/24); but not statistically significant compared to placebo (p=0.494 and 0.601)]. Based on BPI-SF, the n-3 PUFA arm reported less interference of pain symptoms compared to placebo at 12 weeks (-.72, p=0.08). This arm also had a decreased walking, activity and working (WAW) score on BPI-SF at 12 weeks (-.81 p=0.05), and reported significantly greater pain relief from medications at 12 (p=0.043) and 24 weeks (p=0.011). Both arms had similar baseline and wk 24 serum IL-6 levels; levels decreased from baseline to wk 24 in the n-3 PUFA arm (-0.54±0.25, p=0.048). There was a non-significant trend (p= 0.2) toward decreased wrist inflammation by MRI imaging at 24 wks in the n-3 PUFA arm.

Conclusions: This is the first randomized pilot study to show that n-3 PUFA supplementation to prevent AI-induced joint symptoms is feasible and well tolerated. There is preliminary evidence that this intervention may help reduce the burden of AI-induced arthralgias.

OSU Study #11022; ClinicalTrials.gov Identifier: NCT01478477. Grants from the National Cancer Institute (CA037447-26) to the Alliance for Clinical Trials in Oncology supported this pilot study.
Introduction
Chemotherapy-induced peripheral neuropathy (CIPN) is a dose-limiting toxicity of several chemotherapy drug classes, including taxanes. Peripheral neuropathies have been shown to lead to pain, falls, and difficulty in walking and performing activities of daily living in a variety of patient populations. Although the prevalence of CIPN has been noted in cancer patients, the development of self-reported symptoms, gait changes and balance changes during treatment have not been well explored to date. We hypothesized that the use of taxane-based chemotherapy will result in significant changes in spatiotemporal gait and balance parameters, as well as self-reported quality of life and function.

Methods
We characterized the alterations in gait and balance that occur in non-metastatic breast cancer patients during taxane chemotherapy. We evaluated (1) spatiotemporal gait parameters, including cadence and step length, and (2) balance parameters, including time-to-contact and 95% ellipse area, using each patient as her own control. Laboratory assessment of gait and balance was conducted at baseline and at completion of therapy in selected patients. We compared the natural history of changes in gait and balance parameters with changes in CIPN status as measured by validated patient reported outcomes, including EORTC QLQ-C30, CIPN-20, and Brief Pain Inventory Short Form (BPI-SF), and the Duke Activity Status Index (DASI). Time points included pre-chemotherapy, after each cycle of chemotherapy, and one month after the end of therapy to collect information on neuropathy, pain and functional capacity. The preliminary data were illustrated using individual plots; trend lines (changing over time) were based on least square means at each time point, which were estimated using the linear mixed models for repeated measures.

Results
To date, 15 patients with localized breast cancer have been enrolled; patient recruitment is ongoing. The median age is 42 years (range 25-67). Ten patients (67%) received weekly paclitaxel, 1 patient (7%) received paclitaxel every 2 weeks, and 4 patients (27%) received docetaxel every 3 weeks. Preliminary results with these 15 patients, based on least square means at each timepoint, showed trends in several parameters. As treatments progressed, patients tended to develop more difficulty in quiet balance and in their ability to actively shift weight in the sagittal and frontal planes. From the CIPN-20, they also tended to develop increased difficulty with sensory and motor systems. From the QLQ-C30, their global health status also tended to worsen. For most of these parameters, the largest changes were observed between the 2nd and 3rd treatments, though some changes were not observed until the 4th treatment. From the BPI-SF, no trends in pain symptoms or pain interference were observed within this preliminary cohort.

Conclusions
Gait and balance testing is feasible in the clinical setting. Preliminary observations suggest that balance, function and quality of life may all be affected by taxane therapy, even without pain symptoms. The findings of this study will enable us to better characterize the neurotoxic effect of taxanes and to ultimately test the effectiveness of preventative measures and interventions. Funding by NCI R03CA182165-01.
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Title: A prospective longitudinal study of the impact of breast cancer treatment with adjuvant chemotherapy or tamoxifen alone on ovarian reserve

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Body: Background: Many women with breast cancer (BC) of reproductive age would prefer to preserve their fertility and ovarian function, if possible. However, available information is insufficient to predict the likelihood and extent of ovarian damage that will be suffered by an individual woman. In prior studies, menses was used as a surrogate for fertility, but it is a poor marker of future fertility. Preliminary work suggests that anti-mullerian hormone (AMH) may be a better surrogate for ovarian reserve. The goal of this study is to delineate the extent of ovarian damage from specific treatment regimens by using serum AMH. Here we report results from the largest longitudinal study with a pretreatment evaluation to measure the impact of both chemotherapy (chemo) and tamoxifen (tam) on ovarian reserve in BC patients. Methods: A multi-institutional longitudinal IRB-approved study was performed in 207 premenopausal women with stage 0-III BC. AMH levels were evaluated at baseline and 1 yr post completion of adjuvant chemo or 1 yr post initiation of tam. After the exclusions (failure to follow-up, sample inadequacy, presumed PCOS and consent withdrawal), 115 women ages 26-46 (median 38) were available for analysis at 1 yr post treatment (primary endpoint). AMH levels were measured in frozen sera with an in-house ultrasensitive ELISA assay and were log transformed due to non-normal distribution. Results were analyzed with Wilcoxon rank sum test and repeated measures ANOVA to adjust for age, tam use and stage. Results: In the 115 evaluable women, 98 pts had HR positive BC & 17 had triple negative BC. 77 pts received anthracycline-based (ddAC-T/EC-T) chemo, 26 non-anthracycline-based chemo (TC/CMF/TH) & 12 tam only. In the 103 women who received chemo, compared to baseline (median 0.21 ng/ml, 0.001-3.9 ng/ml), AMH levels declined significantly (median 0.11 ng/ml, 0.001-4.47 ng/ml; p<0.0001) at 1 yr post chemo. The type of chemo, stage and adjuvant tam use did not significantly impact the results. In the 12 women who received tam only, compared to baseline (median 0.505 ng/ml, 0.010-1.27 ng/ml), AMH levels declined significantly (median 0.155 ng/ml, 0.010-0.91 ng/ml; p=.0049) at 1 yr. Despite a significant decline in ovarian reserve by AMH, 69% (66/95) of pts who received chemo had return of menses by 1 yr post treatment. Conclusion: This longitudinal study shows that BC chemo is detrimental to ovarian reserve. Although a decline in AMH was also seen in pts treated with tam only, this is a small cohort and the results are hypothesis generating. The decline in AMH may be due to ovarian stimulation from tam, which can cause predominance of larger follicles that produce less AMH rather than actual reduction in ovarian reserve. This needs to be studied in a larger cohort. The fact that the majority of pts resumed their menses despite a significant decline in their ovarian reserve by AMH indicates that resumption of menses is not the best measure of ovarian normalcy/fertility. This new info can be used for counseling pts in childbearing ages so they can make better informed decisions on fertility preservation, if desired. Supported by NIH RO1 HD053112, Jodi Spiegel Fisher Cancer Foundation and Susan G. Komen Foundation.
**Title:** Patient centered support in the survivorship care transition: Outcomes from the patient-owned survivorship care plan intervention

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**Body:** Background: Survivorship care plans will be mandated for use by 2015. Few studies have focused on the care transition process, and few show an impact on the patient experience or health outcomes. This study examines the effect of a patient-owned survivorship care plan (POSCP) intervention on patient outcomes and care coordination. Based on a conceptual model that incorporates IOM survivorship priorities and the chronic care model, the POSCP intervention is a single coaching encounter utilizing motivational interviewing to engage patients in the development of a POSCP that incorporates health goals and strategies related to surveillance, symptom management, and health behavior.

Methods: 79 recent breast cancer survivors Stage I-IIIB were randomized to receive POSCP intervention (n=40) or usual care (n=39). Patient outcomes were assessed using SF-36 Health Survey (SF-36), Social/Role Activities Limitations, Self-Efficacy for Managing Chronic Disease Scale, and Personal Health Questionnaire Depression Scale (PHQ-9) at baseline and 3 month follow up. Care coordination was assessed using confirmed primary care physician (PCP) visits and reported discussion of survivorship plan at follow up. Logistic and linear regressions were conducted to examine the effect of intervention on care coordination, patient experience, and health outcomes.

Results: In this sample age M= 58.35, SD = 10.62, 81% (N=64) non-hispanic white, 16.5% (N=13) African American. A greater proportion of participants in the intervention group reported a confirmed appointment (59.5% vs. 51.4%) and discussed survivorship issues (47.4% vs. 35.1%) with their PCPs compared to those in the usual care group, however these differences did not achieve statistical significance (p= .70 and .65). After controlling for education and perceived financial adequacy, participants in the intervention group demonstrated significantly higher self-reported health (F (3, 70) = 3.57, p< .05), lower social role limitation (F (3, 70) = 3.82, p < .05), as well as a trend toward greater self-efficacy (F (3, 69) = 2.51, p = .07) compared to those in the usual care group. Results of paired-samples t-tests revealed a significant decrease in depression scores from baseline to 3 months follow up in the intervention group, t (36)= 3.21, p < .01, η^2 =.23. No between group differences were identified in symptom burden or patient activation.

Conclusion: This pilot study of a POSCP process utilizing motivational interviewing techniques to enhance patient engagement has a positive impact on patient outcomes and demonstrates promise as a strategy to improve survivor patient experience, care coordination, and health outcomes.
Title: Risk factors for lymphedema are dependent on level of axillary surgery

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Body: Background: Differentiation of lymphoedema (LE) risk factors for those who have undergone a sentinel node biopsy (SNB) from those who have undergone axillary node dissection (AND) is not considered, even though the incidence rates for the two are vastly different. In addition, events women are typically cautioned against have not been well investigated.

Methods: A prospective study was conducted in which women were recruited and assessed prior to surgery, and then seen within 4 weeks following surgery, and at 6, 12 and 18 months following surgery. Women were categorised as having LE if their bioimpedance interlimb ratio exceeded previously established thresholds. Following post-surgery assessment, women were asked to complete weekly diaries regarding events that occurred in the previous week. Risk factors were broadly grouped into demographic, lifestyle, breast cancer treatment-related, arm swelling-related, and post-surgical activities (eg, airplane travel). Crude association between each potential predictor and presence of arm swelling was then identified using unadjusted logistic regression. Those variables with P<0.2 at this initial screen were considered for inclusion in a logistic regression model. The final multivariable model retained all variables with P<0.1 or odds ratio> 2.0, taking into account biological plausibility. The final multivariable models were developed without and with consideration of the presence of swelling in the first year.

Results: 450 women (SNB group: 241; AND group: 209) were recruited and attended the final assessment; a subgroup of 243 women, of whom 112 had AND completed >70% of the weekly diaries. The incidence of LE for the SNB group was 3.3% (n=8) and 18.2% (n=38) for the AND group. The unadjusted risk factors for LE at 18 months for SNB included high BMI and absolute body weight, living alone and presenting at diagnosis with three or more other medical conditions. The final model for the SNB group included a high BMI and not living with a partner, explaining 21.3% of the variance. Inclusion of post-operative swelling in the model explained 48.4% of the variance. The unadjusted risk factors for LE at 18 months for AND included being older, low education, Stage 3, high number of nodes removed and involved, and radiotherapy to the axilla, and receiving taxane chemotherapy. The final risk factors model for the AND group included clinical stage 3, being older, low education, and receiving taxane-based chemotherapy, explaining 20.4% of the variance. The addition of any swelling within the first 6 months following surgery explained 36.8% of the variance. Notably none of the factors related to air travel, arm trauma, medical procedures (eg, blood pressure, injections, blood drawn on the affected side), or exercise which women are typically cautioned against differentiated women who had and did not get LE at 18 months.

Conclusion: Advice to women undergoing SNB should differ to that provided to those undergoing AND, and for both, we should not be burdening them with range of behaviors to avoid. Importantly, for women at high risk, periodic assessment in the first year should occur to identify and manage any arm swelling.

Acknowledgement: Cancer Australia and NBCF.
Title: Determination of the first evidence-based diagnosis of secondary upper limb lymphedema

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Body: Background: The incidence of secondary upper limb lymphedema after treatment for breast cancer is unclear due to the wide variety of measurement tools and diagnostic thresholds that are used in both the literature and clinical practice. Furthermore, this lack of clarity in what constitutes lymphedema or not has prevented the progression of the field of lymphedema. Many of the thresholds have been chosen for ease of use only and have no evidence base to support them. The aim of this study, therefore, was to determine which clinical diagnostic threshold for unilateral upper limb lymphedema has the best sensitivity and specificity when compared to diagnosis by lymphoscintigraphy.

Methods: Women with and without a history of secondary upper limb lymphedema were assessed using lymphoscintigraphy, bioimpedance spectroscopy (BIS) as well as volume and circumference measurements using the perometer. Dermal backflow score was determined as the diagnostic criteria for the lymphoscintigraphy and was assessed by an experienced nuclear medicine physician. Determination of the presence of lymphedema by lymphoscintigraphy was compared with diagnosis by both commonly-used and normatively-determined diagnostic thresholds for circumference, volume and bioimpedance.

Normatively-and commonly-used diagnostic thresholds examined

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<th>Whole arm volume</th>
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<td><strong>Normatively-determined thresholds examined</strong></td>
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<td>3SD perometry threshold*</td>
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<td>3SD frustum threshold*</td>
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<th>Commonly-used thresholds examined</th>
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<td>200 ml interlimb difference</td>
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<td>10% difference</td>
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<td>5 cm SOAC</td>
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* Dylke et al, 2012; Sum of arm circumferences (SOAC); Circumference measure (Circ)

Results: For those with widespread dermal backflow, any clinical diagnostic criteria could differentiate between those with and without lymphedema. In contrast, for those with mild to moderate dermal backflow, only the normatively-determined threshold, set at 2 standard deviations above the norm, for arm circumference and full arm bioimpedance (Cornish et al 2001) had adequate sensitivity and specificity. Both of these thresholds had clinically relevant positive (23 and 10 respectively) and negative (0.2 and 0.3) likelihood ratios.

Conclusion: Evidence-based diagnostic thresholds have been established for the diagnosis of secondary upper limb lymphedema. In determining if lymphoedema is present in those with mild lymphedema, normatively-determined circumference and bioimpedance thresholds that account for limb dominance should be used. Adoption of these evidence-based criteria will allow, for the first time, comparison between studies, clarifying the incidence and risk factors for lymphedema, allowing the field to make meaningful progress forward in determining who is at-risk for lymphedema and how to prevent it from developing.
Acknowledgements: Cancer Australia and National Breast Cancer Foundation.
Title: Impact of radiation on cardiovascular outcomes in breast cancer patients

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Body: Background: Radiation therapy is a key component in the treatment of breast cancer. Recent studies have attempted to detail the potential impact that radiation may have on the development of cardiovascular complications in patients with breast cancer. The results of these studies, however, are conflicting regarding the magnitude and clinical significance of the cardiovascular effects with the use of modern radiation techniques. The aim of our study was to examine cardiovascular outcomes in breast cancer patients who received radiation therapy compared to those who did not.

Methods: After IRB approval, all breast cancer patients who received systemic chemotherapy at University Hospitals Case Medical Center between 1998 and 2006 and with known radiation data were included in this retrospective study. A complete patient medical history including risk factors, comorbidities, and tumor and treatment characteristics were obtained from the institutional tumor registry and were confirmed by the medical record. Billing data provided dates of diagnosis for heart failure (HF), coronary artery disease (CAD), and arrhythmia. The patients were divided into three groups: no radiation, right sided radiation, and left sided radiation. The groups were compared to one another and then further subdivided and analyzed based on timing of diagnosis of CAD and HF. Multivariate analyses using a Cox proportional hazards model were conducted for each outcome adjusting for age and comorbidities.

Results: 1277 patients were included in this study with average follow-up of 7.3 years (SD =3.5). CT based planning was used for the majority of the patients. Radiation start dates were not available for 186 patients; these were estimated at 5.5 months after the initiation of chemotherapy. There was no significant difference between groups in the incidences of the cardiovascular outcomes (p>0.05). Further analysis after accounting for timing of diagnosis of CAD and HF showed there was a significantly shorter time to onset of CAD in the left sided radiation group compared to the right sided radiation group; 4.5 years (SD=2.2) compared to 6.5 years (SD=3.6) respectively, p<0.001. However, multivariate analyses were not statistically significant (p=0.47). The onset to HF was shorter in the right sided radiation group compared to the left sided radiation group; 3.5 years (SD 3.1) compared to 4.3 years (SD=3.5) respectively, p<0.001, but again, multivariate analyses were not statistically significant (p=0.43).

Discussion: In our study, we did not find a difference in the incidence of adverse cardiovascular outcomes among patients who did and did not receive radiation, both before and after correcting for timing of diagnosis. We did find a difference in the time to development of these adverse cardiovascular outcomes between the two radiation groups, but no difference was found in either the incidence or time to development when controlled for age and co-morbid conditions. This study suggests that there is little impact of radiation on cardiovascular outcomes using modern techniques that limit radiation exposure. Prospective longitudinal studies focused specifically on cardiac endpoints may be needed to fully understand the cardiovascular impact of radiation.
Objective markers of fatigue in women undergoing adjuvant chemotherapy for breast cancer

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Background. In breast cancer patients, fatigue is a leading detriment to quality of life. Objective markers of fatigue are needed to advance research into its treatment and prevention. To that end, we evaluated physical and neurocognitive tests as potential markers of fatigue before and after chemotherapy.

Methods. Women about to begin adjuvant chemotherapy for breast cancer were studied prospectively. Before and after chemotherapy, subjects were assessed on self-reported fatigue (Brief Fatigue Inventory, BFI), depression (CES-D), Pittsburgh Sleep Quality Index, body mass index (BMI), and 18 objective measurements: grip strength in dominant and nondominant hands, 6-minute walk, daily total energy expenditure (TEE) per accelerometer averaged over 1 week, and examiner-administered neurocognitive tests: grooved peg board in dominant and nondominant hands; digit symbol coding and symbol search (Wechsler Adult Intelligence Scale, 4th edition); 2 trail-making, 4 color-word interference, and 4 verbal fluency tests (Delis-Kaplan Executive Functioning System, D-KEFS). Due to correlation between markers, each was evaluated individually as a continuous variable in generalized linear models of BFI. Models used generalized estimating equations with independent correlation matrix. Covariates (age, tumor stage, BMI) and potential interaction between marker and time of assessment (pre- or post-chemotherapy) were considered.

Results. Of subjects enrolled (n=28), 3 withdrew before wearing the device; these were similar to evaluable subjects except for lower scores on digit symbol (p<0.002) and color inhibition (p<0.02). Another subject (a vigorous athlete age 36) was excluded, because she alone did not experience the primary endpoint (fatigue) at either assessment. Thus, the final sample was n=24 (age 50.3±9.5 years). BFI before chemotherapy was median 1.17 (interquartile range 0.33-3.50) and increased +2.11(+2.64) after chemotherapy. Worse sleep quality (p=0.003), greater grip strength in dominant (p=0.001) and nondominant hand (p=0.003), and higher daily TEE (p=0.03) were each associated with BFI after chemotherapy but not before. In contrast, better performance on each of 4 neurocognitive tests (D-KEFS color patch p=0.04, color word p<0.001, switch correlation p<0.001, switch accuracy p<0.001) was inversely associated with BFI before chemotherapy but not afterwards. Other neurocognitive tests and 6-minute walk were not associated with BFI. Only depression score was associated with BFI before chemotherapy but not afterwards. In contrast, better performance on each of 4 neurocognitive tests (D-KEFS color patch p=0.04, color word p<0.001, switch correlation p<0.001, switch accuracy p<0.001) was inversely associated with BFI before chemotherapy but not afterwards. Other neurocognitive tests and 6-minute walk were not associated with BFI. Only depression score was associated with BFI before (p<0.0001) and after (p<0.0001) chemotherapy; moreover, a given level of depression was associated with greater BFI after chemotherapy than before (interaction with time, p<0.0001). None of the models fit BFI data better when covariates were taken into account or when markers were considered simultaneously.

Conclusions. Unlike neurocognitive tests, certain physical measures (grip strength in either hand, TEE, sleep quality) can serve as marker of the fatigue that follows cancer treatment. Grip strength has the advantage of being both objective and efficient to measure. No marker currently studied is suitable for comparing fatigue pre- versus post-chemotherapy, however.
Title: Effect of a 12-week supervised physical activity and healthy eating program on body weight, functional capacity and serum biomarkers in survivors of triple-negative breast cancer: A randomized, controlled trial

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Body: Regular physical activity and healthy body composition are important predictors of good outcomes for breast cancer survivors, especially in overweight/obese individuals. However, individualized exercise and healthy eating programs have not focused on lifestyle changes and outcomes among women recovering from triple-negative breast cancer. Our purpose was to examine the associations between baseline levels of inflammatory cytokines and obesity-related adipokines after weight loss, diet and physical activity intervention in survivors of triple-negative breast cancer. We enrolled overweight/obese survivors (average time since diagnosis, 4 years) and randomly assigned them to a 12-week supervised exercise and low-fat diet (n=18) or a usual care group (n=10). The program consisted of supervised exercise 3 times/week, as well as 2 unsupervised sessions per week. The goal of our Get Fit for the Fight® program was to complete 150 min/week of moderate-intensity aerobic exercise. Participants completed a 3-day diet record during baseline testing and the dietitian recommended ways to cut calories from these typical eating patterns. The goal of caloric restriction was to decrease dietary fat in order for the participant to consume 200 kcal/week less. Assessments included aerobic fitness, body composition, and self-reported physical activity, mood and quality of life. Blood cytokines and obesity-related adipokines were also analyzed before and after the intervention period. The intervention group lost an average of 2% body fat compared with the control group (p<0.01). Significant (p<0.05) decreases were seen in the intervention group for body weight, BMI, and all skinfold measures except mid-axillary. Self-reported physical activity and breast-cancer specific quality of life also improved significantly in the intervention group from baseline to 12 weeks, indicating a shift from inactivity toward increasing time spent in moderate physical activity, primarily during weekends. No significant associations were observed between the intervention group and serum levels of inflammatory cytokines. However, BMI was significantly correlated (p<0.05) with serum leptin/adiponectin ratios, an indicator of insulin resistance. These findings indicate that a pragmatic lifestyle intervention with physical activity and healthy eating were consistent with improvements in body composition, functional capacity and quality of life for triple-negative breast cancer survivors. The intervention also evoked favorable changes in blood leptin/adiponectin ratios which are linked to reductions in central adiposity and improved insulin sensitivity.
Title: Risk of breast cancer related lymphedema after treatment with taxane-based chemotherapy: A prospective cohort study

Meyha N Swaroop¹, Cynthia L Miller¹, Nora Horick², Chantal M Ferguson¹, Melissa N Skolny¹, Jean O'Toole³, Lauren S Jammallo¹, Michelle C Specht⁴ and Alphonse G Taghian¹. ¹Massachusetts General Hospital, Boston, MA; ²Massachusetts General Hospital, Boston, MA; ³Massachusetts General Hospital, Boston, MA and ⁴Massachusetts General Hospital, Boston, MA.

Body: Background: Taxane-based chemotherapy is routinely used in the treatment of breast cancer and has been shown to improve both disease-free survival (DFS) and overall survival (OS). A common side effect of taxane-based chemotherapy is fluid retention in the extremities, which may increase the risk of breast cancer related lymphedema (BCRL). BCRL is a chronic swelling of the arms, breast, or trunk due to accumulation of lymphatic fluid in the interstitial tissues, which has a profoundly negative impact on quality of life. Little data exists regarding the impact of taxane-based chemotherapy and fluid retention on risk of developing BCRL. We sought to determine whether receipt of taxane-based chemotherapy for the treatment of breast cancer increases the risk of BCRL development in a large, prospective cohort of breast cancer patients.

Methods: We identified 569 patients diagnosed with unilateral breast cancer between 2005-2012 who underwent surgery and prospective screening for BCRL at our institution. All patients included in this analysis had ≥18 months of post-operative follow-up. Bilateral arm volume measurements were performed using a perometer preoperatively and every 3-7 months postoperatively. BCRL was defined as a relative volume change (RVC) of ≥10%. Clinicopathologic characteristics and treatment details were obtained by medical record review. Cox proportional hazard analyses were performed to analyze risk of BCRL. Arm measurements obtained after contralateral prophylactic surgery or diagnosis of metastasis were excluded to avoid potential confounding.

Results: Arm volume measurements from 569 patients were included with a median post-operative follow-up of 28 months (range 18-75.1). 33% (187/569) of patients received taxane-based chemotherapy in the neoadjuvant and/or adjuvant setting, and 92% (172/187) of these patients received pre-medication with dexamethasone to prevent hypersensitivity and reduce edema. 3% (18/569) received non-taxane based chemotherapy and 64% (364/569) received no chemotherapy. 23% (131/569) had axillary lymph node dissection (ALND), 61% (346/569) had sentinel lymph node biopsy (SLNB), and 16% (92/569) had no nodal surgery. At 24 months, the cumulative incidence of BCRL was 5.0% (95% CI: 3.15-7.81%) among patients who did not receive taxane-based chemotherapy, compared to 13.4% (95% CI: 9.17-19.4%) in the taxane-based chemotherapy group. On univariate analysis, taxane-based chemotherapy was associated with increased risk of BCRL (HR=2.2, p=0.0037), in addition to ALND, higher body mass index, greater number of lymph nodes (LNs) dissected and greater number of positive LNs (p<0.05 for all). However, taxane-based chemotherapy was not associated with increased BCRL risk on multivariate analysis (HR= 0.78, p=0.43), in which only ALND (HR=4.9, p<0.0001) and number of positive LNs (HR=1.1, p=0.02) remained significant.

Conclusion: Our results suggest that patients who receive taxane-based chemotherapy are not at an increased risk of BCRL compared with patients who received non-taxane or no chemotherapy. This data can be used to improve patient education and counsel those who experience temporary fluid retention while on taxane-based chemotherapy.
Title: Improved outcome of breast cancer survivors participating in a multidisciplinary cancer survivorship program at Texas Tech University Health Sciences in El Paso, TX

Zeina Nahleh¹, Rebecca Pasillas¹, Alok Dwivedi¹, Azadeh Nasrazadani¹, Rosalinda Heydarian¹, Luis Sanchez¹, Indika Mallawaarachchi¹, Cecilia Ochoa¹ and Edward Saltzstein¹. 'TTUHSC-Paul L.Foster School of Medicine, El Paso, TX.

Body: Introduction: Breast Cancer (BC) survivors in El Paso, TX include a majority of Hispanics. We have previously reported that these survivors have decreased mental and physical health related Quality of Life (QOL). We sought to determine whether BC survivors would benefit from participating in a comprehensive multidisciplinary BC Survivorship Program at the Garbar Breast Care Center (GBCC), as determined by improvement of performance on the following validated questionnaires: 1) Patient Health Questionnaire 9 (PHQ9), 2) General Anxiety Disorder 7 (GAD 7), and SF36 QOL questionnaires. Methods: After IRB approval, we recruited consecutive patients at our institution over 6 months starting October, 2013, and who are within the first 5 years post-diagnosis with Stages I-III BC and have completed surgery, chemotherapy and/or radiation therapy. Survivors were enrolled in the survivorship program staffed by an oncologist, a nurse practitioner, a nutritionist and a clinical psychologist. They participate in individual as well as group sessions. Survivors are initially screened for depression and generalized anxiety disorder using PHQ9 and GAD 7, in addition to assessing the patient’s QOL at baseline, and every 3 months. Survivors are provided with a personalized summary of all received treatment and follow up care plans; dietary counselling and individual meal plans, in addition to in-depth psychological assessment and an intervention using Mindfulness Based Stress Reduction. Results: 47 patients have been recruited so far and all but one completed baseline questionnaires; 17 and 7 respectively had 3 and 6 months follow up visits. 94% were Hispanics. Mean age 54 years. 38% of participants were younger than 50 years of age; Stage 1 BC (36%), Stage 2 (36%), and stage 3 (28%). 80% received chemotherapy; and 68% had hormone receptor positive BC and received endocrine therapy. The scores at baseline were: PCS representing the mean for the SF-36 QOL Physical Health was 46.0, and the MCS for Mental Health was 44.0, both below the population norm (50.0). Mean scores for GAD 7 was 7.54 and for PHQ 9 7.33, both abnormal (<5 on PHQ9 and GAD 7 are considered normal scores). At 3 months the scores were as follows: PCS 49.71, MCS 45.34, both improved (the higher the better); GAD7 5.18; and PHQ9 6.82 (both improved, the lower the better). At 6 months, same favorable trend continues: PCS 50.07 [SD 5.54, 44.29-53.35]; MCS 49.66 [SD 7.73, 38.93-62.41]; GAD7 5.00 (SD 3.70); and PHQ9 4.57 (SD 3.64).

Conclusion: BC survivors are benefiting from participating in the new BC Survivorship Program launched at Texas Tech in El Paso, TX. They have experienced improved mental and physical health related QOL, improved anxiety and depression by GAD7, and PHQ9, with scores reaching near normal values at 6 months into the program which continues to enroll BC survivors. This program represents a culturally appropriate model for Hispanic BC survivors. It is expected to continue to improve the quality of life of these patients, empower them in their transition from cancer treatment to survivorship and lead to improved psychosocial adjustment and normal social functioning with significant implication not only on survivors, but also on their families and the community.
Title: Randomized clinical trial assessing the impact of survivorship care plans on survivor knowledge

Amye J Tevaarwerk¹, Kevin A Buhr¹, Kari B Wisinski¹, Mark Burkard¹, Mindy Gribble², William Hocking², Wenjun Sun¹, SarahMaria Donohue¹, Jamie Zeal¹, Abigail Terhaar¹, Douglas A Wiegman¹ and Mary E Sesto¹. ¹University of Wisconsin, Madison, WI and ²Marshfield Clinics, Marshfield, WI, United Kingdom.

Body: Purpose: Efforts to inform survivors about long-term risks and planned follow-up after cancer treatment have increased. Survivorship Care Plans (SCPs) and care planning sessions have been recommended since 2005, yet the benefits of both SCP provision and care planning are only now being assessed. The impact of SCPs separate from care planning sessions is unclear, however SCPs alone require less time and cost to provide than SCPs combined with care planning sessions. We hypothesized that SCPs alone might enhance patient knowledge. In a randomized trial, we assessed change in patient knowledge of diagnosis, treatment, late/chronic side effects and followup care pre and post receipt of SCP.

Methods: Patients who completed primary treatment within the past 2 years for Stage 0-3 breast cancer were consented, enrolled, and randomized to immediate (intervention) versus delayed receipt (control) of an individualized SCP without care planning. All participants completed the Wisconsin Survey of Diagnosis and Management in Breast cancer (WiSDOM-B), a test of knowledge about one’s own breast cancer diagnosis, treatment and late effects scored out of 40 points. The survey was completed at both baseline and at four weeks (prior to delayed receipt); the primary outcome was change in score. The study was designed with 90% power to detect a between-group difference of 4 points (out of 40 possible).

Results: Between November 2013 and March 2014, we recruited 64 women aged 36-78 with Stage 0-3 breast cancer. Most participants were Stage 1-2 (n=50, 79%) with n=7 (10.9%) in Stage 3. Most had received chemotherapy (62.5%), endocrine therapy (89.1%), or radiation (76.6%).

At baseline, the average WiSDOM-B score was 28.3 out of 40 (70.8%), range 15.5-38.5. There was no evidence of change in score from baseline to four weeks for either the immediate SCP group (+0.41, 95% CI [-0.97, +1.79]) or delayed receipt control group (+0.95, 95% CI [-0.48, 2.38]). Observed variation was consistent with sample size assumptions, and the observed treatment difference (-0.55, 95% CI [-2.53, 1.44], p=n.s.) ruled out the prespecified clinically significant effect size of +4.0 (+10%). Overall, participants scored better on questions testing knowledge of diagnosis (side, year of diagnosis, lymph node test results, receptor status, and stage) and surgical side effects than on questions testing knowledge of other treatment (chemotherapy, radiation or endocrine therapy) side effects.

Participant satisfaction with knowledge and care team communication was collected pre and post SCP, as well as feedback on SCP content and use of the SCP. These analyses of selected questions shown to change post SCP by Nissen et al will also be presented.

Conclusion: In a controlled, randomized clinical trial, receipt of a SCP without care planning sessions did not appear to significantly increase survivor knowledge about cancer diagnosis, treatment and followup as assessed by the WiSDOM-B survey. Efforts to improve survivor knowledge should investigate the impact of care planning sessions or other interactive health information tools. A second study of patients (n=64) randomized to immediate or delayed receipt of SCPs, in conjunction with care planning sessions, is currently enrolling.
Title: Exercise and the influence of endocrine therapy on cardiac and metabolic outcomes in breast cancer survivors

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Body: Exercise and the Influence of Endocrine Therapy on Cardiac and Metabolic Outcomes in Breast Cancer Survivors

Background: Breast cancer survivors (BCS) are at higher risk for decreased cardiovascular fitness and illnesses due to excess weight and sedentary lifestyle. Exercise has been shown to improve fitness, body composition and helps to maintain weight but sparse data exists on the influence of endocrine therapy on these outcomes.

Methods: The Yale Fitness Intervention Trial (Yale FIT) was a 12 month RCT comparing aerobic-resistance exercise to home based physical activity. Female cancer survivors who were < 3 years of completing primary and/or adjuvant chemotherapy and within the first 5 years of menopause therapy were eligible. The aerobic-resistance intervention was conducted at a community fitness center 3 times/week and included 30 minutes of aerobic and 20-30 minutes of resistance exercise, supervised by an interventionist for first 6 months. The home based group received a flyer outlining recommendations for 30 minutes moderate intensity physical activity most days of week. Data were collected at baseline, 6 and 12 months.

Results: The majority of the 154 women enrolled were white, married, employed and average age was 52 years. At baseline, mean BMI was 29 (± 6.8), waist circumference 86.3 (± 15.3) and subjects reported expending a median of 773 MET minutes per week. A graded exercise stress test was used to measure cardiovascular function. The fitness center group increased time on treadmill (p=0.05) and had improved heart rate recovery (p=0.01) when compared to the home based group. No significant differences were found across time for metabolic outcomes by fitness center or home based group. However, when analyzed by type of endocrine therapy (30% were on an Aromatase Inhibitor (AI), 31% on Tamoxifen (TAM) and 39% were on no endocrine (E) therapy), there was a significant difference in serum insulin and insulin resistance between the fitness center and home based group for women taking TAM at 6 months (p=0.03) and 12 months (p=0.01). Specifically the insulin levels in the fitness center group remained stable while the home based group demonstrated increased serum insulin and insulin resistance. There was a trend for women in the home based groups who gained weight (> 5 lbs) to have higher insulin levels at 12 months (p=0.06). There were no significant changes at 6 or 12 months by group (fitness center or home based), by type of endocrine therapy or by weight change for total lipids, triglycerides, or HDL/LDL. To provide additional insight into the findings, analysis of cytokines and inflammatory markers is in progress.

Conclusions: This study confirms the cardiovascular fitness benefits of exercise but the relationship between exercise, endocrine therapy and weight on insulin levels is more complex. Exercise (aerobic-resistance and aerobic) combined with stable weight over 12 months resulted in relatively unchanged insulin levels. In contrast, only aerobic-resistance exercise (fitness center group) appeared to stabilize insulin levels in women who gained weight.

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2014 San Antonio Breast Cancer Symposium

Publication Number: P1-09-17
Average Grade: 0

Title: Is there a genetic predisposition for cancer-related lymphedema?

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Body: Background: Breast cancer related lymphedema strikes approximately 20% of all breast cancer survivors and is associated with radiation and lymph node dissection. Yet it remains unknown why some women encounter lymphedema after minimally invasive sentinel lymph node biopsy while others who have extensive axillary lymph node dissections and radiation escape the incurable, chronic condition. It has been postulated by many that there exists a genetic predisposition for acquiring lymphedema after cancer treatment. While mutations HGF, cMET, GJC2 and VEGFR3 are thought to be potential predisposing genes for acquired lymphedema, until now, there has been no definitive causal evidence. Acquired lymphedema occurs after trauma, such as cancer treatment or surgical insult, while primary lymphedema is not associated with any known trauma, but attributed to inherited and somatic mutations.

Methods: In a larger study to discover gene variants associated with lymphatic dysfunction, we collected DNA and performed near-infrared fluorescence lymphatic imaging (NIRFLI) to identify abnormal lymphatic phenotypes in a nucleus family with two daughters who were diagnosed with rare primary lymphedema and a mother, who at the time of imaging, was diagnosed with acquired lymphedema with self-reported symptom after injury. Whole exome sequencing and cosegregation analyses was used to find previously known genes as well as new variants that could be predictive of lymphedema symptoms, despite the varied expressivity. Biological relevancy of variants was tested through cell culture studies following siRNA and drug inhibition in lymphatic endothelial cells (LECs), and overexpression of WT and mutant gene variants.

Results: A damaging mutation in HGF was passed to both daughters from the asymptomatic father who showed no abnormal NIRFLI phenotype. Yet the mother, who at time of study was found to have abnormal lymphatics through NIRFLI and diagnosed with both acquired and en tarda lymphedema attributed to minor trauma several years prior, had passed a mutation in SHIP2 to her daughters. Extended family members, including a breast cancer survivor, who agreed to the NIRFLI procedure and harbored the SHIP2 mutation, also possessed abnormal lymphatic phenotypes. Migration and tubulogenesis in LECs was arrested by SHIP2 knockdown, and when compared to WT overexpression, was attenuated following stimulation with mutant SHIP2. Furthermore, we found aberrant PI3K/AKT and MAPK/ERK signaling in LECs expressing mutant SHIP2 or transfected with SHIP2-siRNA.

Conclusions: Our studies identify SHIP2, a regulator of the PI3K/AKT pathway as a potential effector of lymphatic dysfunction. The identification of BCRL biomarkers could affect disease management in a growing cancer-survivorship population where an unmet clinical need with huge economical potential and quality-of-life impact exists. Validation of SHIP2 as a likely therapeutic target to prevent or treat lymphedema could ultimately influence cancer treatment in the following ways: (i) enable genetic screening of cancer patients to determine risk of developing lymphedema based on their PI3K/AKT status; and (ii) ameliorate the risk of lymphedema by seeking alternate cancer treatment options for those patients who harbor a genetic predisposition for lymphedema.
Perceptions and gaps of women living with advanced breast cancer: Results from the “Count Us, Know Us, Join Us” online survey in Latin America

Maira Caleffi

*Hospital Moinhos de Vento, Porto Alegre, Brazil.*

**Body:** Background: Patients with advanced breast cancer (ABC) represent a subgroup of BC patients who have a unique set of needs in their multiprofessional care, as the focus is more concerned with surviving the challenges of the disease, rather than being a survivor. However, little is known about these specific needs. A global Internet-based survey, "Count Us, Know Us, Join Us," was performed to identify and raise awareness of unmet needs of women living with ABC. Herein, the outcomes of respondents in Argentina, Brazil, and Mexico are presented.

Methods: The survey, conducted by Harris Poll between Oct 2012 – March 2013, sponsored by Novartis Oncology, comprised 40 questions to assess respondent demographics and baseline characteristics, diagnosis, metastatic spread of disease, overall health, family history, communication with the health care practitioner (HCP), information sources and resources, treatment, support, quality of life (QOL), satisfaction, emotional state, and economic impact. It was distributed online through locally based patient advocacy groups. Women ≥21 years old with BC that had spread to distant parts of the body beyond the breast and its local lymph nodes (stage IV) were eligible to participate.

Results: Overall, 1,273 participants from 12 countries completed the survey, including 302 respondents from Latin America (n=100, Argentina; n=100, Brazil; n=102 Mexico). Of these, 62% were between 40–59 years old and 59% had metastatic disease at diagnosis. The majority of patients in Brazil (64%) and Mexico (74%), but not Argentina (22%), were very satisfied with the communication they had with their HCP, although overall 74% wanted their HCP to also address their emotional needs. Just over a quarter (28%) felt isolated from the early stage BC community, and two-thirds (67%) felt no one understood what they were going through; 41% experienced a decrease in support from family and friends after being diagnosed. In total, 74% of women felt their QOL had been impaired moderately or strongly following diagnosis. Around half of respondents expressed dissatisfaction with aspects of their mental health, including outlook on life (43%), sense of control over their life (48%), emotional state (50%), and self-esteem (47%) following diagnosis. Moreover, moderate or strong negative impacts on jobs (67%) and finances (71%) were reported by applicable respondents. An impact on work was indicated by 89% of employed women and overall 66% stated that ABC interfered with their ability to work such that they suffered a loss of personal income, with the highest figure for respondents in Mexico (85%).

Conclusions: This survey has identified that ABC has a major impact on many aspects of a sufferer’s life. One of the key findings is the need for a larger psychosocial component in the care of women with ABC in Latin America. Engaging the commitment and collaboration of a multiprofessional care team, caregivers, patient organizations, and governments to improve the quality and breadth of care beyond therapeutic aspects may help to fulfill this unmet need and improve the general well-being of this often invisible patient population.
Title: Patient’s views of follow-up after treatment for breast cancer. A comparison of two approaches

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Body: Background: Routine follow up of patients’ post treatment of breast cancer is standard practice in most countries. Follow up involves regularly scheduled clinical appointments with the aim of detecting early breast cancer recurrence and provision of psychological support to the patient. In the United Kingdom, financial constraint has led individual hospital trusts to revaluate the need for lengthy follow up schedules. Development of novel, less time intensive follow-up services, such as ‘open access follow up’ favour a more patient-led approach.

Aim: To assess patients’ views on breast cancer follow up, as well as the effect on patient satisfaction of transferring current clinical follow up to an ‘open access follow up’.

Method: We report the patients’ views on the basis of pooled data of a detailed survey performed in a large Breast Cancer Centre. All patients receiving regular clinical follow up care over a 6-month period were invited to participate in this prospective study. Patients were provided with a flow-chart, illustrating the current follow up, as well as a proposed ‘open access follow up’ process.

Results: Between November 2013 and April 2014, 304 patients were recruited into the study. 39% of patients were within the first year of their diagnosis with 18% more than 3 years into their follow up. Caucasian women made up the majority of our population group (81%), with 7% Indo-Asian, and 7% Afro-Caribbean.

The main expectation from follow up was surveillance for early detection of recurrence as expressed by 92% and anxiety of treatment side effects. 93% were satisfied with the current follow up they were receiving (satisfaction scores 7-10) and of those, 84% would choose to continue current follow up rather than move toward an ‘open access’ approach.

92% of patients favoured current clinical follow up over ‘open access follow up’, with 66% highlighting ‘open access follow up’ as an ineffective method of follow up. In stark contrast, 94% of patients reported current clinical led follow up to be effective. 91% of patients requested their follow up to be led by a breast surgeon and oncologist, rather than their primary care physician or community nurse. Interestingly, no significant correlation was established between age, ethnic background, distance from hospital and time from diagnosis with the type of follow up preferred.

Conclusion: Following treatment for breast cancer, patients prefer a more regular clinician-led service to a patient-led ‘open access follow up’ process. This may be explained by the observation that patients seek the reassurance of regular clinical review to identify recurrence early, however the psychological support of a clinical consultation cannot be underestimated. It may be inferred that patients who are satisfied in the follow up they were currently receiving are more likely to appreciate the current follow up system rather than moving to an ‘open access’ approach. Further study is warranted which investigates the impact of intensified surveillance on survival based on identification of recurrence and to repeat this study to seek consistency of opinion.
2014 San Antonio Breast Cancer Symposium

Publication Number: P1-09-20
Average Grade: 6.80

Title: Evaluating alcohol and tobacco use in an ethnically diverse sample of breast cancer patients: Implications for survivorship

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Body: Background/Objective: Data suggest that modifiable risk factors such as alcohol and tobacco use may increase the risk of breast cancer (BC) recurrence and reduce survival. According to 2012 data from the Centers for Disease Control (CDC) and Prevention Behavioral Risk Factor Surveillance System (BRFSS), 4.8% of women in South Carolina (SC) are heavy drinkers (>1 drink/day) with 9.6% having ≥4 drinks at least once in the past month (US state medians are 5.2% and 11.4%); 19.1% are current smokers (US state median 17.4%). Female BC mortality in SC is almost 50% higher in African Americans (AAs) than in European Americans (EAs; 27.5/100,000 and 19.1%, respectively). In SC, there is an AA subpopulation, the Sea Islanders, who carry the lowest AA rate of European genetic admixture. Given the substantial racial survival disparity we examined the patterns of alcohol and tobacco use in an on-going, ethnically diverse statewide study of women with recently diagnosed invasive breast cancer.

Methods: Participants were identified within 18 months post-diagnosis through the SC Central Cancer Registry (SCCCR). Women who opted into the study were interviewed via telephone, self-reporting data including race/ethnicity, educational status, alcohol consumption and tobacco use during the past 30 days. Published CDC guidelines were used to categorize alcohol and tobacco use.

Results: During the first 24 months of recruitment, 162 women have opted into our study; 132 have been interviewed and results analyzed (37 EAs and 95 AAs). Age at interview ranged from 38.9 to 90.2 years, with AAs slightly younger (p=0.071). Over half had more than a high school education with no difference by race (p=0.203).

Alcohol: The minority of participants self-identified consuming alcohol (28.8%). Heavy use was infrequent: two (1.5%) reported consuming on average >1 drink/day, and eight (6.1%) consumed ≥4 drinks on any one day. Consumption was less prevalent among AAs than EAs (p=0.063). Use tended to be lower among AAs (statistically not significant).

Tobacco: Smoking (daily or occasional) was reported by 12.1% of participants (AA vs EA: p=0.771).

Alcohol or Tobacco: Use of alcohol and/or tobacco was 1.6 times as prevalent among EAs compared to AAs (p=0.053).

Conclusions: Compared to self-reported state data few participants reported heavy alcohol consumption or current tobacco use, particularly AAs, but 35.6% of participants do use alcohol or tobacco. While these findings suggest that alcohol and tobacco may not contribute to the racial disparities in breast cancer mortality observed in SC, it is nonetheless imperative to reduce these modifiable risk factors and improve breast cancer outcomes for all breast cancer survivors, regardless of race and ethnicity.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>African American (AA) N=96</th>
<th>European American (EA) N=43</th>
<th>p-value</th>
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<tr>
<td>Age (years): mean (±st dev*)</td>
<td>58.8 (±11.6)</td>
<td>62.9 (±13.2)</td>
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<tr>
<td>Education: more than high school</td>
<td>51 (53.1%)</td>
<td>29 (67.4%)</td>
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<tr>
<td>Alcohol in past 30 days</td>
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<tr>
<td>Yes (vs No)</td>
<td>22 (23.2%)</td>
<td>18 (41.9%)</td>
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<td>Among those who drink:</td>
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<tr>
<td>Heavy: average &gt;1 drink/day</td>
<td>1 (4.6%)</td>
<td>1 (5.6%)</td>
<td>1.000</td>
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<tr>
<td>Current tobacco use</td>
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<td></td>
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</tr>
<tr>
<td>Yes (vs No)</td>
<td>7 (7.6%)</td>
<td>3 (7.1%)</td>
<td>1.000</td>
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<tr>
<td>Among smokers: Daily use:</td>
<td>4 (57.1%)</td>
<td>3 (100%)</td>
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<td>Alcohol and/or Tobacco</td>
<td>27 (28.7%)</td>
<td>20 (47.6%)</td>
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<tr>
<td>Both Alcohol and Tobacco</td>
<td>2 (2.2%)</td>
<td>1 (2.4%)</td>
<td>1.000</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------</td>
<td>---------</td>
<td>-------</td>
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<tr>
<td>*standard deviation</td>
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2014 San Antonio Breast Cancer Symposium

Publication Number: P1-09-21
Average Grade: 5.00

Title: Metabolic syndrome is prevalent in women with newly diagnosed breast cancer

Louise C Wong¹, Carolyn Behrendt¹, Robin Smith¹ and Joanne E Mortimer¹. ¹City of Hope, Duarte, CA.

**Body:** Purpose: We estimate the prevalence of metabolic syndrome (MetSyn) among women with newly diagnosed breast cancer and explore potential risk factors.

**Methods:** From January 2012 to May 2014, women with newly diagnosed localized breast cancer were enrolled into a longitudinal survivorship study. At baseline, results of physical exam, use of concomitant medications, serum lipid profile and vitamin D were obtained from the medical record, while quality of life variables were measured via questionnaires in English. MetSyn was defined as at least 3 of 5 criteria: central obesity (BMI >30 or waist >=88 cm); triglycerides >=150 mg/dL; HDL cholesterol <50 mg/dL or cholesterol-lowering treatment; systolic >=130 or diastolic >=85 mm Hg blood pressure or treatment for hypertension; fasting glucose >=100mg/dL or diagnosis of Type 2 diabetes. Risk factors for MetSyn (age, education, serum vitamin D, self-reported energy in past month) were evaluated using multivariate logistic regression.

**Results:** Subjects (n=154) were age 52.0 ±10.1 years. Prevalence of MetSyn at baseline was 46.1(±4.0)%; for another 1.9(±1.1)% (n=3), MetSyn status was indeterminate due to lack of data on HDL and triglycerides. Among subjects with MetSyn (n=71), most had been diagnosed with hypertension (81.7%) and diabetes (61.4%). Without MetSyn (n=80), the latter conditions were much less prevalent (31.3% and 22.5%, respectively, both p<0.0001). Adjusted for race and year of accrual, the likelihood of MetSyn at baseline was higher per year of age (Odds Ratio (O.R.) 1.05, 95% CI 1.01-1.10, p=0.015), lower per increasing educational attainment (from less than high school to high school/some college, to bachelor’s, to graduate degree: O.R. 0.58, 0.36-0.93, p=0.024), and higher per increasing “time in past month with a lot of energy” (from little/no time to most of the time, to all of the time: O.R. 2.30, 1.11-4.75, p=0.025). An inverse association with serum Vit D (p=0.14) did not reach statistical significance.

**Conclusions:** Metabolic syndrome is present in nearly half of women newly diagnosed with breast cancer and is associated with age, less education, and more energy in past month.
Title: Improving quality of life among Latino breast cancer survivors: A national randomized control trial of patient navigators using LIVESTRONG’s Cancer Navigation Center

Amelie G Ramirez¹, Edgar Muñoz¹, Sandra San Miguel¹, Kip Gallion¹, Arely Perez¹, Leo Castillo², Sarah R Arvey³ and Frank Penedo². ¹University of Texas Health Science Center, San Antonio, TX; ²Northwestern University, Feinberg School of Medicine, Chicago, IL and ³LIVESTRONG, Austin, TX.

Body: Purpose: Latina breast cancer survivors experience an unequal burden of unmet needs after treatment, which compromise their health care and wellness, experts recommend providing psychological services as an integral part of quality cancer care. Methods: Redes En Accion: The National Latino Cancer Research Network and LIVESTRONG partnered to conduct a randomized control trial utilizing trained, bilingual, bicultural patient navigators to improve wellness and access to psychosocial services among non-metastatic Latino breast cancer survivors from Texas and Chicago. The trial tests the efficacy of patient navigation (PN) in improving general and disease-specific quality of life (QoL), treatment compliance, and identification of mechanisms that may promote quality of life. The study involves a 2 X 4 randomized repeated measures design with an experimental condition (combined PN over three months with access to the LIVESTRONG Cancer Navigation Center [LCNC] services [PN+LCNC]) versus a control condition (PN only) as the between-groups factor, and time-point (baseline/pre-randomization [T1]; post-PN [3-months post T1; T2], and 6 [T3] and 12 months [T4] follow up after T2) as the within groups factor. LCNC provides free, bilingual support to U.S. cancer survivors throughout the cancer journey. LCNC also refers to survivors to services addressing their medical, economic and psychosocial needs, and monitors client contact and access outcomes. Preliminary Results: A total of 128 Latina women with early stage breast cancer have been randomized into either our control (Standard Patient Navigation; PN) or our experimental (Enhanced Patient Navigation; EPN) conditions. Complete data for multiple regression analysis on 76 randomized Latina women who completed baseline and first follow up assessment is available. At baseline, we identified major stated needs by our participants. These included fear of recurrence, more information regarding their disease, and assistance in communicating with the medical team. The top three physical functioning concerns stated by our participants included lack of energy/fatigue, interference with daily routines and poor physical well-being. Consistent with prior work with Hispanic cancer survivors, our sample reported significantly lower (over 1.5 standard deviation below) general health related quality of life, physical well-being and emotional well-being as measured by the FACT-G. Exploratory analyses have begun to reveal that there are several significant moderators of the effects of the intervention across several outcomes. At conference, we will present quality of life data on breast cancer participants, exploratory analysis, and lessons learned. Discussion: Limited work has addressed the psychosocial needs of Latina breast cancer survivors. Culturally sensitive patient navigation (PN) could address these needs and significantly improve breast cancer survivorship.
Title: Symptoms, desire for help, and quality of life among recent breast cancer survivors

Steven C Palmer\textsuperscript{1}, SarahLena L Panzer\textsuperscript{1}, Karen Glanz\textsuperscript{1}, Marilyn M Schapira\textsuperscript{1}, Angela M DeMichele\textsuperscript{1} and Linda A Jacobs\textsuperscript{1}.
\textsuperscript{1}University of Pennsylvania, Philadelphia, PA.

Body: Introduction:
Advances in detection and treatment have led to improved survival among breast cancer (BC) patients and 89% can expect $\geq$5 year survival. Progress comes at a cost, however, and patients experience lasting effects from disease and treatment. It is not clear how these effects impact quality of life (QoL) achieved by BC survivors. We examined relationships between symptoms, desire for help, and QoL among recent BC survivors.

Method:
Eligibility included non-metastatic BC treated $\leq$ 1 year prior and attendance at a survivorship clinic. QoL was assessed with the SF-12. 19 common symptoms were assessed on a 0-5 bother scale. Participants reported desire for help item for each symptom rated $\geq$ 1.

Results:
171 primarily white (73%), middle aged (M=54.9 yrs), and Stage I (58%) BC survivors were recruited. Both Physical (M=48.1) and Mental (M=53.8) QoL were similar to national norms. Survivors reported an average of 12 symptoms, most commonly Fatigue (90%), Insomnia (75%), Hot Flashes (73%), and Joint Pain (70%). Most bothersome symptoms included Joint Pain (M=2.9), Decreased Sexual Drive (M=2.8), Hot Flashes (M=2.8), and Vaginal Dryness (M=2.7). Participants desired help with few symptoms (M=2.3), primarily Weight Gain (50%), Joint Pain (45%), and Numbness in Hands/Feet (44%). Both Physical and Mental QoL were negatively associated with number of symptoms experienced ($r = -.46$ and $-.41$, respectively) and number of symptoms for which help was desired ($r = -.16$ and $-.41$, respectively).

Conclusion:
Symptoms are common among recent breast cancer survivors and negatively impact Physical and Mental QoL. Desire for help is less common, though similarly associated with impairment in QoL. Assessing QoL in isolation from symptom burden may miss important areas for which remediation is possible and could result in improved QoL. Patient education regarding the potential for and value of symptom reduction may be needed in BC survivors.
Title: Oral health-related quality of life in women with early stage breast cancer

Linda S Taichman1, William G Giannobile1, Thomas M Braun1, Marita R Inglehart1 and Catherine H Van Poznak1. 1University of Michigan, Ann Arbor, MI.

Body: Introduction: Aromatase inhibitors (AI) are a well-established component of the adjuvant therapy in postmenopausal (PM) women with hormone receptor positive (HR+) early breast cancer (BCA). AIs are associated with side effects that may adversely affect the quality of life (QoL). The impact of AIs and on oral health-related QoL (OHRQoL) in women with BCA is unknown. To generate data on the impact of adjuvant AIs on OHRQoL we performed a prospective, longitudinal, cohort study assessing patient reported outcomes.

Methods: PM women with early BCA were eligible if they were on an adjuvant AI for 3 to 12 months. AI patients were recruited from the Breast Medical Oncology Clinic. The Control group consisted of PM women without BCA who were not on AI therapy and were recruited at the time of screening mammography. Study participants provided socio-demographic information and completed questionnaires OHRQoL which included the Michigan Oral Health-Related Quality of Life (MOHRQoL) Scale, and Oral Health Impact Profile (OHIP-14) which both measure discomfort, dysfunction and disability resulting from oral problems on a 5 point scale. Presence of saliva status as a measure of oral health was measured by the saliva flow rate. Data was collected at baseline and at 6, 12 and 18 months. The baseline data is presented here. The student t-test and chi-square test were used to analyze outcomes. The Pearson correlation coefficient was used to evaluate the correlation between the OHIP-14 and the amount of saliva.

Results: The study met its target accrual of 58 PM women; 29 with BCA on AI and 29 controls. Median time on AIs at study entry was 5.7 months. Demographics were similar regarding age, education, income level, frequency of dental visits, and dental insurance status across both groups. The OHIP-14 score varied by AI use (p=0.02) and duration of AI use (p=0.03) where the lowest poor OHRQoL was found in AI users less than 6 months. Patients receiving AI therapy had significantly lower perception of OHRQoL for the individual OHIP-14 items: "I have had pain in the mouth", "I have had to limit my diet", "I have had painful aching in my mouth", "I have had to limit foods I eat" and "I have felt tense because of problems with my mouth" compared to controls.

Conclusions: This study is the first to report on the OHRQoL among PM women with early BCA using AIs. The baseline data demonstrates a significantly lower OHRQoL in those on AIs compared to controls. Dimensions that were particularly relevant were physical pain, psychological discomfort and physical disability. Analysis of serial time points is ongoing.
Title: Determinants of weight gain after breast cancer diagnosis: Results from the prospective SU.VI.MAX cohort

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Body: PURPOSE To identify correlations between lifestyle related risk factors and weight gain after breast cancer. MATERIAL AND METHODS The SU.VI.MAX randomized trial was initially designed to assess the effect of a daily antioxidant supplementation on the incidence of cardiovascular disease and cancer. A total of 13,017 subjects were enrolled in 1994–1995. Health events occurring during the follow-up (1994-2007) were self-reported by participants. Pathological reports were used to extract cancer characteristics. Anthropometric measurements were taken one year after inclusion in the study and repeated every two years during follow-up. Participants were invited to provide a 24-h dietary record every two months. Among the 258 invasive breast cancers, 169 had complete weight data before and after cancer diagnosis and were used for analysis. When measured weight and height were not available before diagnosis (n=28), self-reported weight and height were used instead of measured data. After cancer diagnosis, all anthropometric data were measured by study nurses. Women with weight gain (WG) ≥5% (moderate WG) were compared to those with WG <5% by multivariate logistic regression, according to the following factors: menopausal status, educational level, physical activity (irregular, <1h equivalent walking/day, ≥1h equivalent walking/day), smoking status, supplementation group of the trial, weight status before diagnosis, chemotherapy, endocrine therapy, tumor size, pN, mitoses and grade, ER and PR status, mean daily energy intake before diagnosis (<, ≥ median=1865 Kcal/d). The same analysis was performed to compare women with WG ≥10% (severe WG) to those with WG <10%. We also calculated the difference between weight before diagnosis and weight at the age of 20 (“lifetime weight change”) and we tested its association with the risk of moderate or severe WG. RESULTS Mean age at diagnosis was 54.4 y. Mean duration between pre- and post-diagnosis weight measurements was 5.8 years. Among the 169 women included, 59.8% were post-menopausal at diagnosis and 28.4% were already overweight before cancer diagnosis. 66 women had a WG ≥5% and 25 had a WG ≥10%. Women who practiced more physical activity at baseline were less inclined to face moderate WG (OR=0.42 (0.18-0.97), P=0.04). Moderate WG was also directly associated with hormone receptors status. All other studied factors (including treatment type or energy intake) were not associated with the risk of moderate WG. Surprisingly, tumor size was directly associated with the risk of severe WG (mean tumor size = 14.6mm among women with <10% WG versus 20.1mm among women with ≥10% WG, P=0.04). All other studied factors were not associated with the risk of severe WG. Lifetime weight variation was not associated with the risk of moderate or severe WG. CONCLUSION Physical activity seems to have a protective effect against moderate WG. Severe WG raises risk for recurrence (Caan B, et al. 2012), but since it is also linked with tumor size, we hypothesize that this relation is more complex than a direct causal effect of WG on recurrence risk.
Title: Surgery for breast cancer in the elderly is associated with better outcomes

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Body: Background
Breast cancer often presents differently in the elderly with more neglected disease. There is patchy evidence to support surgery as a preferred management tool with a positive survival outcome. The objective of this study was to 1. Understand disease presentation and management of patients diagnosed with breast cancer over the age of 80, and 2. To review survival outcome between conservative and surgically managed elderly breast cancer patients.

Method
A retrospective single centre case series was conducted from 2008 to 2014 on patients aged 80 and over at breast cancer diagnosis. Survival outcome between conservative and surgical treatment groups were reported by Kaplan Meier analysis, with comparison by log-rank test.

Results
There were 156 patients, with a median age of 86.6 years (range 80 – 107). 67.9% had invasive ductal carcinoma. At presentation, 9.6% had T4 fungating lesions and 11.9% had distant metastatic disease. The median tumour size was 25mm (range 4 – 85mm) and 81.5% were oestrogen receptor positive.
Conservative treatment (44.9%, n = 70) was accounted for in 80% of cases by patient choice and/or pre-morbid status precluding surgery. 55.1% of patients underwent surgical resections, the majority of which were performed with curative intent (96.4%). 11 patients had surgery under local anaesthetic with no complications. Median duration of hospital stay was 1 day.
Conservative vs. surgical cohorts were comparable in co-morbidities aside from age (89.6 +/- 5.1 vs. 85.2 +/- 4.2 years, p = 0.005) and dementia (18 vs. 9 (p = 0.015). The overall survival for those treated conservatively was 29 +/- 4.1 months compared to 40 +/- 6.1 months (p = 0.004) for those having surgery.

Conclusion
The elderly are more likely to present with advanced or neglected breast cancer. Those undergoing surgery had a significant survival advantage and this modality should be offered unless co-morbidities are severe.
Title: High serum levels of 25-hydroxyvitamin D are associated with better quality-of-life, and lower levels of perceived stress, depression, and fatigue among breast cancer survivors

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Body: Background: Recent evidence suggests a role for vitamin D in breast cancer etiology and survival. Although data are not definitive, breast cancer survivors might derive additional benefits from adequate vitamin D levels, including better quality-of-life (QoL) and psychosocial well-being.

Objective: Using a prospective study design, we examined associations between QoL and psychosocial functioning among breast cancer survivors and circulating 25(OH)D levels at the time of initial breast cancer diagnosis and 1 year post diagnosis.

Methods: Women (n=504) with incident breast cancer (stages 0-III) were recruited prior to initiating breast cancer treatment at Roswell Park Cancer Institute (RPCI), with 372 patients also providing data 1 year later. Demographic and clinical data were obtained from the RPCI Surgical Oncology Breast Database. At each time point, participants provided blood samples and completed surveys on mental and physical health (SF36 Health Survey), perceived stress (Perceived Stress Scale), depression (Center for Epidemiologic Studies Depression Scale), and their experiences with fatigue (Multidimensional Assessment of Fatigue Scale). Serum 25(OH)D levels were measured by immunochemiluminometric assays.

Results: Median 25(OH)D levels at diagnosis and 1 year post were 23.8 ng/ml (range 0.1-67.4) and 26.6 ng/ml (range 3.0 – 80.0), respectively. At the time of diagnosis, multivariate logistic regression analyses indicated that women with sufficient levels of 25(OH)D (≥ 30 ng/ml) had ~50% lower odds of reporting poor physical health (OR=0.52, 95% CI: 0.29, 0.90) or poor mental functioning (OR=0.54, 95% CI: 0.31, 0.95) compared to women with deficient levels (<20 ng/ml). Women with higher 25(OH)D were also less likely to report high levels (>median) of perceived stress (OR=0.45, 95% CI: 0.26-0.80), depression (OR=0.46, 95% CI: 0.25-0.83) or fatigue (OR=0.54, 95% CI: 0.31, 0.92). Findings were similar 1 year later, with reduced odds of poor physical health (OR=0.47, 95% CI: 0.24-0.93), poor mental health (OR=0.62, 95% CI: 0.33, 1.13), high levels of perceived stress (OR=0.67, 95% CI: 0.36, 1.25), depression (OR=0.33, 95% CI: 0.15, 0.71), and fatigue (OR=0.45, 95% CI: 0.24, 0.84). Changes in vitamin D levels over the 1 year period were also assessed. While none of the relationships were significant, women in the upper tertile of vitamin D change (>3.99 ng/ml) were more likely to report low mental health and higher levels of perceived stress at 1 year compared to patients with declines in 25(OH)D of >0.95 ng/ml, but were less likely to report poor physical health or fatigue. This may have been due, in part, to women being somewhat more likely to use vitamin D supplements if they reported lower mental health QoL scores. Changes in circulating 25(OH)D levels during this 1 year period were strongly associated with use of supplements (p=0.001).

Conclusions: Breast cancer survivors with adequate vitamin D levels were less likely to report poor QoL compared to women with deficient vitamin D levels. Maintenance of optimal vitamin D levels may help improve breast cancer patients’ QoL as they go through breast cancer treatment.
Title: What is a healthful diet? – Cancer survivors' interpretations of their own dietary recall data

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Body: Background
The number of long-term cancer survivors will continue to grow, and developing strategies for tertiary prevention of recurrence, cancer-related comorbidities, and general chronic disease-related health burden is critical. Nutrition and energy balance are increasingly recognized to play important roles in both decreasing recurrence risk, and improving health and well-being throughout survivorship. To inform strategies for nutrition education and behavior change for cancer survivors, we used a mixed methods approach to explore concepts about healthful diet and eating patterns among long-term cancer survivors.

Methods
We collected three ASA-24 dietary recalls from 53 breast, prostate and non-Hodgkins lymphoma survivors. Utilizing qualitative interviews, we provided participants with feedback on how their eating patterns compared to dietary guidelines, and asked respondents to reflect upon their diet and dietary changes.

Results
Like most American adults, respondents were not eating in accordance with many dietary recommendations. In terms of adequate intake of recommended nutrients, 68% met fruit and vegetable guidelines, but only 15% reported adequate intake of fiber. In terms of following recommendations for limits on intake, 89% reported diets that met guidelines for limiting alcohol intake, but only 55% met limits for cholesterol, 40% for saturated fat, 19% for caloric intake, and only 2% limited their sodium intake to within recommended levels. Respondents were often surprised by their own dietary patterns, and had differing conceptualizations of a "healthful" diet.

Conclusions
Cancer survivorship may provide motivation for dietary change, but survivors need nutrition education to identify specific strategies for dietary improvement.
Body: Background
Breast cancer (BC) is the most common cancer diagnosed in women. With advances in diagnostics and therapy, early stage disease has an estimated 85% 10-year overall survival. Cancer survivors have unique needs including screening for recurrence, psychosocial concerns, as well as routine health maintenance. There is growing evidence to support the safe transition from oncologist to primary care. This is challenging in the era of complex adjuvant and extended adjuvant programs, which are standard of care for women with hormone receptor positive BC.
In February 2013 the Wellness Beyond Cancer Program (WBCP), developed at the Ottawa Regional Cancer Program, started accepting BC patients. A risk stratification system enables the treating oncologist to determine the most appropriate care provider stream based on patient complexity and/or risk of relapse. There are three possible streams: primary care provider (PCP), nurse practitioner (NP), or oncologist. The program offers education on general and breast specific cancer survivorship issues. Unique to our program is a hormone re-assessment review (HRR), scheduled in advance, to discuss switching hormonal therapies and/or extended adjuvant strategies. A rapid re-entry system is in place for those with evidence of disease recurrence or a new primary.

Specific aims
The aims of our study are:
1. To describe unique aspects of WBCP developed for early stage BC patients
2. To assess efficacy of the WBCP
3. To evaluate safety of the HRR
4. To examine patient and primary care giver satisfaction with the program

Methods
All BC patients eligible for discharge through the WBCP will be included in the evaluation. We will evaluate patients by examining needs assessments completed at time of referral and one year later, as well as completed patient and PCP satisfaction surveys. The proportion of patients with changing scores, increasing or decreasing, will reflect improvements or worsening of needs. The proportion of both patients and PCP that are/are not satisfied with the program will be recorded and examined further.
For the HRR we will code the proportion of patients where the consult was requested and was "scheduled vs. not scheduled". Finally, the number/proportion of patients and reasons for re-entry will be captured.

Results
February 2013–June 2014, 1339 BC patients were referred to the program, 584 patients to PCP, 740 to NP, and 15 remaining with their oncologist. Early results are reported here with results of the surveys expected by December 2014. 7 patients have required re-entry for disease recurrence and 30 HRR have been booked. No cancer specific negative outcomes have been reported. Survey results addressing patient needs, empowerment and concerns are being collated, with PCP satisfaction results to be reported.

Conclusion
Our institution has endorsed evidence-based recommendations for an organized survivorship program and has taken it a step further by developing a strategy to address the unique long term needs of hormone positive early stage breast cancer. Early feedback supports that this program has increased patient and caregiver knowledge of survivorship issues and anticipates improved patient empowerment, without compromising cancer specific outcomes.
Title: Relationship of dietary and red blood cell polyunsaturated fatty acids to inflammatory markers in breast cancer survivors taking aromatase inhibitors

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Body: Introduction: A large body of literature supports modulation of inflammation by polyunsaturated fatty acids (PUFA). Inflammation may play a role in the painful joint symptoms commonly experienced by breast cancer survivors taking aromatase inhibitors (AI). AI-induced joint symptoms negatively impact drug adherence and quality of life in many breast cancer survivors. N-3 PUFAs, especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) have anti-inflammatory effects; in contrast, excess very long chain n-6 PUFAs may promote peripheral and joint inflammation. We hypothesized that breast cancer survivors with greater EPA+DHA or n-3/n-6 PUFA in their diet would have decreased inflammation and be less likely to develop AI-induced joint symptoms.

Materials and methods: This is a secondary analysis of PUFA intake self-reported from food frequency questionnaires and red blood cell (RBC) PUFA data from a randomized placebo-controlled pilot study comparing n-3 PUFA supplementation vs placebo in 44 postmenopausal women with breast cancer initiating first line adjuvant AIs. Primary outcomes were feasibility and tolerability; secondary outcomes were differences in clinical symptoms and inflammatory markers. Participants were randomized to 4.8 g/day DHA+EPA vs matched placebo for 24 weeks (wks). Serial peripheral blood samples were drawn for RBC PUFA levels and inflammatory cytokines (IL-6, TNFα-R2 and IL-17). Clinical symptoms were assessed by the Brief Pain Inventory short form (BPI-SF), FACTB-ES, and Stanford Health Assessment -Disability Index (HAS) at 12 and 24 wks. Compliance and toxicity were evaluated monthly.

Results: Median age of all 44 women enrolled and randomized was 60 years (range 43-76); breast cancer stage I (n=29), II (n=10), III (n=5); prior taxane (n=15, 34%). Baseline mean dietary intakes of n-3 PUFAs were: total n-3 PUFAs = 1.43 ± 0.86 g/d; EPA = 0.05 ± 0.05g/d; and DHA = 0.10 ± 0.11 g/d. There was a trend toward lower TNFα-R2 with higher dietary intake of EPA (p=0.09) and DHA (p=0.10). Baseline mean RBC EPA+DHA (n-3 Index) was 4.36% and the RBC n-3/n-6 ratio was 0.20. RBC total n-3 PUFAs were similar at baseline between n-3 treatment and placebo groups (6.6 ± 1.6%, 7.2 ±1.9%, p=0.20). RBC PUFAs were not predictive of baseline IL-6 or IL-17. However, the baseline RBC n-3 Index trended toward an inverse relationship with TNFα-R2 (r = -0.23, p 0.14)

Mean dietary intake of n-3 PUFAs did not change over 24 wks for the 30 women with complete FFQ data and blood samples: total n-3 = 1.38 ± 0.70 g/d; EPA = 0.06 ± 0.07 g/d; and DHA = 0.13 ± 0.14 g/d. Dietary EPA and DHA were significantly associated with lower TNFα-R2 (r = -0.40, p=0.02 for both) at 24 wks. RBC total n-3 PUFAs were significantly higher in the n-3 treatment vs placebo group at wk 24 (6.5%±1.0% vs 15.0%±3.3%, p<0.001). RBC EPA+DHA and RBC n-3/n-6 ratio were not predictive of inflammatory cytokines at 24 wks.

Conclusions: There was a trend toward lower TNFα-R2 in breast cancer survivors with higher dietary and RBC EPA and DHA before starting AIs, but only dietary intake of EPA and DHA was significantly related to lower TNFα-R2 after 24 wks of AI therapy.
INTRODUCTION: Although survivorship research focuses on quality of life after breast cancer treatments, it is usually initiated by providers and focused on previously reported side effects and consequences of treatment. Patients are often reluctant to report side effects they may experience during and after cancer treatment and providers are often reticent to ask. In an effort to explore the patient perspective, the Dr. Susan Love Research Foundation convened a collaboration of advocacy groups to "crowdsource" women’s questions regarding collateral damage from treatment. These concerns will then be incorporated into the Health of Women [HOW] Study, an ongoing, online cohort study of breast cancer open to anyone aged 18 or older.

METHODS: Emails to current HOW participants were sent out July 2013 to solicit questions about collateral damage. In October 2013, we undertook the design of a new website landing page for the submission of questions, partnered with other breast cancer advocacy organizations, and began collecting responses. Responses were then categorized to guide questionnaire development.

RESULTS: Sixteen breast cancer and other advocacy organizations came together to support this project. Emails to current HOW participants resulted in 1191 responses. The website landing page resulted in an additional 3000 responses. Overall, 16.8% of respondents complained of fatigue, 16.3% of memory problems, 15.7% of anxiety and/or depression, and 14.0% of numbness/neuropathy. Other less frequently reported problems included problems with nail growth, vision, hearing, urinary tract infections, and allergy-like symptoms.

CONCLUSION: Through this approach, we received an overwhelming number of responses about collateral damage from treatment. Many of the issues are known side effects, while others are less commonly reported, but debilitating nonetheless. We will next compare responses to previously validated questionnaires, develop a comprehensive collateral damage questionnaire for inclusion in the HOW Study, and generate a report for publication to be distributed to the participants.
A novel candidate for chemotherapy induced alopecia (CIA) through local modulation of apoptosis and inflammation

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INTRODUCTION
CIA, with an incidence of 65%, is considered by the sufferers as the most emotionally distressing side effect of cancer therapies. Cooling devices, the only option for CIA, have variable efficacy, and requires trained staff. To improve patient’s quality of life, the solution needs to address not only prevention, but also recovery (faster, non-patchy, in original quality) of hair, nails, eyebrows and eyelashes, in a self-medication setting.

Normal hair cycle mainly consists of growth phase (anagen), regression phase (catagen) and resting phase (telogen) that precedes the shedding phase (exogen). The rapidly dividing anagen cells are damaged by chemotoxic agents and undergo apoptosis, inducing premature onset of the apoptosis-driven catagen phase. The intrinsic apoptosis pathway is essentially mitochondria dependent and executed by members of the Bcl-2 family. Androgenetic alopecia (AGA) subjects show remarkably low level of Bcl-2; Moreover p53 knock-out mice do not undergo CIA. These indicate that pharmacological inhibition of apoptosis pathways is key to effectively manage excessive hair loss, including CIA.

In addition, apoptotic cells or cell debris will trigger and sustain scalp inflammatory condition by stimulating production of pro-inflammatory mediators, delaying hair regrowth process. Dampening inflammation is therefore essential to stimulate hair regrowth.

APOPTOSIS MODULATION (STUDY N°1)
OBJECTIVE: To demonstrate the molecular basis for proof of concept and safety through assessment of the candidate’s potential in modulating Bcl-2 towards normal level in AGA subjects.

METHOD: After 3 months’ topical application, analyze, via immunohistochemistry, Bcl-2 level in scalp biopsies of male AGA subjects (n=20), and compare with Bcl-2 in non-AGA volunteers (n=25).

RESULT: AGA subjects' Bcl-2 level is depressed (1.7). The candidate restored Bcl-2 (3.2) towards normal level (4.7).

ATTENUATION OF INFLAMMATION (STUDY N°2)

OBJECTIVE: To test, in vitro, the anti-inflammatory potential of the candidate.

METHOD: To evaluate TNFα-induced expressions of the pro-inflammatory mediators (E-selectin, ICAM-1 and il-8) in endothelial cells (HUVECs) using specific Antibody Binding Capacity (sABC) technology, measured by flow cytometry.

RESULT: The candidate is able to inhibit TNFα-induced expression of inflammatory marker/cytokine: E-selectin, ICAM-1 and il-8 in HUVECs.

OPEN CLINICAL INVESTIGATION (STUDY N°3)

OBJECTIVE & METHOD: To assess the candidate’s efficacy and tolerance, through topical application on female cancer patients, under supervision of oncologists/nurses (n = 11)

RESULT: Candidate showed promising results in faster / non-patchy / original-quality recovery and prevention of CIA. Nail damages were generally prevented.

CONCLUSION AND DISCUSSION
In-vivo and in-vitro studies demonstrate that the candidate normalizes scalp apoptosis, a key to prevent CIA, and attenuates scalp inflammation, a key to promote faster/non-patchy/original-quality hair recovery.

In cancer patients under chemotherapy, the candidate showed promising results in faster/non-patchy/original-quality recovery and prevention of CIA, although a higher dose may be required. Nail damages were prevented.
Title: Weight loss and bone health in postmenopausal breast cancer survivors

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Body: Background:
The importance of bone health among postmenopausal breast cancer survivors cannot be overemphasized. In addition to bone loss due to aging, secondary bone loss resulting from treatment is a major concern. Although weight loss has adverse effects on bone health in postmenopausal women without cancer, the impact of weight loss on bone health in postmenopausal women with breast cancer is not known. Our objective in this study was to evaluate the effect of weight loss on bone health among overweight/obese postmenopausal breast cancer survivors participating in a weight loss trial.

Methods: We conducted this study in a subset of women (postmenopausal, N=81) enrolled in the multi-site Exercise and Nutrition to Enhance Recovery and Good health for You (ENERGY) study. The ENERGY study is a randomized controlled clinical trial designed to achieve a sustained ≥7% loss in body weight among overweight/obese breast cancer survivors with stage I, II or IIIA disease. Weight loss was achieved largely through dietary modification with the addition of physical activity. Bone health was assessed in a subset of women at a single site using bone turnover markers (BTMs) and dual energy X-ray absorptiometry (DXA). Markers of bone formation: osteocalcin, bone-specific alkaline phosphatase (BALP), procollagen type I N-terminal propeptide (PINP) and bone resorption: N-telopeptides of type-I collagen (NTx), C-terminal telopeptide (CTX) were quantified in blood samples collected at baseline, 6 month and 12-month follow-up. DXA T-score was used to measure bone mineral density at baseline and at 12-month follow-up. Generalized estimating equations were used to assess differences in mean values over time as well as from baseline to 6- and 12-month follow-up. Data from intervention and control arm women were used and treated as a cohort.

Results: The mean age and body mass index (BMI) of women enrolled in our sub-study were 56 years and 31.6kg/m2, respectively. Majority (54.3%) had stage 2 disease. Mean weight decreased by 5.6 pounds between baseline and 6-month follow-up and 4.3 pounds between baseline and 12-month follow-up. There were statistically significant decreases in mean osteocalcin (2.0ng/ml, p-value<0.001), PINP (7.9ng/ml, p-value<0.001) and NTx (3.65nM BCE/L, p-value<0.001), but not BALP and CTX-1 levels between baseline and 12-month follow-up. No significant changes were observed in mean T-scores, pelvis and lumbar spine bone mineral densities between baseline and 12-month follow-up. Weight change from baseline to 12-month follow-up was weakly inversely associated with changes in DXA T-score (r=-0.25, p-value=0.04) and CTX (r=-0.22, p-value=0.07) but not with other BTMs over the same time period.

Conclusion: Weight loss over a 12-month period was associated with decreases in markers of bone formation and resorption but no changes in BMD in overweight/obese postmenopausal women with stage I, II or IIIA breast cancer. Further studies on the association of weight loss and the possible modifying effect of physical activity are needed in order to personalize weight control strategies in overweight/obese breast cancer survivors.
Title: Are physicians "choosing wisely" when imaging for distant metastases in women with early stage (1 or 2) breast cancer? A population study

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Body: Background: The probability of detecting radiologically evident distant metastases in asymptomatic patients with early stage (stage 1 and 2) breast cancer (BC) is low. Published guidelines, including the 2012 American Society of Clinical Oncology (ASCO) "Top-5" recommendation for "choosing wisely" in oncology, recommend against imaging in these patients. Imaging is also associated with significant false positive results and the need for additional confirmatory imaging and/or invasive procedures. Despite this evidence-base, imaging continues to be over-utilized. We decided to quantify the rates of staging imaging in women with early breast cancer in Canada's largest province to determine whether provincial practice patterns are in keeping with the spirit of the guidelines and the 2012 ASCO "Top-5" recommendation.

Methods: Provincial registry data available through the Institute for Clinical Evaluative Sciences were used to identify all patients with a first diagnosis of stage 1 and 2 breast cancer who underwent breast cancer surgery. For each patient, all imaging of the most common metastatic sites (i.e. skeleton, thorax and abdomen) was captured from the date of diagnosis up until 3 months after definitive breast cancer surgery. Patients who received neoadjuvant therapy or with a prior history of any invasive malignancy were excluded.

Results: Between 2007 and 2012, 27,236 patients with stage 1 and 2 BC were identified. Overall data are shown in the table:

<table>
<thead>
<tr>
<th>Stage</th>
<th>No. of patients</th>
<th>% having at least one imaging test (total)</th>
<th>Total no. of imaging tests (mean per patient imaged)</th>
<th>% of patients imaged who required confirmatory imaging (total)</th>
<th>No. of confirmatory imaging tests performed (% of total imaging)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>14,113</td>
<td>83% (11,713)</td>
<td>40,464 (3.5)</td>
<td>55% (6,420)</td>
<td>11,202 (28%)</td>
</tr>
<tr>
<td>2</td>
<td>13,123</td>
<td>94% (12,330)</td>
<td>53,729 (4.4)</td>
<td>65% (7,983)</td>
<td>16,292 (30%)</td>
</tr>
</tbody>
</table>

The most common initial (i.e. not confirmatory) imaging modalities of the skeleton, thorax and abdomen, for both groups were, bone scan (19%; 12,799/66,699), chest x-ray (28%; 18,789/66,699) and abdominal ultrasound (20%; 13,387/66,699) respectively. Use of advanced imaging (isotope bone scans, CT, MRI, PET) to look at potential sites of metastasis represented 38% (11,063/29,262) and 46% (17,180/37,437) of initial imaging tests in stage 1 and 2 BC patients respectively.

Conclusion: Imaging for distant metastatic disease in patients with stage 1 and 2 BC is commonly performed in Ontario despite multiple guidelines recommending against this. Advanced imaging (isotope bone scans, CT, MRI, PET) is commonly used as the initial imaging modality of choice. Of particular concern is the fact that 60% (14,403/24,043) of BC patients with early stage disease who were imaged required some form of confirmatory imaging. These results show the importance of the recent ASCO "Top-5" recommendation against staging imaging in such patients. The reasons for this disconnect between evidence and practice are not fully understood, but knowledge translation strategies beyond publishing guidelines or recommendations are required if we are to elicit a meaningful and sustained change in physician practice.
Title: Adjuvant radiation after lumpectomy: A cost comparison of treatment patterns in 43,247 women from the National Cancer Data Base

Body: BACKGROUND: Breast cancer treatment contributes the greatest proportion of cancer-related health care spending in the United States. Locoregional therapy comprises a significant share of these costs. We hypothesized that among eligible women with early-stage breast cancer treated with lumpectomy, evidence-based utilization of hypofractionated whole breast radiation or omission of radiotherapy could substantially reduce cancer-related treatment costs.

METHODS: Using the National Cancer Data Base which captures approximately 70% of all newly diagnosed cancers in the United States, we identified 43,247 women with clinically node-negative, T1-T2 invasive breast cancers treated with lumpectomy during 2011. Women with DCIS, those treated with mastectomy, accelerated partial breast irradiation (APBI), or unknown or questionable radiation regimens were excluded. Adjuvant radiation was categorized into the following regimens: conventionally fractionated whole breast irradiation therapy (CF-WBI) [25-40 fractions, 45-66 Gy], hypofractionated whole-breast irradiation (HF-WBI) [15-24 fractions, 40-58 Gy], and lumpectomy without radiation (no RT). Women were considered eligible for no RT if ≥70 years with T1N0, ER+ breast cancers, and for HF-WBI if ≥50 years, with T1-T2 N0 invasive breast cancer. Treatment costs were calculated using Medicare Physician Fee Schedule payment information for 2011, and based on average current procedural codes billed per regimen. Costs per patient were estimated as follows: CF-WBI $13,358.37, HF-WBI $8,327.98, and lumpectomy without RT $0. Actual treatment costs were compared to evidence-based, reduced-cost radiation regimens for which patients were potentially eligible.

RESULTS: Median patient age was 63 years (range 19-90). Median tumor size was 1.2 cm. Of the total study cohort, 84.5% was eligible for HF-WBI, and 22.3% for no RT. Among the 36,562 (84.5%) patients eligible for treatment with HF-WBI, 28,383 (77.6%) received radiation therapy. Of these, 22,653 (79.8%) received CF-WBI, 5,289 (18.6%) received HF-WBI, and 441 (1.6%) received accelerated partial breast irradiation (APBI). Among 9,651 women ≥70 years with ER+ tumors eligible for no-RT, 4,245 (44.0%) received CF-WBI, 1,768 (18.3%) received HF-WBI, 153 (1.6%) received APBI, and 3,485 (36.1%) received no RT. 26% of women received the least expensive evidence-based radiation regimen for which they were eligible, while 67% of patients were treated with more costly radiation regimens. Estimated costs of actual treatment were $420.2 million during 2011, compared to $256.2 million had women been treated with the least expensive radiation regimen for which they were eligible. This translates into an annual cost savings of $164.0 million, a 39% reduction in costs.

CONCLUSIONS: Utilization of evidence-based adjuvant radiation following lumpectomy is associated with reductions in cancer-related costs in the locoregional treatment of early-stage breast cancer. Although treatment decisions should not be driven by health care costs alone, consideration of hypofractionated regimens or omission of radiotherapy for patients that fit evidence-based eligibility criteria could translate into dramatic reductions in annual health care spending.
Title: NSABP B-43 is unlikely to produce a cost-effective treatment strategy for HER2+ DCIS

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Body: Background. National Surgical Adjuvant Breast and Bowel Project (NSABP) B-43 is an ongoing randomized clinical trial that compares breast conserving surgery, radiation therapy, and two infusions of trastuzumab to breast-conserving surgery and radiation only for HER2+ ductal carcinoma in situ (DCIS). We sought to identify, from a societal perspective, the level of risk reduction observed in this trial that would be required to make treating HER2+ DCIS patients with trastuzumab cost-effective.

Methods. We developed a Markov decision-analytic model to evaluate these treatment strategies in terms of life expectancy, quality-adjusted life years (QALYs), costs, and the incremental cost-effectiveness ratio (ICER) over 15 years. The results of NSABP B-43 are pending. We therefore assumed the hazard reduction to be 36%, based on the statistical considerations of the trial protocol and explored this in sensitivity analyses. A series of one and two-way sensitivity tests were conducted.

Results. The baseline ICER for switching from a breast-conserving surgery and radiation strategy without trastuzumab treatment to a breast-conserving surgery and radiation strategy with trastuzumab treatment is $1.57 million per life-year gained and $299,616.73 per QALY gained. Compared to a societal willingness-to-pay threshold of $100,000 per QALY gained, adding Herceptin treatment is not cost-effective in the treatment of DCIS under these baseline conditions. In a one-way sensitivity test, we found that the Herceptin strategy only became cost-effective at an 88.3% risk reduction for local recurrence. The model was not sensitive to any costs or probabilities within 20% of baseline values. The model was sensitive to the utilities of having had breast-conserving surgery and radiation with and without trastuzumab treatment. It was not sensitive to other utilities within 0.05 of baseline values.

Conclusions. High risk DCIS treated with breast-conserving surgery and radiation has a low risk of local recurrence as demonstrated in multiple randomized trials. The addition of a costly drug in order to reduce this small risk further is very unlikely to be cost-effective and could be considered overtreatment of a non-fatal disease that is sufficiently managed with local treatment. Prospective cost-effectiveness analyses have the potential to guide allocation of valuable resources for breast cancer research, and should be regularly integrated into the protocol design of large clinical trials.
Title: Post-operative imaging after atypical ductal hyperplasia excision: The findings and costs

Jennifer K Plichta¹, Adrienne N Cobb¹, Gerard J Abood¹, Constantine Godellas¹ and Claudia B Perez¹. ¹Loyola University Health Systems, Maywood, IL.

Body: Introduction: With a reported incidence of 2-12% in breast biopsy specimens, the appropriate management of atypical ductal hyperplasia (ADH) remains in evolution. At present, the optimal screening guidelines for patients with high-risk breast lesions such as ADH remain unclear. Current practices often parallel the surveillance of cancer patients and include a 6 month interval mammogram prior to resuming annual screening, which may result in unnecessary procedures and financial costs. This interval mammogram is typically a diagnostic study, which is an additional cost to the patient and healthcare system. The purpose of this study was to identify interval pathology following initial surgical resection and review associated costs.

Methods: Following institutional review board approval, the pathology database from a single institution was queried for patients who underwent surgical excision for ‘atypical ductal hyperplasia’ from 2008 to 2013. Those who did not have follow-up data available were excluded. Subsequent clinical care was reviewed, including interval imaging and need for additional intervention. Based on a review of hospital charges from 2013, the average charge for a unilateral diagnostic mammogram (out-patient, digital) was $382.

Results: There were 55 patients who underwent an excisional biopsy that were diagnosed with ADH and had subsequent follow-up. The median age was 57 years (range 38-82 years), and the median breast cancer risk assessment score was 2.3% at 5 years (range 0.5-17.9%) and 12.5% lifetime risk (range 2.2-37.6%). Pathology included concurrent lobular carcinoma in situ (n=1), atypical lobular hyperplasia (n=3), flat epithelial atypia (n=14), and papillary lesions (n=19). In addition to a routine clinical breast exam, a short-term follow-up diagnostic (ipsilateral) mammogram was performed in 35 patients. Of the 35 interval mammograms obtained, 31 yielded benign findings on initial imaging, while 4 patients required additional imaging that ultimately resulted in benign findings. The overall hospital charges for the 35 short interval mammograms alone during this 6 year period were roughly $13,370. For the patients that resumed annual surveillance, 3 had abnormal mammograms requiring additional imaging, and no malignancies were identified in this subset of patients. To date, the median physician follow-up is 3 years, and 52 patients have undergone at least one mammogram since their initial imaging; all subsequent findings have been benign for all patients. When extrapolated to national data, cost savings to the healthcare system from eliminating short interval mammograms would exceed $12 million annually without compromising clinical outcomes.

Conclusions: Based on our findings, a 6 month follow-up mammogram is not recommended and incurs unnecessary costs to the patient and healthcare system. In the post-surgical breast, imaging may be misleading and result in additional procedures and significant charges that ultimately do not affect clinical outcomes. Although a clinical exam is still recommended at 6 months following surgical excision for a diagnosis of ADH, patients should forego short interval (6 month) imaging and resume annual mammogram surveillance.
Title: A cost utility analysis from a Chinese health care perspective of Nab-paclitaxel or docetaxel, both as alternatives to solvent-based paclitaxel in metastatic breast cancer (MBC)

George Dranitsaris¹, Bo Yu², Jennifer King³, Adams Zhang³, Satyn Kaura³ and Zhai Qing². ¹Augmentium Pharma Consulting; ²Fudan University Shanghai Cancer Center and ³Celgene Corporation, Summit, NJ.

Body: Background: Paclitaxel and docetaxel are commonly used for the treatment of MBC in China. However, one important drawback, particularly with docetaxel, is the potential for dose-limiting toxicity. To improve the side effect profile and efficacy of paclitaxel, an albumin-bound formulation (nab-paclitaxel) is currently available in China (Abraxane®). To provide health economic data for China, a cost utility analysis comparing nab-paclitaxel to docetaxel, both as alternatives to paclitaxel was conducted.

Methods: Clinical data was obtained from a meta analysis of randomized trials comparing either nab-paclitaxel (260 mg/m² q3wk) or branded docetaxel (100 mg/m² q3wk) to solvent-based branded paclitaxel (175 mg/m² q3wk). Health care resource use for the delivery of chemotherapy and the management of grade 3/4 toxicity was collected from a time and motion study in three Chinese cancer centers, from a survey of clinicians and from the oncology literature. Using the Time Trade-off technique, treatment preferences and utility estimates were obtained from interviewing 28 cancer patients from two centres in China. All costs were reported in 2014 $U.S.

Results: Nab-paclitaxel had the most favourable safety profile characterized with the lowest incidence of grade 3/4 neutropenia, febrile neutropenia, anemia, emesis and stomatitis. This translated to lower costs for managing the grade 3/4 side effects of nab-paclitaxel relative to both docetaxel and paclitaxel ($21 vs. $166 vs. $81) In the preference assessment, 22 of 28 (78.6%) patients selected nab-paclitaxel as their preferred agent. As an alternative to paclitaxel, the incremental cost per quality adjusted life year (QALY) gained was determined to be more favourable with nab-paclitaxel than docetaxel ($57,900 vs. $130,600).

Conclusions: Nab-paclitaxel is an economically attractive alternative to both paclitaxel and docetaxel in MBC patients, providing a substantially lower cost per QALY. Additionally in the patient preference survey, the majority of patients surveyed selected nab-paclitaxel as their preferred agent.
**Title:** Health economic evaluation of: Bevacizumab plus paclitaxel vs. bevacizumab plus metronomic cyclophosphamide and capecitabine as first-line therapy in patients with HER2-negative advanced breast cancer: A multicenter, randomized phase III trial - SAKK 24/09

Klazien W Matter-Walstra, Martin Bigler, Matthias Schwenkglenks, Daniela Bertschi, Jörg Brechbühl, Ursula Hasler-Strub, Roger von Moos, Andreas Müller, Ralph Winterhalder and Christoph Rochlitz. ¹ECPM / University Basel, Basel, Switzerland; ²SAKK Coordinating Center, Bern, Switzerland; ³Cantonal Hospital, St.Gallen, Switzerland; ⁴Cantonal Hospital, Chur, Switzerland; ⁵Cantonal Hospital, Winterthur, Switzerland; ⁶Cantonal Hospital, Luzern, Switzerland and ⁷University Hospital, Basel, Switzerland.

**Body:** Background: Bevacizumab combined with chemotherapy has been shown to improve response rate and progression free survival in metastatic breast cancer. The aim of the health economic analysis (HEA) of this study was to demonstrate that the combination regimen of bevacizumab with cyclophosphamide and capecitabine (metronomic chemotherapy) compared to bevacizumab and paclitaxel treatment decreases overall treatment costs in patients with breast cancer without suffering any losses in effectiveness.

Methods: In this multicenter, randomized phase III trial, we compared bevacizumab (10 mg/kg i.v. q 2 weeks) with either paclitaxel (90 mg/m²) i.v. on days 1, 8, and 15 of a 4 week cycle (arm A) or daily oral capecitabine (3x500 mg) and cyclophosphamide (50 mg, arm B) as first-line treatment in patients with HER2-negative advanced breast cancer. Primary endpoint of the health economic analysis was the total incurred treatment costs until patients stopped trial treatment (time to trial treatment stop (TTS)). TTS was defined as treatment stop due to progressive disease, symptom deterioration, unacceptable adverse events, patient refusal, death or other reasons for withdrawal. The HEA adopted a health system perspective including all substantial direct medical costs incurred in the treatment of the patient. Health-related quality of life was measured by means of the EQ-5D utility instrument. Statistical differences between costs in the treatment arms were tested by the Wilcoxon rank-sum test. A global multivariable linear model, with a gamma distribution and a logarithmic cost transformation was used to analyze the costs for the two arms controlled for age.

Results: Between September 2010 and December 2012, 147 patients were included at 22 centers in Switzerland, 73 (intention to treat (ITT) n=71) in arm A and 74 (ITT n=68) in arm B. The clinical study results will be presented at ASCO 2014. In January 2014, 66 patients in arm A and 63 in arm B of the ITT patients had reached TTS and were analyzed. Mean TTS was 7.3 month in arm A (95%CI 6.3–8.2, median 5.9; quality adjusted mean 5.9, median 5.1) versus 8.5 month (95%CI 6.7–10.2, median 6.8; quality adjusted mean 6.5, median 5.0) in arm B. Total incurred mean costs per patient were CHF 69'474 in arm A (95%CI 60'624–78'324, median CHF 61'815; mean cost per month CHF 10'044) versus CHF 80'324 in arm B (95%CI 62'975–97'672, median CHF 61'751; mean cost per month CHF 10'229). There were no significant differences in costs between the treatment arms and age had no significant effect on the results.

Conclusion: Metronomic chemotherapy plus bevacizumab compared to bevacizumab and paclitaxel treatment showed no substantial reductions in treatment costs. In view of the clinical results the HEA does not favor the metronomic approach. An incremental cost-utility analysis is planned.
Patients’ perspectives on using a genomic test for their breast cancer treatment decisions: Disparities in information exchange

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Research Objective
Genomic tests predicting tumor recurrence risk may help inform treatment decisions and improve quality of care. Yet differences in information exchange and patient decision style about genomic tests may further exacerbate disparities in care, particularly if recommendations conflict with patients’ preferences or other prognostic information. This study looks at the information exchange between patients and physicians and patients’ preferences in decision-making in the context of receiving a genomic test for early-stage breast cancer.

Study Design
Retrospective study of women covered by a large national health insurer, who had received the most common, commercial genomic test for early-stage breast cancer prognosis. All eligible minority women (n=705) and a random sample of eligible white women (n=719) matched by age category, geography and diagnosis year, were invited to a mailed survey later linked to claims data. We used random-effects logistic regression models (cluster=state) to examine the association of patient self-reported race/ethnicity with 3 outcomes: 1) knowledge of being tested; 2) patient decision style (assessed with the Control Preference Scale and categorized as active (patient-based), shared, or passive (physician-based); 3) amount of information received on the test. Models were adjusted by patient age, education, income, family structure/support, year of testing, and plan type.

Population Studied
Privately insured women aged 64 years old or less, enrolled for at least 9 continuous months, with an insurance claim for the genomic test in 2009–2012.

Principal Findings
The 62% response rate yielded 896 respondents, including 108 Hispanics, 112 Black, 97 Asians, and 549 whites. 10% of the respondents were unaware that they had received the genomic test, with significant differences in this knowledge by race/ethnicity after controlling for age, education and income (OR= 3.8 for Hispanic and OR = 2.7 for Black women compared to White, both p < .010). Decision style was reported predominantly as active (47%) and shared (40%), and varied significantly with age, income, education and race. In particular, Blacks were more likely than Whites to report a passive rather than shared decision (OR = 1.6; p = .065). Black women were also more likely to rate the information received on the test’s clinical significance as insufficient (OR=2.1; p = .001).

Conclusions
While most women reported more active styles of decision-making, we found significant differences in information exchange that may lower the odds of a well-informed decision for minority patients. Variation in patient reports may reflect variation in understanding and retaining information about the genomic test, or in information delivery by physicians.
Title: Telemedicine: Expanding access to cancer genetic services to underserved populations

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Body: Background: Given the increasing demand for genetic services and limited genetic workforce, many patients do not receive recommended pre- and post-test genetic counseling. Telemedicine has been used to expand specialized medical services to low access populations. The feasibility and outcomes of telemedicine in clinical genetics are not well described.

Methods: Patients at 3 community sites without genetic counseling services received real-time pre-test (V1) and post-test (V2) counseling for cancer susceptibility with a genetic counselor (GC) at a center of expertise via community sites’ and host institution’s computers equipped with web cameras and videoconferencing software. Mixed-methods surveys assessed patient knowledge, satisfaction, psychosocial responses and experiences at baseline (BL), post-V1 and post-V2. We used paired T-tests to assess change between time points and linear regressions.

Results: Of 100 patients approached, 83% consented to telegenetic services. To date, 57 have completed BL and V1, and 70% proceeded with genetic testing, 31 patients have received results, including 3 carriers (BRCA2, MSH2, PMS2). Patient characteristics did not differ between those who agreed to and declined telegenetics. 4% of sessions were aborted due to technology failures. 30% experienced disconnections but were completed. Nearly all (94%) were satisfied with their telegenetic experience. Knowledge and satisfaction with telegenetic services significantly increased and general anxiety and depression significantly decreased. Event related (state) anxiety did not change significantly.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>BL Mean (sd)</th>
<th>Post-V1 Mean (sd)</th>
<th>Post-V2 Mean (sd)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge (6-28)</td>
<td>20.9(2.8)</td>
<td>22.0 (3.0)</td>
<td>21.5(3.1)</td>
<td>0.007</td>
</tr>
<tr>
<td>General Anxiety (0-21)</td>
<td>7.4(4.1)</td>
<td>6.6 (4.1)</td>
<td>5.7 (3.5)</td>
<td>0.06</td>
</tr>
<tr>
<td>General Depression (0-21)</td>
<td>3.9 (3.9)</td>
<td>3.5 (3.4)</td>
<td>2.9 (3.5)</td>
<td>0.07</td>
</tr>
<tr>
<td>State Anxiety (20-80)</td>
<td>36.0(15.2)</td>
<td>35.7(13.7)</td>
<td>32.1(12.5)</td>
<td>NSS</td>
</tr>
<tr>
<td>Satisfaction with Genetic Services</td>
<td>39.5(3.0)</td>
<td>39.8 (4.0)</td>
<td>42.2(3.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>Satisfaction with Telemecine</td>
<td>51.3(5.6)</td>
<td>51.5(5.7)</td>
<td>53.0(5.3)</td>
<td>0.008</td>
</tr>
</tbody>
</table>

Patients reported several advantages to telegenetics (e.g. decreased travel burden) and few disadvantages (e.g. audio challenges and technical glitches). Conclusions: Telemedicine delivery of cancer genetic services is feasible, identifies genetic mutation carriers, increases knowledge, decreases anxiety and depression and is associated with high satisfaction, suggesting an innovative model for delivery of genetic services for patients and community practices without access to local genetic providers.
Title: Receipt of risk reduction strategies among an underserved population of BRCA mutation carriers

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Body: Introduction: Risk reduction strategies for women with deleterious BRCA mutations include risk-reducing mastectomy (RRM) or screening with annual mammograms and MRIs, chemoprevention and prophylactic bilateral salpingo-oophorectomy (RRBSO). The objective of the study was to describe the receipt of various risk reduction strategies in an underserved and largely understudied minority population of BRCA mutation carriers.

Methods: BRCA mutation carriers detected in our center between October 2005 and January 2014 were included in the study. Participants who had bilateral mastectomies prior to genetic testing were excluded from the analysis of breast cancer reduction strategies. Women age 40 or older, with at least one ovary intact at baseline and no history of ovarian malignancy or metastatic cancer were included in the analysis of uptake of RRBSO. Compliance with annual screening mammogram and MRI was defined as receipt of 2 screening mammograms and 2 MRIs within 2 years of the disclosure of the test results. Medical records were reviewed to collect demographic data, personal history of cancer and receipt of mammography, MRI, RRM and RRBSO. Uptake of chemoprevention was not assessed.

Results: Of the 92 BRCA mutation carriers, 89 were women and 3 were men. 35% were Caucasian, 34% were African American and 26% were Hispanic. Sixty eight percent were uninsured and 24% had public insurance. The mean age at the time of testing was 44 years (range: 18-71 years). BRCA1 positivity was seen in 54% and BRCA2 positivity was seen in 46%. Of the 89 women, 7 had bilateral mastectomies prior to testing and 15 were lost to follow up. Among the remaining 67 women, 53 (79%) had a personal history of breast and/or pelvic cancer (affected) and 14 (21%) had no personal history of breast or pelvic cancer (unaffected). Of the 53 affected women, 14 (26%) received RRMs. The median duration from disclosure to RRM was 104 days (IQR: 21- 477 days). Among the 39 affected women who did not undergo RRM, 31 had follow up care at our institution for at least 2 years after disclosure, during which 25 (81%) had at least 2 screening mammograms and 13 (42%) had at least 2 screening MRIs. Among the 14 unaffected women, only 1 (7%) woman underwent RRM. Ten unaffected women had follow up care at our institution for at least 2 years after testing, during which 4 (40%) had at least 2 mammograms and 2 (20%) had at least 2 MRIs. Of the 44 women eligible for RRBSO, 8 (18%) women were lost to follow up. Among the 36 remaining, 23 (64%) underwent RRBSO. Twenty-one out of the 23 who underwent RRBSO had a previous history of cancer. The median duration between disclosure to RRBSO was 170 days (IQR: 90-454 days). Thirteen women (36%) have not yet received the recommended RRBSO after a median follow up of 976 days (IQR:465-1591 days).

Conclusions: Among a diverse group of medically underserved minority women who tested positive for a deleterious BRCA mutation, the receipt of surgical risk reduction strategies and MRI screening is low, especially among women who do not have a personal history of cancer. Additional studies are needed in this population to examine the factors associated with acceptability and compliance with standard of care recommendations as well as institutional barriers to receipt of these risk reduction strategies.
Title: Implementation of cancer risk assessment and genetic testing in underserved patients

Ian K Komenaka¹, Lisa M Winton¹, Jesse N Nodora², Lisa Madlensky², Meredith A Heberer¹, Richard Schwab², Marcia E Bouton¹, Jeffrey N Weitzel³ and Maria Elena Martinez². ¹Maricopa Medical Center, Phoenix, AZ; ²University of California, San Diego, La Jolla, CA and ³City of Hope National Medical Center.

Body: PURPOSE: There is great disparity in genetic testing for breast cancer. Hispanic/Latina women with breast cancer are more likely to have adverse clinical features and have a high prevalence of BRCA mutations. We propose that Academic-Community clinic partnerships offer great potential to provide access to genetic cancer risk assessment (GCRA) for underserved communities, including Hispanic/Latina women. The study also evaluated the willingness of these patients to participate in biospecimen collection.

METHODS: This study assessed the implementation of a limited GRCA and testing service at a safety net institution from July 1, 2011 to December 31, 2013. In the 10 years prior, only two breast cancer patients had undergone genetic testing and both were insured. The inability to perform GCRA was recognized as a critical area of need. Therefore, a breast surgical oncologist received training with City of Hope National Medical Center. The goal is to provide clinicians the appropriate skills to provide GCRA services in areas where these are not available.

Three generation pedigrees and sociodemographic information were collected including health literacy, education, self-reported income, employment status, and insurance status. We conducted a comparison of the patient characteristics along the continuum of GCRA, genetic testing, and mutation carriers, and for the latter group, we describe the BRCA mutation profile.

RESULTS: 125 patients were offered GCRA and all accepted, of which 70% of this patient population was Hispanic and 66% did not have health insurance. Of the 125 patients, 84 (67%) were recommended to undergo genetic testing and 81 (96%) agreed. Of the 81 patients who underwent genetic testing, 68 were also asked to participate in the City of Hope Cancer Screening and Prevention registry and all but one (94%) agreed.

Significant differences between patients who had genetic testing and those who did not were shown for race/ethnicity, insurance, and family history. A higher percentage of Hispanic patients and patients with no insurance underwent testing. Additional trends in differences between patients who were tested vs. those who were not were observed for education and health literacy but these were not statistically significant. Few differences were observed between women who had genetic testing and mutation carriers; however, the number of carriers was too small to merit statistical testing. Twelve of 81 (15%) patients were found to have deleterious mutations, seven BRCA 1 and five BRCA 2. Of the 12 mutation carriers, one patient had ovarian cancer and therefore had already undergone bilateral salpingo-oophorectomy and two others underwent RRSO. Six are either considering RRSO or getting financial assistance for the operation. The last three are still undergoing breast cancer treatment.

CONCLUSION: Results of our experience at a safety net hospital with a largely minority and uninsured population show that limited GCRA and testing can be successfully implemented. The great majority of patients agree to undergo counseling, testing, and participate in biospecimen research registries. Current recommendations for genetic counseling are far from being met across the country and this model could be considered for similar safety net populations.
Title: Women's opinion on when to start screening mammography and reasons for not undergoing screening

Ian K Komenaka¹, Meredith Heberer¹, Jesse Nodora², Chiu-Hsieh Hsu³, Lisa Winton¹, Marcia Bouton¹ and Maria Elena Martinez². ¹Maricopa Medical Center, Phoenix, AZ; ²University of California, San Diego, La Jolla, CA and ³University of Arizona, Tucson, AZ.

Body: Background: In late 2009 significant controversy arose when some screening recommendations were changed to advocate screening mammography starting at age 50 rather than the long standing recommendation of starting at age 40 years. More recently some studies have called into question the benefit of screening mammography. The current study was performed to evaluate patients' opinion on when women should start screening mammography and reasons for not undergoing screening.

Methods: Maricopa Medical Center is the safety net hospital in Phoenix, Arizona. 1,157 consecutive patients were seen at the Breast Clinic from May 2013 to May 2014. Sociodemographic variables were collected including health literacy assessment using the Newest Vital Sign (NVS) validated screening instrument. Patients were asked when they felt women should start screening mammography. In addition, in women at least 40 years of age, if they did not undergo screening mammography, they were asked for the primary reason for not undergoing screening. Differences in patient characteristics were evaluated based on a Fisher's exact test for categorical variables and one-way ANOVA for continuous variables.

Results: Thirteen of the 1,157 consecutive patients were male and excluded. The average age of the 1,144 consecutive female patients was 45 years. Most patients were Hispanic, underinsured, and had limited health literacy. Overall use of screening mammography was poor at only 24%. 402 women (35%) felt that age 40 years was the most appropriate time to start screening. Only 30 women (3%) felt that age 50 years was the most appropriate age. More women, 470 (41%), chose an age younger than age 40 to start screening. More than half (55%) of these women who chose an age 50 years or younger, however, did not undergo screening because they felt they had “no problems” or “didn’t know” they should get a mammogram despite choosing an age to start that was below their current age. Only 187 women (32%) cited cost as the reason for not undergoing screening. Other reasons for not undergoing screening were: physician did not recommend and other medical problems/forgot/too busy. Few patients (4%) cited problems with mammograms for not undergoing screening and none cited concerns about false positives. Multivariate analysis showed that patients with adequate health literacy and insurance were more likely to use screening mammography than patients who were uninsured or had limited health literacy. Family history of breast cancer was not associated with use of screening mammography.

Conclusions: Use of screening mammography was poor in this underinsured population. Most women felt that screening mammography should start at age 40 years or younger. More than half of women who did not undergo screening did not do so because they had "no problems" or "didn't know" they should. Although many women feel that screening should start at age 40, most women in this population do not understand the concepts of screening and early detection. Interventions to increase use of screening mammography should focus on the concept of screening as well as the age.
Title: Mammograms on-the-go: Predictors of repeat visits to mobile mammography vans in St. Louis, Missouri

Bettina F Drake¹, Salmafatima S Abadin¹, Sarah Lyons¹, Su-Hsin Chang¹, Lauren T Steward¹, Susan Kraenzle² and Melody S Goodman¹. ¹Washington University, St Louis, MO and ²Joanne Knight Breast Health Center, The Alvin J. Siteman Cancer Center, St Louis, MO.

Body: Background: Among women, breast cancer is the most common noncutaneous cancer and second most common cancerous cause of death. The American Cancer Society recommends that all healthy women over age 40 have a mammogram annually because early detection and treatment of tumors has been associated with a 15% decrease in breast cancer mortality. African American women have higher mortality rates than white women and, in general, uninsured women have low rates of screening. To increase screening rates, mobile mammography has been implemented in many cities. Previous studies have investigated women’s self-reported adherence to screening guidelines at the time of participation in mobile mammography, but no study has examined if women use it as an annual screening tool.

Objective: The purpose of this study was to determine if women are using mobile mammography vans as their established source of medical care for breast cancer screening and the factors that predict repeat visits to these vans.

Methods: A prospective cohort study was conducted from 2006 to 2013 in which 8450 women who received a mammogram as part of Siteman Cancer Center’s Breast Health Outreach Program responded to surveys and provided access to their clinical records. Only visits on the mammography van were included. The predictor variables explored in this study were: urban status, insurance coverage, age group, race, marital status, mammography experience at baseline visit, employment status, and year of screening. Data were analyzed using chi-square tests, logistic regression, and negative binomial regression.

Results: Among the study participants, 25.3% (N=2134) had multiple visits to the mobile mammography van. Of these women, 57.2% had good mammography experiences at baseline, 48.2% were from urban settings, 70.6% were uninsured, 51.2% were ages 50-65, 69.7% were Black, 76.4% were not currently married, and 63.3% were unemployed. Women who were ages 50-65, uninsured, or Black had a higher odds of a repeat visit to the mobile mammography van compared to women who were ages 40-50, insured, or White (OR=1.135, 95% CI: 1.013-1.271; OR=1.302, 95% CI: 1.146-1.479; OR=1.281, 95% CI: 1.125-1.457), respectively. However, the odds of having a repeat visit to the van was lower among women who reported a rural zip code or were unemployed compared to women who provided a suburban zip code or were employed (OR=.503, 95% CI: .411-.616; OR=.868, 95% CI: .774-.972), respectively.

Conclusion: This study has identified key characteristics of women who are either more or less likely to use mobile mammography vans as their primary source of medical care for breast cancer screening and have repeat visits. It is important that mobile mammography is maintained and remains easily accessible to women who continuously use the service. Further research should be done to discover ways to make mobile mammography a more effective resource for those more likely to use it for routine screening.
Title: Multivariable analysis of repeat utilization of mobile mammography units: 10 year analysis of a comprehensive cancer center

Tezo Karedan, Elizabeth Riley, Lane Roland, Laura Barkley, Jianmin Pan, Shesh Rai and Sarah Mizuguchi. 1University of Louisville, Louisville, KY.

Body: Introduction: Mobile Mammography Units (MMU) are a model of outreach. This study analyzes the association of race, insurance and location to MMU utilization. Our group previously reported these variable as independent predictors of MMU repeat use. This study explores if race, age, insurance and location jointly can predict the utilization of MMU. Methods: From Jan 2001-Dec 2010, 48,324 screening mammograms were performed with 21,587 unique subjects. Demographic data was retrospectively reviewed to identify race/ethnicity, insurance and location for each encounter. Locations were grouped as Corporate (C), Partnership Clinic (PCI), Partnership Community (PCo). Insurance type was classified as Private Insurance (PI), Medicaid (Maid), Medicare (MCare) and Uninsured (UI). Insurance type was classified based on the primary insurer. Utilization was defined as 1x or more than 2x in 10 yrs. Descriptive statistics related to different predictors were produced and statistical comparisons were conducted. P-values were calculated using Chi-square test for comparison between the two groups. Odds ratio and its 95% confidence interval were provided. Logistics regression analysis is used to jointly model the effect of independent factors on repeat utilization of MMU. Reference range for race, insurance and location were chosen based on prior work. Results were declared significant at significance level of 5%. Results: Univariable analysis of race, insurance status, location and age were predictive of repeat utilization of the MMU over a 10 yrs. (p= value <.001, <.001, <.001 <.002) respectively. All variables, race, insurance, location and age remained statistically significant for repeat use in multivariable analysis. Blacks (B) were more likely to repeat compared to Whites (W) and Hispanics (H). MCare patients remained most likely to repeat compared to PI, UI or MCaid patients in multivariable analysis. Location, PCI and Age 50-64 continued to predict for highest utilization rate within the respective cohorts. The estimated OR are shown in Table 1.

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<tr>
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<th>Univariable</th>
<th>Multivariable</th>
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<tr>
<td></td>
<td>pvalue</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Race</td>
<td>&lt;.001</td>
<td>0.880 (0.829-0.934)</td>
</tr>
<tr>
<td>White</td>
<td>&lt;.001</td>
<td>0.643 (0.555-0.745)</td>
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<tr>
<td>Hispanic</td>
<td>&lt;.001</td>
<td>0.834 (0.770-0.904)</td>
</tr>
<tr>
<td>Insured</td>
<td>0.003</td>
<td>0.804 (0.698-0.926)</td>
</tr>
<tr>
<td>Location</td>
<td>0.006</td>
<td>0.828 (0.828-0.968)</td>
</tr>
<tr>
<td>Corporate</td>
<td>&lt;.001</td>
<td>0.856 (0.805-0.911)</td>
</tr>
<tr>
<td>PCo</td>
<td>&lt;.001</td>
<td>0.714 (0.666-0.766)</td>
</tr>
<tr>
<td>Age</td>
<td>.002</td>
<td>0.849 (0.679-1.061)</td>
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<tr>
<td>25-39</td>
<td>.015</td>
<td>0.895 (0.844-0.949)</td>
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<tr>
<td>40-49</td>
<td>.083</td>
<td>0.928 (0.654-1.010)</td>
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</table>
Conclusions: To our knowledge this is the reported largest database of MMU. Race, insurance status and location remained independent predictors of repeat utilization. Interestingly an insured subset (MCare) was more likely to repeat utilize the van regardless of race and location. This may reflect the average age of screening mammography however a better understanding of the uninsured subset may be important given the intended outreach of the MMU.
Title: The impact of social inequalities on breast cancer mortality in Brazil

Ruffo Freitas-Junior¹, Carolina M Gonzaga¹, Maria-Paula Curado², Ana-Luiza L Sousa¹ and Marta R Souza¹. ¹Federal University of Goias (UFG), Goiania, Goias, Brazil and ²International Prevention Research Institute (IPRI,), Ecully, France.

Body: Introduction: Female breast cancer mortality has decreased considerably in developed nations. In contrast, an increase has been observed in developing countries. Objective: To describe the impact of social inequalities in female breast cancer mortality in Brazil, between the years of 1990 and 2011. Methods: Breast cancer mortality data and estimates for the resident population were obtained from the Brazilian National Health Service (SUS) database for the 1990-2011 period. Age-standardized mortality rates were calculated (20-39, 40-49, 50-69 and ≥70 years) by direct standardization using the 1960 standard world population. Trends were modeled using linear regression, with mortality rates as the dependent variable and the year of death as the independent variable. The Social Exclusion Index (SEI) and the Human Development Index (HDI) were used to classify the 27 Brazilian states. Pearson's correlation was used to describe the association between the SEI and the HDI and the variations in mortality rates in each state. Results: Age-standardized mortality rates in Brazil were found to be stable (annual percent change [APC] = 0.32; 95%CI: -0.1 – 0.7). Statistically significant decreases in mortality rates were found in the states of Rio Grande do Sul, Rio de Janeiro and São Paulo. Increases in mortality rates were most notable in the states of Maranhão (APC = 11.2; 95%CI: 5.8 – 16.9), Piauí (APC = 9.8; 95%CI: 7.6 – 12.1) and Paraíba (APC = 9.3; 95%CI: 6.0 – 12.8). There was a statistically significant correlation between SEI and a change in female breast cancer mortality rates in the Brazilian states between 1990 and 2011 and between HDI and mortality between 2001 and 2011. This reduction was most notable in the Brazilian states with better socioeconomic conditions. Conclusions: It was observed a direct impact of social inequalities in female breast cancer mortality rates in Brazil. Reductions in these rates were found in the more developed states, possibly reflecting a better local healthcare.
Early discontinuation of adjuvant chemotherapy in women with early stage breast cancer: The BQUAL study

Alfred I Neugut, Grace C Hillyer, Lawrence W Kushi, Lois Lamerato, Jinjoo Shim, Dana H Bovberg, David Nathanson, Christine B Ambrison, Jeanne S Mandelblatt, Carol Magai, Wei Yann Tsai, Judith S Jacobson and Dawn L Hershman.

1Columbia University Medical Center, New York, NY; 2Kaiser Permanente of Northern California, Oakland, CA; 3Mailman School of Public Health, New York, NY; 4Henry Ford Health Center, Detroit, MI; 5University of Pittsburgh Cancer Institute, Pittsburgh, PA; 6Roswell Park, Buffalo, NY; 7Georgetown University, Washington, DC and 8Long Island University, Long Island, NY.

Adjuvant chemotherapy for early stage breast cancer decreases recurrence and increases survival. However, early discontinuation of chemotherapy occurs frequently and has a negative influence on patient outcomes.

METHODS
The Breast Cancer Quality of Care Study (BQUAL) is a prospective cohort study designed to investigate factors associated with early discontinuation of adjuvant chemotherapy among women diagnosed with non-metastatic breast cancer at three sites in the U.S between 2006 and 2010 (Columbia University Medical Center, Kaiser-Permanente of Northern California, Henry Ford Health System). Chemotherapy regimens were classified based on NCCN guidelines. Regimens were further categorized as standard and non-standard/experimental. Early discontinuation for standard treatments was defined as missing 20% of the recommended number of treatments for the prescribed regimen. We used multivariate analysis to examine the association between early discontinuation and sociodemographic factors, tumor characteristics, and baseline psychosocial factors.

RESULTS
Of 1157 women recruited, 478 patients initiated chemotherapy; 35 women received non-standard/experimental chemotherapy and an additional 17 did not complete all interviews and were excluded from the analysis. Of the remaining 426 patients, 59 (13.9%) did not complete the full course of prescribed chemotherapy. In multivariate analysis, compared to those who completed their full prescribed course of adjuvant chemotherapy, those who discontinued were more often >50 years of age (p=0.04). Early discontinuation of chemotherapy was less likely among Asian women (OR 0.12, 95% CI 0.01-0.96), those who held positive beliefs related to the efficacy of chemotherapy (OR 0.43, 95% CI 0.22-0.81), and those who were more optimistic (OR 0.93, 95% CI 0.86-0.99). Women prescribed chemotherapy regimens that had more cycles (>5 cycles) or contained paclitaxel/docetaxel were significantly more likely (OR 7.54, 95% CI 2.68-21.20 and OR 5.02, 95% CI 1.59-15.83, respectively) to discontinue chemotherapy treatment early than regimens with 6 or less cycles.

CONCLUSIONS
Women prescribed longer regimens were significantly more likely not to complete the full course. Positive beliefs about the efficacy of treatment were associated with continuation of treatment. Educational interventions focused on the importance of completing therapy may increase chemotherapy adherence.
Title: Young women with breast cancer: Needs and experiences

Punam Rana¹, Jonathan Sussman¹, Jenna Ratcliffe¹, Margaret Forbes¹, Mark Levine¹ and Nicole Hodgson¹. ¹McMaster University, Hamilton, ON, Canada.

Body: Background
About 11% of all breast cancers in the United States are diagnosed in women younger than the age of 45. Young women with breast cancer (YWBC) may experience unique physical and psychosocial problems. Meeting the needs of these young women is often dependent on a patient’s oncology team. There is a lack of data about the experiences of YWBC in our region including whether their needs are met and which resources are available and offered to them.

Objectives
To explore the unique physical and psychosocial needs of YWBC in our region (Hamilton and Niagara) and to describe what information YWBC receive, and which decisions are made, regarding: fertility, breast reconstruction, mastectomy, sexuality and emotional support.

Methods
This study includes women 40 years of age or less who were diagnosed with invasive breast cancer within 12 months. Using a qualitative design, participants attended a 1 hour focus group or a 30 minute interview moderated by the research coordinator, JR. Recordings were transcribed verbatim, with anonymization of any identifying information. Fourteen participants (ages 30 to 40) were included. Co-PI (PR) and JR reviewed the transcripts, complied recurring categories, and discrepancies were resolved via consensus.

Results
Most participants underwent a timely work-up and diagnosis (64%) but some experienced a delay in diagnosis (37%) as they were seen as unlikely to have cancer due to age. A majority of the participants felt they were provided enough information for informed decisions about treatment. However, over one third of the women were not provided any information about social or community supports and a similar proportion also felt that fertility and sexuality issues were not adequately addressed. Over one third planned on having bilateral mastectomy and/or immediate reconstruction, regardless of physician recommendation. The most distressing aspects of the cancer experience included: telling children and parents about the diagnosis; waiting for test results; fear of recurrence; and re-starting life after breast cancer.

Recurring Themes From YWBC

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<tr>
<th>Recurring Themes</th>
<th>FG1</th>
<th>FG2</th>
<th>FG3</th>
<th>INT1</th>
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<td>Delay in Diagnosis</td>
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<td>Poorly Communicated</td>
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<td><strong>Treatment</strong></td>
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<td>Undergoing reconstruction</td>
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<td><strong>Communication</strong></td>
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<td>Not well-informed about Rx plan</td>
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<td>Well informed about fertility</td>
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### Needs met re: sexuality

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### Needs unmet re: sexuality

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### Info provided re: supports

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### Info not provided re: supports

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### Supports Utilized

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<tr>
<th>Supports Utilized</th>
<th>Social Worker</th>
<th>Community Services</th>
<th>None</th>
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</table>
| FG=Focus Group; INT=Interview

### Conclusion

YWBC feel that they are provided with adequate medical information for treatment decision-making. However, there is a gap in supports for psychosocial, fertility, and sexuality-related needs. Interventions targeted to address these unmet needs should be evaluated. In the second phase of this study, we will create an instrument to further assess the needs of young women with breast cancer in our region.
Title: Enhancing compliance with national nutrition recommendations in breast cancer survivors. Experience in an underprivileged community

Laurent Zelek\textsuperscript{1,2}, Chloe Bodere\textsuperscript{1}, Delphine Bourlier\textsuperscript{1} and Anne Festa\textsuperscript{1}. \textsuperscript{1}Oncologie 93, Bobigny, France and \textsuperscript{2}Avicenne Hospital, Bobigny, France.

Body: BACKGROUND: To enhance compliance with national nutrition recommendations in breast cancer survivors (BCS), a 3-year program granted by the Regional Health Authority began in 2013 in an area (Seine-Saint-Denis, SSD) which is among the poorest in France. Median household income is 68\% lower than in Paris (+68\%), a gap growing with time. Whereas it is widely admitted in France that 25\% of patients are faced with financial difficulties after breast cancer, this proportion reaches 40\% in SSD. We present here intermediate 1-year results.

PATIENTS AND METHODS: Oncologie 93 is a non-profit organization whose aim is to provide supportive care, health education and individualized assistance to patients and families, and to facilitate timely access to quality medical and psychosocial care. Vulnerability was evaluated using an 11-item standardized score (EPICES) previously investigated by French Health Examination Centers. Strictly speaking this score was aimed at measuring precarity, a concept referring to a social condition assumed to face worsening. This score is more strongly related to health status than the administrative classification of poverty (Sass, Sante Publique 2006). Vulnerability was defined by a score $>30$ and considered as severe when $>40$. Between March 2013 and December 2013, 27 BCS were enrolled in a 3-month education program including 3 sessions of a professionally led support group (with dieticians and social workers). Given the high level of poverty in the area and the incidence of financial difficulties in cancer survivors, a particular attention was paid to comparing the costs of different foods in order to promote affordable dietary changes. Mean age was 52. Median vulnerability score was 51.8 (0-93.48) and 64\% of patients had a score $>30$. Dietary intakes were assessed at baseline, and 1 and 6 mos. after the last session. At baseline, although 13 BCS correctly answered the questionnaire, 17 BCS had difficulties to prepare meals and fatigue was the main reason for 10 of them; 4 BCS were lost for follow-up before the end of the program. At 1 mo. 15 BCS had knowledge of healthy dietary choices and 11 were ready to translate it into practice. Of note, 9 BCS decided to enroll in a tailored physical activity program or planned to do it. At 6 mos. only 10 BCS still had knowledge of healthy diet but all of them turned it into practice. Barriers were reported in 10 BCS and included anxiety or depression (n=8), family (n=6) and asthenia or other treatment side effects (n=5).

CONCLUSION: A short-term dietary intervention is feasible in vulnerable BCS living in an underserved area and improves adherence to higher quality diet in a meaningful number of patients. In spite of the attention paid to the affordability of dietary modifications, numerous barriers still exist in this population, mainly related to psychosocial reasons. Furthermore, fatigue negatively influences the ability to prepare meals. A total of 100 BCS will be included over 3 years, and further studies will evaluate the impact of the vulnerability level and psychosocial comorbidities on BCS' compliance.
Title: Religion-related factors and breast cancer screening among American Muslims

Aasim Padela¹, S Murrar¹, S Mallick¹, Z Hosseinain¹, C Liao¹, C Ajax¹, F Marfani¹ and M Peek¹. ¹University of Chicago, Chicago, IL.

Body: Background: Cancer disparities research often overlooks the influence a shared religion may have across race and ethnicity, and thereby misses opportunities to leverage shared religious networks to promote screening. While American Muslims have low rates of mammography and their health behaviors are strongly influenced by religion, a description of such relationships is lacking in the literature. Our study fills this gap.

Methods: We conducted a mixed-methods exploration of how religion-related factors impact breast cancer screening practices. We sampled an ethnically and racially diverse group of Muslim women frequenting mosques and community sites. A survey incorporated measures of fatalism, religiosity, religious discrimination, and Islamic modesty, while subsequent focus groups elicited perspectives on how religious beliefs, values and identity impact breast cancer screening intentions. Survey analyses involved logistic regression models, while focus group data were analyzed using a team-based framework content analysis approach.

Results: Of 240 survey respondents, 72 were Arabs, 71 S. Asians and 59 African Americans. Seventy-five percent had insurance, while 85% had a PCP. 77% reported ever having a mammogram while only 37% a mammogram within the past 2 years. In multivariate models, positive religious coping (OR= 0.21; P < .05) and perceived religious discrimination in healthcare (OR=0.74; P < .05) were negatively associated with having a mammogram in the past two years, while having a primary care physician (OR=20; P < .01) was positively associated. Ever having a mammogram was positively associated with increasing age (OR=1.1; P < .05), years of US residency (10-20 yrs OR=11; 20 yrs OR=4.3; P < .05) and knowing someone with breast cancer (OR=3.5; P < .01). Importantly, ethnic/racial affiliation did not influence mammography rates.

Of 50 focus group participants there were nearly equal numbers of S. Asians, Arabs, and African Americans, 74% reported ever having a mammogram, with 56% having a mammogram within the past two years. Focus group data revealed that family support and encouragement strongly impacted screening intentions, and that obtaining screening in a way that accommodated notions of religious modesty was paramount and prior experiences with such accommodations influenced subsequent intentions. Focus group participants believed that the mosque is a critical community venue for setting religious mores but is underutilized for health education and for motivating theological responses to illness. Participants expressed the need for mosque administration and religious leaders to openly discuss breast health and mammography screening among the Muslim community.

Conclusions: Aspects of religion appear to influence cancer screening behaviors similarly across the socioeconomic, ethnic, and racial diversity of American Muslims. Promoting biennial mammography screening among American Muslims requires addressing ideas about religious coping as related to preventive cancer screening and empowering women to combat perceived religious discrimination. Mosques are underutilized in breast cancer screening interventions but are a ripe setting for religiously-tailored programming that can address barriers to screening and promote a culture of health in this community.
An evaluation of mobile mammography outreach in urban and rural communities

Lauren Steward¹, Susan Kraenzle², Bettina Drake¹, Sarah Lyons¹ and Melody Goodman¹. ¹Washington University School of Medicine, St Louis, MO and ²Alvin J. Siteman Cancer Center/Barnes Jewish Hospital, St Louis, MO.

Background: Mobile mammography has been used to reach underserved women in a diverse number of settings. In this work, we demonstrate similarities and differences between rural and urban communities served by a single mobile mammography unit (MMU) affiliated with a comprehensive cancer center in Missouri.

Methods: An outreach registry of patients serviced by the MMU was created and includes data from medical records and responses to a brief questionnaire completed at each visit. Data was examined by point of care (urban/rural) to assess the efficacy of mobile mammography as an outreach strategy in each of these environments. Bivariate analyses were used to examine the relationships between demographic characteristics such as age, income, race/ethnicity, education, employment status, marital status, insurance status, and living environment proxy.

Results: Between 2006 and 2013, 9480 women received their care on the mobile mammography van. The sample was stratified by point of care (urban vs. rural) served, with majority of the women (86%) residing in urban/suburban St. Louis City/County, and 14% in rural regions. Urban zip codes had a lower percentage of women with income greater than $20,000 (12% v. 21%) and higher percentage of women with income less than $10,000 (49% v. 37%) in comparison to rural communities(p=0.01). There were higher proportions of black women in urban communities (63%) compared to rural communities (10%; p<0.001). Almost half (47%) the women that received mobile mammography in rural zip code were married compared to less than a quarter of women from urban zip codes (24%; p<0.001). Rural communities (83%) had a higher percentage of uninsured women compared to urban communities (67%; p<0.001). Women in urban and rural communities were similar in respect to age, employment status, and education.

Conclusions: Mobile mammography has the potential to reach a large population of women with limited educational, financial, and healthcare resources. Future studies will be needed to determine if increasing the range and extent of mobile units, such as this one, would be effective in increasing the screening rates of not only women in Missouri, but also in other portions of this country.
Evidence-based programs to reduce breast cancer mortality in Panama City

Anna Cabanes¹, Maria Roquebert², Tauane Cruz¹, Blanca Benaglio¹ and Becky Royer¹. ¹Susan G. Komen, Dallas, TX and ²Gestion Social, Panama City, Panama, Panama.

Body: In Panama, as in other Latin American countries, breast cancer is the most common cause of cancer-related deaths among women, and is becoming a major public health problem. Breast cancer mortality rates have steadily risen since 2002 and reflect not only breast cancer incidence and risk factors, but also late-stage diagnosis, quality of cancer care and reduced access to health care.

Susan G. Komen® developed an evidence-based strategic plan for reducing breast cancer mortality focused on increasing access to breast cancer screening and care. Komen’s approach resulted from an analysis of the breast cancer continuum of care in Panama City, aimed at understanding the factors that determine whether women enter the breast cancer continuum of care, and the barriers that prevent women from completing the continuum. Komen utilized the results of the study to drive programs and develop evaluation plans.

We conducted four focus groups and individual interviews with 35 breast cancer survivors living in Panama City to assess the factors that determine access to the breast cancer continuum of care, as well as personal perceptions of breast cancer treatment and follow up. In addition, we held 69 in-depth interviews to capture the community’s perceived barriers in accessing cancer care from three stakeholder groups: public and private medical professionals, non-profits, and private insurers. Interviews and focus groups were audio taped, transcribed and the content analyzed for emergent themes and patterns.

Based on the data, we drafted three different paths of breast cancer care depending on the type of health insurance: Seguro Social (Social security system), private insurance, or no insurance. In all scenarios we identified health care system deficiencies within the primary, secondary and tertiary care levels, in particular low medical breast cancer training and lack of delineated referral guidelines. Community level gaps identified include lack of information, awareness, and social support networks.

To address these gaps, Komen implemented strategic activities that included a program to fund interventions to conduct awareness campaigns, train primary care medical professionals, and reduce barriers to access breast health and cancer care that in Panama is mostly localized in the capital. Impact was measured using an impact chart based on evidence-based and best practice interventions in the areas of public awareness, community and professional education, and continuum of care services. With an investment of $500,000 over two years we were able to reach 2 million people with breast health and breast cancer messaging through 92 television and 268 radio events, and 2,500 views through social media. We funded a total of 36 medical trainings on standard breast health care guidelines by level of care, protocols and referral processes reaching a total of 1,328 medical providers and 65 community leaders, some from indigenous communities (caciques). Finally, we provided housing assistance to 130 women from rural areas that had traveled to receive breast health and breast cancer services, including treatment and follow-up if needed.

Our results show that evidence-based programs provide the opportunity to improve service in areas of most need.
Title: Breast cancer prevention awareness and breast examination attitudes among Hong Kong women who work in a medical environment – A pilot study

Janice Tsang¹, Siu Ting Yung¹, Alston Chiu¹, Henry Sze¹, Cindy Lai Shan Wong¹, Wendy Chan¹, Agnes Cheung¹, Eve Lai¹, Sin Ting Wong¹, To Wai Leung¹ and Dora Kwong¹. ¹Queen Mary Hospital, University of Hong Kong, Hong Kong, China.

Body: Background: Breast Cancer is the commonest female cancer worldwide and the most common female cancer in Hong Kong. Overseas studies have explored the knowledge and attitudes towards breast cancer, but most are targeted at general public. Similar studies were done among female health care personnel but mainly overseas with paucity of data within the Chinese community. This is a pilot study aiming at exploring the breast cancer prevention awareness and breast examination attitudes among Hong Kong women who work in a medical environment. Methods: This is a cross-sectional, qualitative pilot study targeting female nurses working at the Queen Mary Hospital, Hong Kong. We planned for 300 samples as pilot study to validate the questionnaire. This involved self-administrated questionnaires during 1st October 2013 to 30th November 2013. Altogether, 1800 questionnaires were distributed to nurses at Queen Mary Hospital through the Central Nursing Department. Results: A total of 317 completed self-administered questionnaires were collected. The age of the respondents ranged between 20-60, with the majority in the age group of 40-49 (28.4%), followed by 20-29 (27.4%). The mean years in practice was 17. Among the six diseases given for ranking (diabetes mellitus, breast cancer, hypertension, depression, cardiovascular disease, cerebrovascular disease), breast cancer is ranked as the most important disease for their age (37.2%). However, many respondents perceived the degree of risk of breast cancer during their life time to be average (34.1% with median score 5) or even lower (35% under median score 5). The knowledge towards breast cancer risk factor remained limited. Most of them did not identify late menopause (74.5%), advanced maternal age (70.6%) and young age at menarche (58.7%) as risk factors. For breast examinations, most respondents had performed breast self-examination (BSE) (91.2%), less than half of them performed BSE on a regular basis (only 39.4% performed BSE once every month). Ñ The need of my age Ñ (72.2%) and Ñ I am more aware of breast cancer Ñ (54.6%) are claimed to be the major factors encouraging them to perform BSE. Relatively few respondents had ever undergone clinical breast examination (CBE) (41.0%). Similar to the case of BSE, those who had undergone CBE are found to practise it irregularly (23.3%), comparing to 10.1% practising CBE once every year. More respondents with secondary educational level or above had mammography (p=0.000) or ultrasound (p=0.007) done than respondents with primary educational level or below which is statistically significant. Conclusion: The general breast cancer risk awareness among nurses in this pilot study is of concern. There is an unmet need to develop breast cancer preventive intervention program targeting at nurses. This pilot study helps to validate the proposed survey, and serves as the basis for our future design of appropriate intervention to enhance breast cancer awareness and improve early breast cancer prevention for female healthcare workers. Further study is warranted to be extend to female doctors, healthcare assistants and ward clerks etc.
Title: CYP19A1 and ESR1 polymorphisms and selected early-onset side effects during combined endocrine therapy in the IBCSG TEXT trial for premenopausal women with hormone receptor-positive (HR+) early breast cancer

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Body: Background: Single nucleotide polymorphisms (SNPs) of the aromatase enzyme (CYP19A1) and estrogen receptor alpha (ESR1) may be associated with breast cancer susceptibility and endocrine-mediated side effects. The IBCSG Tamoxifen (Tam) and Exemestane (Exe) Trial (TEXT) includes a translational research project to assess whether selected SNPs may influence treatment efficacy and toxicity. We report on early-onset hot flashes and sweating (HF/S) and musculoskeletal symptoms (MS; myalgia, arthralgia, stiffness) with respect to CYP19A1 and ESR1 SNPs under combined endocrine therapy.

Patients and Methods: 2,672 premenopausal women with HR+ early breast cancer were randomized to treatment with the GnRH-agonist triptorelin (Trip)+Tam or Trip+Exe for 5 years. Randomization was stratified according to intended use of chemotherapy (yes/no) and lymph node status (N- vs N+). Estrogen-depletion side effects (HF/S and MS) were recorded at baseline, 3-monthly during the first year and 6-monthly thereafter using the NCI CTCAE v3.0. DNA was centrally extracted from whole blood with Qiagen kits. SNPs of CYP19A1 (rs4646 and rs10046) and ESR1 (rs207764, rs2234693 and rs9340799) were analyzed by a pyrosequencing method (Diatech Pharmacogenetics S.r.l., Jesi, Italy). Control genotypes (wt/wt; wt/v; v/v) for all SNPs were processed in each run. Logistic regression was used to analyze two endpoints: presence or absence of grade (gr) 2-3 HF/S during first 6 months and gr 2-4 MS during first 12 months. Four genetic models were used to explore associations with side effects: genetic (wt/wt; wt/v; v/v), additive, dominant and recessive.

Results: DNA was isolated and genotyped for 1970 (74%) consenting women. Clinical characteristics and outcomes of this cohort were consistent with the overall trial. At baseline median age was 44, median BMI 24 kg/m2 and 86% had regular menses. During follow-up, 43% reported gr 2-3 HF/S and 27% reported gr 2-4 MS. The 5 SNPs did not deviate from Hardy-Weinberg equilibrium (p>0.30) and minor allele frequency ranged from 29%-48%. The CYP19A1 SNP rs10046 (C>T) was associated with gr 2-3 HF/S. Specifically, women with variant homozygote genotype (T/T) had a reduced risk of HF/S (39% T/T vs 45%). The univariate odds ratio (OR) was 0.77 (95%CI: 0.62-0.96; p=0.02) compared with other variant groups. The multivariate model showed consistent results after adjusting for age, BMI, menstrual status, chemo use, treatment allocation (Tam vs Exe) and baseline HF/S. The other 4 SNPs were not associated with the selected side effects.

Conclusions: Our analysis indicates association of HF/S with CYP19A1 rs10046 genetic variants. The lack of association between early-onset of selected estrogen-depletion side effects and the other 4 SNPs may be masked by the concurrent Trip. Ovarian reserve before treatment may also influence the impact of SNP genotypes on early-onset side effects. Further analysis with details of concurrent medications and treatment adherence will contribute to the interpretation of these results.

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Title: Effect of using LHRH analog during chemotherapy (CT) on premature ovarian failure and prognosis in premenopausal patients with early-stage, hormone receptor-positive breast cancer: The primary analysis of a randomized controlled phase III trial

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Body: Background: Whether administration of LHRH analog during CT in premenopausal patients with early-stage, hormone receptor-positive disease would reduce CT-induced premature ovarian failure (POF) is still controversial. Moreover, whether LHRH analog would influence the prognosis of patients is unknown yet. This randomized study is to evaluate whether administration of LHRH analog during CT would reduce POF and effect the prognosis of breast cancer.

Methods: This is a randomized, controlled phase III clinical trial. Premenopausal patients age <46 with stage I-IIIA, ER/PR-positive BC to be treated with CT were randomized into two arms. Concurrent arm received CT with goserelin (GN) 3.6 mg SQ starting 0-7 days prior to the first CT dose, and after CT, the researchers determined whether continuous GN to 2-5years or cessation. Sequential arm received CT without GN, and after CT, the researchers determined GN 2-5 years immediately or after restoration of ovarian function or bilateral ovariectomy. Neoadjuvant CT was allowed. Five-year tamoxifen after CT was administered to all patients. The primary endpoint is POF, defined as amenorrhea for the prior 12 months and post-menopausal FSH or not assessed after last CT dose or last GN dose. Other endpoints include efficacy of neoadjuvant CT and relapse-free survival (RFS), defined as time to the first of these events: loco-regional recurrence, contralateral breast cancer or distant metastasis.

Results: Between 2/09 and 5/13, the trial has finished enrollment, 216 patients were enrolled. The median age were 37.5 in combined arm (n=108) and 39 in sequential arm (n=108), respectively. The median follow-up time was 27.4 months and 25.7 months, respectively. 15 patients and 21 patients received neoadjuvant CT, respectively. There were no significant difference in age, tumor stage and CT regimens (p>.05). The median cycles of GN were 25, respectively. 47% had complete primary endpoint data. POF rate were 5/42 (11.9%) in the combined arm and 16/60 (26.7%) in the sequential arm. POF rate (and post-menopausal FSH) rate were 1/42 (2.4%) in the combined arm and 8/60 (13.3%) in the sequential arm. In neoadjuvant CT subgroup, each has 1 patient achieved pathological complete remission, and there was no significant difference in objective clinical response. There were 9 patients in the combined arm and 3 patients in the sequential arm had occurred RFS events (including 2 and 0 deaths, respectively, OR=3.18, 95%CI:0.84-12.09, P=.075).

Conclusions: LHRH analog administration with CT might be associated with less POF and did not affect the efficacy of neoadjuvant CT, however, had no RFS benefit, it may need longer follow-up. We will conduct an interim analysis in November 2014.

Clinicaltrials.gov Registry Number: NCT01712893.
**Title:** Factors influencing on discontinuation of adjuvant anastrozole in postmenopausal Japanese breast cancer patients: Results from a prospective multicenter cohort study of patient-reported outcomes

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**Body:** Background: Adjuvant five-year treatment with aromatase inhibitors is standard for postmenopausal women with estrogen receptor positive breast cancer. However, aromatase inhibitor-related adverse events including joint symptoms and vasomotor symptoms have a strong impact on patients’ quality of life and sometimes result in treatment discontinuation. The aim of this study is to determine risk factors for discontinuation of endocrine therapy in Japanese postmenopausal breast cancer patients treated with adjuvant anastrozole in a prospective cohort study based on patient-reported outcomes (PROs).

Patients and Methods: A total of 391 postmenopausal Japanese women with estrogen receptor-positive breast cancer and treated with adjuvant anastrozole were enrolled from 28 centers in this prospective cohort study (SAVS-JP, UMIN000002455). PROs assessment was obtained at baseline, 3, 6, 9 and 12 months which included joint and vasomotor symptoms. Long-term adherence of anastrozole was obtained from 364 out of 391 patients (median follow-up: 44 months, range: 5-105months). We analyzed the relationship of discontinuation of anastrozole with joint and vasomotor symptoms induced by treatment, and patients’ characteristics.

Results: Among 364 patients, 64 (17.6%) discontinued, 297 (81.6%) are ongoing and 3 (0.8%) have completed five-year anastrozole treatment. The reasons for discontinuation were recurrence: 20 (31.3%), secondary malignancies: 5 (7.8%), death from non-breast cancer: 1 (1.6%) and adverse events: 38 (59.4%). These 38 patients who stopped treatment caused by adverse events were compared with other 323 patients. Joint and vasomotor symptoms were categorized into grade 0 (no symptom or no change from baseline), grade 1+2 (mild-moderate) and grade 3 (severe). Grades of joint symptoms were significantly associated with discontinuation of anastrozole (Grade 0: 9.7%, grade 1+2: 7.8%, grade 3: 25.0%, p=0.02). Patients with longer time after menopause (16 years or longer) were significantly higher frequency of discontinuation as compared with shorter time after menopause (0-15years) (14.9% vs 8.0%, p=0.04). Univariate analysis revealed that grade 3 joint symptoms (odds ratio: 3.67, 95% confidence interval: 1.34-10.04, p=0.01) and longer time after menopause (OR: 2.01, 95%CI: 1.01-4.00, p=0.04) were significant risk factors for discontinuation. By multivariate analysis, both grade 3 joint symptoms and long time after menopause were independently associated with discontinuation.

Conclusion: In the present study, we have identified that grade 3 joint symptoms and longer time after menopause were risk factors for discontinuation of adjuvant anastrozole. These data might give us useful information for counseling in patients with adjuvant aromatase inhibitors for postmenopausal Japanese women.
Title: Aromatase inhibitor induced musculoskeletal syndrome (AIMSS) in Australian women with early breast cancer: An Australia and New Zealand Breast Cancer Trials Group (ANZBCTG) survey of members of the Breast Cancer Network Australia (BCNA)

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Body: Background: AIMSS is experienced by approximately half of women taking an aromatase inhibitor (AI), impairing quality of life and in some leading to AI discontinuation. There is a lack of evidence for effective AIMSS treatments. Aim: To investigate the importance of AIMSS in Australian women with early breast cancer. Method: A survey invitation was distributed to 2390 members of the BCNA Review and Survey Group in April 2014. The online questionnaire consisted of 45 questions covering demographics, AI use, clinical manifestations and risk factors for AIMSS, reasons for AI discontinuation and efficacy of interventions used for AIMSS. AIMSS was defined as joint pain or stiffness that developed or worsened after commencing an AI. Results: Of 594 respondents, 370 (62\%) were eligible. Reasons for exclusion were: preinvasive disease, locally advanced/metastatic breast cancer, or other reason. Eligible respondents had a median age range of 50-59 years. Duration of AI use varied (26\%1 year, 64\% 1-5 years, 10\% 5 years). 57\% had received adjuvant chemotherapy. 43\% of these commenced AI within 3 months of chemotherapy and 30\% within 3-6 months of chemotherapy. A vitamin D test was performed in 64\% of women and 68\% were currently using vitamin D supplements. Joint pain during menopause was reported by 22\% of respondents. AIMSS occurred in 302/370 women (81\%). Of those who developed AIMSS, sites affected were feet (68\%), hands or wrists (65\%), knees (62\%), hips (56\%), shoulders or elbows (49\%), back (46\%), or neck (3\%). 34\% of women had considered stopping an AI because of AIMSS. 99 (27\%) of respondents had discontinued AI for any reason and of these 68\% discontinued because of AIMSS. Non-AIMSS symptoms identified as reasons for discontinuation included fatigue, vaginal/urinary symptoms and hot flushes. In respondents who discontinued AI, 20\% ceased use in the first 3 months, 30\% during months 3-12 and 38\%12 months. 42\% of respondents who discontinued an AI restarted the same or a different AI after a treatment break. To manage AIMSS 23\% of respondents used doctor prescribed medications (eg anti-inflammatories, codeine, morphine,), 55\% over the counter (OTC) or complementary medicines (eg low dose anti-inflammatories, paracetamol, chondroitin, fish or krill oil, glucosamine, and vitamin D) and 29\% alternative therapies (eg acupuncture, massage, Tai Chi and yoga). Respondents identified the following in each of the above categories as most successful in relieving AIMSS symptoms: doctor prescribed anti-inflammatories, paracetamol and yoga. Doctor prescribed medications and OTC/complementary medicine either completely or significantly relieved AIMSS in 12\% and 25\% of cases respectively. 27\% of respondents found that one or more of the interventions that they had used to manage AIMSS helped prevent AI discontinuation. Conclusion: AIMSS is a significant issue for Australian women and is an important reason for AI discontinuation. Women use a number of interventions to manage AIMSS, however their efficacy appears limited. Effective AIMSS interventions are needed, to improve quality of life and reduce AI discontinuation.
Title: Co-SOFT: The cognitive function substudy of the suppression of ovarian function trial (SOFT)

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Body: Background: Cognitive impairment is a potential side-effect of breast cancer (BC) treatment. Estrogen is an important neuromodulator that affects cognition. Estrogen depletion by oophorectomy or GnRH agonists may adversely affect cognition in non-oncological settings, but there are few data regarding the cognitive effects of ovarian function suppression (OFS) in women with breast cancer.

Patients and Methods: Between November 2003 and January 2011, 3066 premenopausal women with hormone receptor-positive BC were randomised on the SOFT trial to 5 years of adjuvant endocrine therapy with tamoxifen alone, tamoxifen+OFS or exemestane+OFS. OFS was achieved by the GnRH agonist triptorelin, oophorectomy or ovarian irradiation. Prior chemotherapy was allowed, provided women had premenopausal estradiol levels at enrolment. Women eligible for Co-SOFT must not have received any prior adjuvant endocrine therapy. At study entry (t1), and approximately 1 year after SOFT randomisation (t2), objective cognitive function was assessed with a brief computerized test battery comprising 7 tasks (CogState Ltd: cogstate.com). Subjective cognitive function, psychological distress, fatigue, insomnia and quality of life were also assessed. Co-SOFT recruited 86 of a planned 321 patients from 27 of 426 SOFT centres between November 2007 and January 2011, when Co-SOFT was closed as the SOFT trial completed accrual. The protocol-specified primary comparison was the change in the composite score of the CogState tasks over 1 year for women randomised to tamoxifen alone compared with OFS+oral endocrine therapy. However, due to low accrual this was modified, prior to any analysis, to compare the tamoxifen versus the pooled tamoxifen+OFS and exemestane+OFS groups.

Cognitive test scores were standardized according to age-specific norms, averaged to compute the composite score and then change between t1 and t2 calculated; a negative change in composite score indicates deterioration in cognitive function. Change in composite score was compared using Wilcoxon rank sum test.

Results: Of 86 Co-SOFT enrolled patients, 74 underwent both t1 and t2 CogState testing and were included in the primary analysis (7 withdrew consent/declined assessment, 5 missed testing due to scheduling). Of these 74 women, 20 were randomised to tamoxifen and 54 to OFS+tamoxifen (28) or OFS+exemestane (26). Baseline characteristics were well balanced between the 2 groups. During the first year 49 women utilised GnRH alone for OFS, 4 had GnRH followed by oophorectomy and 1 had oophorectomy alone. There was no significant different in the changes in the CogState composite scores from t1 and t2 for patients randomised to tamoxifen alone compared with OFS+oral endocrine therapy (median, -0.057 versus -0.146 respectively, p=0.51). There were no significant between-group differences in the changes from t1 and t2 for any of the 7 individual cognitive tasks comprising the composite score.

Conclusions: The results of this 1-year longitudinal substudy suggest that the addition of OFS to oral endocrine therapy does not significantly affect cognitive function in the setting of adjuvant BC treatment. Co-SOFT was limited by small sample size, so further investigation of the impact of OFS on cognitive function in BC patients is warranted.
Title: Characteristics of recurrence after completing adjuvant tamoxifen therapy for 5 years

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Body: Background Treatment with tamoxifen (TMX) reduces the recurrence rate and increase overall survival in patients with hormone receptor positive breast cancer. Up to now, 5-year TMX therapy is generally accepted, but it is demonstrated that the rate of late recurrence after 5 years is considerably higher in hormone receptor positive type than in other subtype. Several clinical trials such as ATLAS and aTTom showed the benefit of continuing tamoxifen up to 10 years instead of stopping at 5 years without increasing mortality due to the effect of extended tamoxifen medication.

Method We collected data of 1633 hormone receptor positive breast cancer patients who received surgery at Seoul National University Hospital from 1997 to 2007, and had completed 5-year TMX therapy with no recurrence within 5 years after diagnosis. Mean age of the patients was 43.3, and the patients have estrogen receptor or progesterone receptor. We included from the stage I to stage IV patients underwent curative surgery and received adequate adjuvant therapy such as chemotherapy or radiation therapy after surgery. We excluded the cases treated aromatase inhibitor (AI) or switched to AI.

Result Among these patients, recurrences after 5 years of TMX therapy were found in 93 patients (late recurrence group). Local recurrences and distant metastases were found in 43 and 50 patients, respectively. Electronic medical records were retrospectively reviewed for clinicopathological factors. When comparing between patients with no recurrence and patients with late recurrence, p53 and HER-2 expression were significantly related to late recurrence (p=0.01, p<0.001 respectively). Also when subgroup analysis was done for distant metastasis of late recurrence group, distant metastasis was significantly associated with HER-2 expression and high nuclear grade (p=0.005, p=0.006 respectively). There are no relation between late recurrence and age, stage and ki-67.

Conclusion our data shows that p53 and HER-2 expression is associated to late recurrence and especially HER-2 expression is related to distant metastasis after completing TMX for 5 years. On the basis of the result of large clinical trials, extending TMX therapy significantly reduces recurrence rate and increase survival. Our result support continuing TMX in patients with HER-2 expression and high nuclear grade is considerable after 5 years of TMX medication.
Title: Bortezomib enhances the efficacy of fulvestrant by promoting the aggregation of the ER in the cytoplasm

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Body: Background: Aromatase inhibitors (AIs) are standard treatment for Estrogen Receptor (ER)+ breast cancers in post-menopausal women where the main source of estradiol comes from adipose tissue when the aromatase enzyme converts androgens to estrogens. However, in premenopausal women, functioning ovaries flood the body with estrogens, and aromatase inhibitors used alone offer no therapeutic benefit. In addition to tamoxifen and aromatase inhibitors, estrogen receptor down-regulators, are a third type of anti-estrogen. The first of this class to be FDA approved is Fulvestrant, which acts by promoting the proteosomal degradation of the ER. Like tamoxifen, fulvestrant binds directly to the ER but while tamoxifen has both antagonist and agonist effects on the ER, fulvestrant is a pure antagonist. Other important advantages of fulvestrant over tamoxifen are that 1) fulvestrant prevents the ER from binding DNA, 2) fulvestrant is not linked to increased risk of endometrial cancer, 3) fulvestrant promotes permanent degradation of the ER.

The molecular machinery leading to the degradation of the ER in the nucleus following fulvestrant treatment is well described and correlates with its ubiquitination in the nucleus. A less well-recognized mechanism, is fulvestrant’s ability to promote the aggregation of the newly synthesized ER in the cytoplasm. Understanding that protein aggregates are toxic when not eliminated by the proteasome, we took advantage of this effect of fulvestrant to ask whether combining fulvestrant with the proteasome inhibitor Bortezomib could enhance the efficacy of Fulvestrant.

Results: We found that bortezomib enhances the aggregation of the ER in the cytoplasm following treatment with fulvestrant. Further, these aggregates were found to be insoluble and to activate the unfolded protein response (UPR), a stress response that leads to cell death. Further, bortezomib is able to prevent the activation of cytoprotective responses linked to the acquisition of fulvestrant resistance. Furthermore, in a breast cancer mouse model of tamoxifen resistance, the combination induced tumor regression. We currently are testing new generation proteasome inhibitors and the results will be presented at the meeting.

Conclusion: We conclude that adding bortezomib to fulvestrant enhances its efficacy by taking advantage of a previously poorly recognized mechanism—fulvestrant’s induction of ER aggregation in the cytoplasm. Further, our data suggest that this strategy will block the ability of cells to acquired resistance to fulvestrant. Our group has developed a clinical trial that has tested this combination and the results of this trial presented at the meeting.
Title: Possibility of GnRH agonist plus tamoxifen as an alternative treatment to AC (adriamycin plus cyclophosphamide) chemotherapy plus tamoxifen treatment in premenopausal hormone responsive HER2 negative breast cancer patients

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Body: Background: The purpose of this study is to compare the treatment outcomes using GnRH agonist plus tamoxifen, and adriamycin and cyclophosphamide (AC) containing chemotherapy plus tamoxifen in hormone responsive premenopausal node negative breast cancer patients.

Methods: Total 1027 premenopausal women with node negative, hormone receptor positive, Her-2 negative breast cancer were included in this retrospective cohort study. 595 patients (57.9%) were treated with GnRH agonist together with tamoxifen, and 432 patients (42.1%) were treated with adriamycin and cyclophosphamide containing chemotherapy with tamoxifen.

Results: A median follow up period was 81 months. In pT1 disease, 7 year DFS (disease free survival) was 98.9% for GnRH agonist plus tamoxifen group and 97.4% for chemotherapy plus tamoxifen group, and in pT2 disease, 7 year DSS was 99.1% for GnRH agonist plus tamoxifen group, and 96.9% for chemotherapy plus tamoxifen group with no statistical significance (p=0.252 & 0.230). In pT1 disease, 7 year RFS was 95.4% for GnRH agonist plus tamoxifen group and 92.9% for chemotherapy plus tamoxifen group, and in pT2 disease, 7 year RFS was 91.6% for GnRH agonist plus tamoxifen group, and 91.7% for chemotherapy plus tamoxifen group with no statistical difference between two treatment groups (p=0.353 & 0.836). In subgroup analysis, ER+/PR+ patients and patients younger than 40 years old showed slightly better survival in GnRH agonist plus tamoxifen group when compared to chemotherapy plus tamoxifen group with p-value of 0.046 & 0.018.

Conclusion: Adding GnRH agonist to tamoxifen is reasonable alternative to adding AC chemotherapy to tamoxifen in premenopausal luminal type node negative breast cancer patients.
Title: Tamoxifen treated patients have a better survival than patients treated with aromatase inhibitors - A population based registry study in Sweden

Håkan Olsson¹,², Rickard Einefors¹ and Per Broberg¹. ¹Clinical Sciences, Lund University, Lund, Sweden and ²Lund University, Lund, Sweden.

Body: Background. Randomised trials suggest that therapy with aromatase inhibitors improves survival in breast cancer compared with tamoxifen therapy in postmenopausal cases with hormone receptor positive breast cancer. Whether these results from randomized studies transform into the general population is unknown. We have therefore compared survival for all breast cancer cases in Sweden diagnosed 2000-2008 (n=54406) who received adjuvant antihormonal therapy.

Material and methods. The study includes all women with BC diagnosed in Sweden between 2000 through 2008 (n=54406). The women had no previous cancer diagnosis during the period of 1958-1999. Dates of birth, BC diagnosis and TNM-stage were directly extracted from the cancer registry. The women’s antihormonal therapy was gathered from the Swedish Prescription Registry (22213 women were on antihormonal therapy). Information regarding the cause of death and date of death was obtained from the Cause of Death Registry and the Swedish Population Register up until the 31st of December 2012 and 31st of December 2013 respectively. The breast cancer death and overall death have been calculated and the survival was compared between tamoxifen and aromatase inhibitor treated breast cancer patients. Analyses were adjusted for TNM-stage and age at diagnosis and restricted to women aged 50 and above.

Results. Patients being treated with tamoxifen had a better breast cancer prognosis compared with aromatase inhibitor treated patients (HR 0.54, 95%CI 0.48-0.61). Restricting the analysis to stage 1 disease confirmed a better prognosis for tamoxifen treated women (HR 0.48, 95%CI 0.34-0.66). A better prognosis could be seen in all age strata studies, 50-60,61-70,71-90. The findings for overall survival gave similar results.

Conclusion. This population based observational study show that women treated with aromatase inhibitors have a worse overall and breast cancer specific survival compared with tamoxifen treated women regardless of age and tumor stage.
Title: Prospective study of aromatase inhibitor-induced bone loss and lipid levels in early hormone sensitive breast cancer treated with AI during 8 years

Christel Fontaine¹, Lore Decoster¹, Denis Schallier¹, Leen Vanacker¹, Jacques De Grève¹, Marian Vanhoeij², Guy Verfaillie² and Jan Lamote². ¹Oncologisch Centrum, UZ Brussel, Jette, Brussels, Belgium and ²Breast Surgery, Oncologisch Centrum, UZ Brussel, Jette, Brussels, Belgium.

Body: Introduction: AI’s are the preferred adjuvant hormonal treatment for postmenopausal(PM) estrogen receptor positive (ER+) disease because of the improved efficacy over tamoxifen in terms of disease free survival and reduction in distant recurrence. AI’s have been associated with increased bone loss by blocking peripheral tissue estrogen synthesis, arthralgia and a negative effect on lipid metabolism. In this prospective study we wanted to assess the amount of bone loss and the changes in lipid levels in early breast cancer(EBC) patients (pts) treated with 3yrs of extended letrozole as part of the SOLE study after 5 yrs of adjuvant AI. Objectives: the primary objective of the study is to calculate the mean percentage change in bone mineral density(BMD)(g/cm²) after 8 yrs of letrozole and to compare between the continuous(C) and intermittent(I) intake of letrozole. The secondary objectives are to correlate the mean change in BMD with the initial value, BMI and to describe the evolution of the lipid levels in both groups. Patients and methods: BMD was measured at the lumbar spine (L2-4) and hip by dual energy X-ray absorptiometry (DEXA) in PM pts. with ER+ EBC. We also measured the fasting lipid levels baseline and after 8 yrs of an AI. Differences between the two groups were assessed by the independent samples T-test and the correlation by the linear regression analysis. Results: Fifty four pts were included in the study with a mean age of 62.5 yrs(+/−8.6 yrs). Seventeen pts are too early to be evaluable, 8 pts stopped letrozole due to adverse events and 2 pts had no baseline DEXA values. Two pts received tamoxifen 2 to 5 yrs before inclusion in the SOLE study. The remaining 25 pts showed a mean change in BMD for the lumbar spine of -1.1 (10.6SD) and for the hip -4.4 (7.04SD). Currently 13 pts in the C arm demonstrated a mean decrease in BMD for the (LS) of -2.7(10.2SD), and for the hip -4.4 (7.04SD). Twelve pts included in the I arm experienced a mean increase in BMD for the LS of 0.65(SD11.2) and a mean decrease in BMD for the hip of -4.9(9.1SD). The difference between the two treatment groups was not significant for the LS measurements after 8yrs (p=0.4) nor for the hip (p=0.23) neither was it at baseline (p=0.9; 0.1). Only three pts had fractures due to trauma. The overall fracture rate was only 0.05%.There was no correlation with the BMI, but there was a significant correlation with baseline BMD.(p=0.002)The mean fasting cholesterol levels at 8yrs in the C arm was 214mg/dl(41.3SD) and in the I arm was 218mg/dl(35.6SD)and was not significantly different(p=0.8), nor was it at baseline(p=0.5).

Conclusion: This first prospective long term BMD and lipid follow-up study in PM EBC pts taking adjuvant letrozole beyond 5 yrs shows a decline in BMD. Until now no significant differences were observed between continuous and intermittent letrozole. An updated and detailed follow-up on more patients will be presented.
Title: The insulin-like growth factor axis and development of tamoxifen resistance in breast cancer

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Body: Background
Insulin-like growth factors (IGF). IGFs are potent mitogens for breast epithelial cells that modulate the action of AKT, which has been reported to be associated with tamoxifen resistance. To date, however, anti-IGF strategies have proved disappointing in clinical trials We have investigated whether the IGFBP family of proteins, which modulate the activity of IGF, may play a role in tamoxifen resistance, opening up the route for alternative anti-IGF based therapies.

Methodology
We investigated the expression of IGF axis genes in parental and tamoxifen-resistant (TamR) MCF-7 cells using qRT-PCR. Gene and protein expression was confirmed using ELISA, Western, and ligand blotting. shRNA transfection was used to silence candidate IGF axis genes in both cell lines. Cell sensitivity 4-hydroxytamoxifen (4-HT) was investigated by WST-1 cell proliferation assay.

Results
IGF-IR, IGF-2R, IGFBP-2, -4 and -5 genes had the highest expression levels. IGFBP-5 was down-regulated 7-fold while IGFBP-2 was up-regulated by 2-fold in TamR v wt cells. Changes in IGFBP-5 and IGFBP-2 gene expression were mirrored in protein levels measured in conditioned media by ELISA, Western and Ligand blot. Importantly knock down of IGFBP-2 in TamR cells restored sensitivity to 4-HT suggesting a causal role for IGFBP-2 in the acquisition of tamoxifen resistance.

Conclusion
IGFBP-5 and IGFBP-2 are reciprocally regulated on the acquisition of tamoxifen resistance by MCF-7 cells. Preliminary studies suggest that IGFBP-2 may play a role in the development of tamoxifen resistance in vitro.
Factors associated with adherence to adjuvant endocrine therapy in patients with hormone receptor positive breast cancer

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Background/Purpose
Adjuvant endocrine therapy in patients with hormone receptor positive breast cancer reduces recurrence and mortality, but many patients are non-adherent to anti-hormonal medication. In order to increase the adherence, it is important to know about factors associated with adherence. So we investigated factors associated with adherence to anti-hormonal medication using variable questionnaires.

Methods
We carried out a cross-sectional survey of a sample of women who underwent surgery due to breast cancer in the Seoul National University Hospital Breast Care Center from 2007 to 2011 and treated with anti-hormonal medication. Questionnaires were sent to 1,000 patients. The questionnaire booklet included the Medication Adherence Report Scale-5(MARS-5), Women's Health Questionnaire(WHQ), Beliefs about Medicine Questionnaire(BMQ), Satisfaction with Information about Medicines Scale(SIMS). And to identify patient's clinical characteristics, we reviewed electronic medical records, retrospectively.

Result
The response rate of questionnaire was 40.8%(408/1000). Of the answered patients, 263 patients were treated with tamoxifen and 145 patients were treated with aromatase inhibitors(AIs). 197 of 408 answered patients(48.3%) were classified as non-adherence. The rate of non-adherence was 132/263(50.1%) and 65/145(44.8%) in patients treated with tamoxifen and AIs. Of the all answered patients, non-adherent patients had more depressed mood (p<0.001). Non-adherent patients scored lower on positive beliefs as measured on BMQ-necessity (OR = 0.65, 95% CI 0.51 to 0.82) and higher on negative beliefs as measured on BMQ-overuse (OR=1.81, 95% CI 1.29 to 2.54). Non-adherent patients also scored lower on satisfaction with information about action and usage of anti-hormonal treatment as measured on SIMS-action and usage (OR = 0.47, 95% CI 0.38 to 0.65). Of the patients treated with tamoxifen, non-adherent patients had more depressed mood (p=0.003), scored higher on BMQ-overuse (OR=1.97, 95% CI 1.22 to 3.20) and scored lower on SIMS-action and usage (OR = 0.33, 95% CI 0.22 to 0.50). Of the patients treated with AIs, non-adherent patients had more depressed mood (p=0.014), scored lower on BMQ-necessity (OR=0.52, 95% CI 0.36 to 0.75).

Conclusion
This study showed associations between depressive mood of breast cancer patients treated with anti-hormonal therapy and adherence. And beliefs and satisfaction with information about medication also associated with adherence. To improve adherence, we should evaluate and correct patient's mood. And we should provide proper information about medications.
**Title**: Retinoic acid sensitizes triple-negative breast cancer cells to tamoxifen treatment

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**Body**: Tamoxifen, an estrogen receptor (ER) antagonist, is often used as an adjuvant endocrine therapy in the successful treatment of ER+ breast tumors. Tumors that lack ER, progesterone receptor (PR) and HER2 expression (i.e. triple-negative breast cancers) cannot be treated with adjuvant endocrine therapies, like tamoxifen, and are often more aggressive. Inducing ER expression is a potential strategy for sensitization of triple-negative breast cancers to adjuvant endocrine therapies. Given recent evidence suggesting cross-talk between the retinoic acid (RA) and estrogen signaling pathways, we investigated if RA induces expression of ER in triple-negative breast cancer cells. We hypothesize that this would lead to sensitization of the cells to tamoxifen treatment. Quantitative PCR of mRNA isolated from triple-negative MDA-MB-231 cells treated with RA and estradiol had increased ER transcript levels. Furthermore, treatment with estradiol and RA synergistically induced increased expression of RA-inducible genes. In cell proliferation studies, neither RA nor estradiol treatment alone significantly altered the growth of MDA-MB-231 cells; however, when treated with both estradiol and RA together, the growth of the cells increased significantly. This suggests that the RA-mediated increase in ER expression sensitizes MDA-MB-231 cells to estradiol-induced cell growth. Next, we investigated whether the increased ER expression sensitized MDA-MB-231 cells to tamoxifen treatment. Tamoxifen did not decrease the growth of MDA-MB-231 cells; however, when applied in combination with both estradiol and RA, tamoxifen significantly reduced MDA-MB-231 proliferation. Furthermore, tamoxifen treatment reduced the synergistic effects of estradiol/RA on RA-inducible gene expression. Together, these results suggest that the use of RA in combination with tamoxifen warrants further investigation as a potential treatment for triple-negative breast cancers. The success of the combination treatment of tamoxifen and RA in the reduction of triple-negative breast cancer cell tumor xenografts will provide further justification for this strategy in the treatment of triple-negative breast cancers.
2014 San Antonio Breast Cancer Symposium

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Title: Intermittent dosing of aromatase inhibitors (AI) to improve tolerance in post-menopausal women

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Body: Clinical rationale: A significant proportion of post-menopausal, patients treated with AI reports side-effects, especially bone pain. In such patients, the difficulties to treat pain and to clearly identify its causes may lead to treatment discontinuation. Individual cases reported an improvement in AI tolerance when dosage is reduced. The aim of this work was thus to analyse pharmacological data in order to validate this concept ie. ensuring that intermittent dosing would not result in a possibly deleterious under-dosing of AI.

Pharmacological rationale: Ageing is associated with physiological modifications that may impair drug pharmacokinetics (PKs). The elimination can be altered, with decreased drug clearance (CL), resulting in an increased exposure to the drug, reflected by increased AUCs. Drugs benefit-risk balance can thus be modified. The major sources of PKs alterations in the elderly are hepatic and/or renal impairments (HI/RI).

It has been shown that AI PKs are altered in the elderly and/or in case of HI or RI. Exemestane AUC is increased 3-fold in the elderly and in case of RI [1,2]. Letrozole AUC can double in case of HI [3] and a 42%-increase has been reported in the elderly [4]. The renal CL of anastrozole is reduced by 50% in RI, resulting in a 10% decrease in total body CL. In HI, total body CL is 30% lower as compared to normal [5].

These data demonstrate that elderly patients may be overexposed to AI when treated with the usual dosage, resulting in safety issues occurring in some patients. As a result, a dosage adjustment approach could help prevent over-exposure and reduce side-effects incidence/severity.

Proof-of-Concept studies: The reported increases in AI exposure being around 50%, an intermittent dosing schedule of 1 administration every other day could result in a similar drug exposure as compared to the usual daily schedule. AI elimination half-lives in the absence of any alteration also are consistent with this dosing schedule (24, 48, and 50 hours for exemestane, letrozole, and anastrozole, respectively). In order not to impair plasma concentrations, such a schedule is preferably suggested as compared to a half-dose daily schedule. Prospective studies are needed, in which the PKs, efficacy, and safety of this intermittent dosing schedule should be conducted.

References:
Efficacy of adjuvant tamoxifen in hormone receptor-positive premenopausal breast cancer patients according to the body mass index

Kadri Altundag¹, Mehmet AN Sendur², Sercan Aksoy¹, Taner Babacan¹ and Yavuz Ozisik². ¹Hacettepe University Cancer Institute, Ankara, Turkey and ²Yidirim University, Ankara, Turkey.

**Body:** Introduction: Obesity is an independent risk factor for the development of breast cancer and has been associated with poor breast cancer outcomes. But, this association usually depend on hormone-receptor positivity and ovarian activity. Obesity was confirmed as an adverse prognostic factor in patients treated with aromatase inhibitors, but the adverse effects in patients treated with tamoxifen was not known exactly. Thus, we aimed to examine the efficacy of adjuvant tamoxifen in hormone receptor-positive premenopausal breast cancer patients according to the body mass index (BMI).

**Material-Methods:** Newly diagnosed hormone receptor-positive breast cancer patients who were premenopausal and non-metastatic were enrolled to the study. Patients with BMI ranging between 18.5 and 24.9 kg/m² as normal weight patients (Arm A, n = 408), and patients with a BMI ranging ≥ 25 kg/m² were overweight and obese patients (Arm B, n = 418).

**Results:** The median follow-up time for this analysis was 36 (6-327) months. The median age was 39.5 (22-57) and 43 (20-56) in Arm A and Arm B, respectively (P<0.0001). The mean BMI was 22.1 ± 1.8 kg/m² and 29.2 ± 3.3 kg/m² of Arm A and Arm B, respectively (P = <0.001). In both normal weight and overweight patients, the other baseline clinico-pathologic properties and the treatment history with radiotherapy and chemotherapy were similar and not statistically significant. In overweight and obese patients the history of diabetes mellitus and hypertension was significantly higher compared to normal weight patients. In patients with normal weight patients DFS rate was 88.5% and 78.2% whereas in overweight and obese patients DFS rate was 87.2% and 70.9% in the third and fifth years respectively (Figure 1) (P = 0.43). In patients with normal weight patients OS rate was 98.5% and 93.2% whereas in overweight and obese patients OS rate was 94.6% and 87.4% in the third and fifth years respectively (Figure 2) (P = 0.02).

**Conclusion:** Our study showed that BMI have no worse effect on recurrence risk in patients treated with tamoxifen in hormone-receptor positive premenopausal breast cancer. Poor survival outcome was observed in overweight and obese patients can be due to dose limitations of chemotherapeutic agents and higher rate of comorbid diseases.
Title: Phase I study of ARN-810, a novel and potent oral selective estrogen receptor degrader, in postmenopausal women with metastatic estrogen receptor positive (ER+), HER2- breast cancer

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Body: Background: Evidence that ER can signal in both ligand-dependent and independent manner in endocrine resistant breast cancer (BC) provides rationale for therapies that are not only functional antagonists of ER but also reduce ER levels, thus targeting both modes of signaling. Furthermore, mutations in ESR1 affecting the ligand-binding domain (LBD) that drive ER-dependent transcription and proliferation in the absence of estrogen suggest that LBD-mutant forms are involved in mediating clinical resistance and next generation ER modulators with robust activity in both wild type and mutant ER tumors are needed. ARN-810 is a novel, orally bioavailable, ER antagonist that induces proteasomal ER degradation in BC cell lines at picomolar concentrations and tumor regression in tamoxifen-sensitive and resistant BC xenograft models.

Methods: ARN-810 was tested using standard 3+3 dose escalation to assess safety, PK, and Recommended Phase 2 Dose (RP2D). Key eligibility criteria included ER+ (HER2-) metastatic BC progressing ≥ 6 months (m) on endocrine therapy and ≤ 2 prior chemotherapies. Pre- and on-study tumor biopsies were obtained when feasible. Pharmacodynamics was assessed by functional imaging with [¹⁸F]-fluoroestradiol (FES)-PET, tumor-based ER/PR/Ki67 IHC, and ER target gene expression. Plasma PK was assessed following a single dose and at steady-state. Anti-tumor activity was assessed by clinical benefit rate (CBR) [complete response, partial response, or stable disease ≥ 6m] and progression-free survival (PFS).

Results: From April 2013 to June 2014, 32 patients (pts) (median age 61 (range 43 – 75); median number of prior therapies = 3 (range 1 – 7); visceral metastases 54%) were enrolled at 5 doses (100, 200, 400, 600, 800 mg) and 2 different regimens (once [QD] and twice daily) given orally with and without fasting. Increases in ARN-810 exposure were dose-dependent with no apparent food effect. At 4 weeks of treatment, complete reduction in FES uptake consistent with full receptor saturation and/or degradation was seen in 95% pts (21/22 scanned to date), including 2 pts with ESR1 mutations, suggesting ARN-810 exhibits greater ER occupancy than that recently reported for fulvestrant 500 mg (van Krutchen et al, ASCO 2014). Evidence of reduced ER levels and Ki67 staining was observed on treatment. To date, 19 pts (59.4%) remain on study with a preliminary CBR of 41%. RP2D, PFS and gene expression results will be provided at time of meeting. The most common adverse events were grades 1/2 nausea, diarrhea, fatigue, and abdominal pain. There was 1 dose limiting toxicity (grade 3 diarrhea) at 800 mg QD which led to expansion of that cohort, while in parallel, evaluation of the other dose regimens continues. No patients have discontinued the study due to toxicity.

Conclusions: ARN-810 appears to be safe and tolerable, with predictable PK, promising anti-tumor activity, and pharmacodynamic evidence of target engagement, ER degradation and reduced tumor proliferation in heavily pre-treated metastatic ER+ BC. In Phase II, ARN-810 will be studied in patients previously treated with aromatase inhibitors and fulvestrant, including those with ESR1 mutations.
**Title:** Visceral metastases from hormone receptor positive breast cancer are as sensitive to endocrine therapy as non-visceral metastases

John FR Robertson¹, Robert Paridaens², Jan Bogaerts³, Yuri Rukazenkov⁴, Christine Campbell⁵ and Ian Bradbury⁶. ¹University of Nottingham, Derby, Derbyshire, United Kingdom; ²Universitair Ziekenhuis Gasthuisberg, Leuven, Belgium; ³European Organisation for Research and Treatment of Cancer, Brussels, Belgium; ⁴AstraZeneca Pharmaceuticals, Macclesfield, United Kingdom; ⁵Frontier-Science, Grampian View, Kincraig, United Kingdom and ⁶Frontier-Science, Grampian View, Kincraig, United Kingdom.

**Body:**

**Background:** There remains a perception among many clinicians that visceral metastases (VMs) from hormone receptor positive (HR) breast cancer (BC) respond less well to endocrine therapy (ET) than non-VMs and so should receive chemotherapy as first line treatment.

**Patients & Methods:**

Four phase 3 randomised controlled trials (RCTs) of first line ET, all with tamoxifen (TAM) as their control arm) have been reviewed – Exemestane (Exe), fulvestrant 250mg (F250) and two with anastrozole (Ana); the latter, were study 27 in the Rest of the World (ROW) & study 30 in North America (NA). All reported objective response (OR), clinical benefit (CB), time to progression (TTP) / progression free survival (PFS); all have been published (1-5). Only HR positive tumors were included in this review. Data have been analysed both for Tam control arms alone and also for the four different endocrine agents combined.

**Results:**

CB & OR for TAM alone in each study individually and then combined are shown in the Table. The OR and CB rates were similar for non-VMs versus VMs in all studies except study 30 (NA) where CB rates were 59% for non-VM and 33% for VM (Test for heterogeneity of CB rates was p=0.047). For the four studies combined, the CB rates for non-VM versus VMs were 64% and 57% respectively (p=0.06) while the OR rates were 34% versus 30% respectively (p= 0.28).

When all endocrine agents were combined the OR rate between non-VMs and VMs was significantly different (p = 0.038) as was the CB rate (p = 0.0015). Rates of CB and OR in study 30 (NA) again appear different between non-VMs and VMs (data not shown).

The Median Duration of CB on Tam appear similar between non-VMs versus VMs, both for each study individually and when combined (see Table); when combined the Medians were 420 versus 418 days respectively with a Hazard Ratio (HR)=0.952 (0.748-1.211) (p=0.69). When all endocrine therapies for the combined four studies were assessed the HR for DoCB between non-VMs and VMs was 0.922 (0.779-1.093) (p=0.35).

For the TTP the HR of non-VMs versus VMs on Tam alone was 0.851 (0.715-1.011) (p=0.07) and for all endocrine therapies the HR was 0.821 (0.727-0.926) (p=0.001).

**Conclusions:**

HR+VMs which achieve clinical benefit on ET remain controlled for as long as non-VMs as shown by the DoCB results for both Tam and all endocrine therapies combined.

There was no significant difference in OR rates between VMs and non-VMs with Tamoxifen. There was when all endocrine agents were combined and the difference appears to be primarily due to one study (30 â– NA). There is no confirmed explanation for these differences.

TTP differences appear to be due primarily to the difference in initial CB rates in study 30 (see Table).

HR+ VMs have hormone sensitivity similar to non-visceral mets – they respond as well and for as long as non-VMs. In the absence of visceral crisis (ie immediately life-threatening disease) ET would appear to be the treatment of choice for VMs in the same way as it is for non-VMs.

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**Clinical Outcome by VM and Non VM (Tamoxifen only patients) N (%)**

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<table>
<thead>
<tr>
<th></th>
<th>Exe</th>
<th>Ana (EUR)</th>
<th>Ana (NA)</th>
<th>F250</th>
<th>Combined</th>
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<tr>
<td>N</td>
<td>168</td>
<td>144</td>
<td>162</td>
<td>209</td>
<td>683</td>
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<tr>
<td>Without VM (55%)</td>
<td>N = 88</td>
<td>N = 71</td>
<td>N = 82</td>
<td>N = 135</td>
<td>N = 376</td>
</tr>
<tr>
<td>CB (%)</td>
<td>66 (75)</td>
<td>42 (59)</td>
<td>47 (59)</td>
<td>87 (64)</td>
<td>242 (64)</td>
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<tr>
<td>OR (%)</td>
<td>41 (47)</td>
<td>26 (37)</td>
<td>15 (19)</td>
<td>45 (33)</td>
<td>127 (34)</td>
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<tr>
<td>TTP (Median in days)</td>
<td>287</td>
<td>254</td>
<td>305</td>
<td>234</td>
<td>277</td>
</tr>
<tr>
<td>DoCB (Median in days)</td>
<td>343</td>
<td>360</td>
<td>445</td>
<td>451</td>
<td>420</td>
</tr>
<tr>
<td>With VM (45%)</td>
<td>N = 80</td>
<td>N = 73</td>
<td>N = 80</td>
<td>N = 74</td>
<td>N = 307</td>
</tr>
<tr>
<td>CB (%)</td>
<td>64 (80)</td>
<td>38 (52)</td>
<td>27 (33)</td>
<td>46 (62)</td>
<td>175 (57)</td>
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<tr>
<td>OR (%)</td>
<td>36 (45)</td>
<td>21 (29)</td>
<td>13 (16)</td>
<td>21 (28)</td>
<td>91 (30)</td>
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<tr>
<td>TTP (Median in days)</td>
<td>340</td>
<td>230</td>
<td>152</td>
<td>281</td>
<td>238</td>
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<tr>
<td>DoCB (Median in days)</td>
<td>376</td>
<td>505</td>
<td>411</td>
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<td>418</td>
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</table>
Title: Association of PIK3CA mutation with clinical response to specific endocrine therapies in metastatic hormone receptor positive (HR+) breast cancer

Douglas S Micalizzi¹, Dejan Juric¹, Andrzej Niemierko¹, Kerry L Reynolds¹, Darrell Borger¹, Sadhna R Vora¹, Steven J Isakoff¹, Beverly Moy¹, Leif W Ellisen¹ and Aditya Bardia¹. ¹Massachusetts General Hospital, Boston, MA.

Body: Background: Phosphatidylinositol 3-kinase (PI3K) gene (PIK3CA) mutations are the most common somatic mutations in breast cancer. Preclinical models suggest that activating mutations in PIK3CA may mediate resistance to endocrine therapy in breast cancer, and multiple clinical trials testing combinations of endocrine therapy with PI3K inhibitors are ongoing. However, the role of PIK3CA in modulating the clinical response to endocrine therapies is less clear, with some studies suggesting that PIK3CA mutations are associated with improved prognosis in HR+ breast cancer. The primary objective of this study was to evaluate the association of PIK3CA mutation with clinical response to endocrine therapies in metastatic HR+ breast cancer.

Methods: We identified patients with metastatic HR+ /HER2 negative breast cancer, including ER+/PR+ (estrogen receptor/progesterone receptor) and ER+/PR-, to determine the time to progression (TTP) on first-line endocrine therapy for metastatic disease. PIK3CA mutations, including 8 common hotspot mutations, were assessed by a robust, high-throughput tumor genotyping assay (Snapshot), developed at our institution, using DNA derived from formalin-fixed, paraffin-embedded (FFPE) tissue. Actuarial analysis of TTP was performed using Cox proportional hazard method to compute Hazard Ratio (HR) and 95% Confidence Intervals (CI).

Results: Between 2009 and 2012, we identified 188 patients with HR+ metastatic breast cancer who had tumor genotyping performed. PIK3CA mutations were identified in 32.2% of tumors, including mutations in both helical (exon 9) and kinase (exon 20) domains (60% and 40%, respectively). The PIK3CA mutant and wild type patients had a similar median age at diagnosis of metastatic disease (55.8 and 56.7 years; p=0.2), ER+/PR+ tumors (75.0% vs 76.4%; p=0.8), and median TTP (8.2 versus 11.4 months; p=0.6). After adjusting for age at diagnosis and ER+/PR+ versus ER+/PR-, the TTP on first-line endocrine therapy did not vary among patients with PIK3CA mutations versus wild type (HR: 0.94; 95% CI:0.62-1.43; p=0.8), but did vary by type of endocrine therapy. Patients with PIK3CA mutations, as compared to wild type patients, had shorter TTP with fulvestrant (HR: 3.6; 95% CI:1.2-11.0; p=0.03), but not with aromatase inhibitors (AIs) (HR: 0.70; 95% CI:0.44-1.1; p=0.1), suggesting that mutant PIK3CA may specifically modulate the response to fulvestrant therapy. We did not observe any difference in TTP for exon 9 versus 20 PIK3CA mutations, though numbers were small resulting in limited statistical power.

Conclusion: Among patients with metastatic HR+ breast cancer in this study, PIK3CA mutations are associated with decreased time to progression with fulvestrant, but not with AIs, suggesting that these mutations may mediate resistance to specific endocrine therapies. Further studies are needed to confirm these findings and provide mechanistic insights to help guide optimal selection of endocrine therapy and combination with PI3K directed therapy in metastatic HR+ breast cancer.
Title: Enobosarm for the treatment of metastatic, estrogen and androgen receptor positive, breast cancer. Final results of the primary endpoint and current progression free survival

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Background: Historically, androgens have been utilized for the treatment of breast cancer (BC) as the androgen receptor (AR) is the most highly expressed steroid receptor in BC (75-95% of estrogen receptor positive (ER+) and 50% of ER negative). Reports of the use of androgens in metastatic BC (MBC) indicate that women progressing on tamoxifen have the ability to respond to synthetic androgens with overall response rates in the range of 20-60%; however, these steroidal androgens also exhibit virilizing side effects, thus limiting clinical use. A non-steroidal, tissue-selective, AR modulator (SARM), such as enobosarm, offers a targeted approach of AR activation without virilization or estrogenic effects.

Methods: This is a phase II proof of concept study examining the efficacy and safety of once daily enobosarm 9 mg in post-menopausal women with ER+ MBC who had responded to adjuvant and/or salvage endocrine therapy. Therapy is continued until patients display evidence of disease progression. The proportion of AR+ patients with clinical benefit response (CBR) at 6 months is the primary endpoint; defined as patients with a complete response (CR), partial response (PR), or stable disease (SD) as detailed in modified RECIST 1.1. AR status of metastatic disease will be correlated with CBR. Serum prostate specific antigen (PSA) will be assessed as a biomarker of AR activation by drug. Secondary endpoint is progression free survival (PFS).

Results: Patient demographics: mean age 63.7 years, mean time from diagnosis 11.0 years, 72.7% prior chemotherapy, 89% (17/19) AR+. After a median follow-up of 81 days (range 7-304 days), preliminary results of 22 patients: 9 SD as best response, median duration 212 days. Current disposition of patients: 15 PD after a median 80 days (range 15-304 days), 4 SD (1 on treatment for < 6 months), and 3 early discontinuations (days 7, 28, 255). Five patients have died due to PD off study. Among the 17 evaluable patients, 6 reached the primary endpoint (35.3%; 95% CI=16.6% to 59.4%) with increased PSA, thereby exceeding the pre-defined statistical threshold requiring that at least 3 of 14 patients with an AR+ metastatic lesion demonstrate clinical benefit. CR or PR has not been observed. Current six month Kaplan-Meier estimate of PFS is 43.8% (95% confidence interval=19.5% to 68.1%). Enobosarm is well-tolerated with common grade 1/2 toxicities of nausea (8%), menopausal symptoms (13%), pain (25%), fatigue (10%), weight change (4%); 5 (4%) grade 3 toxicities (3 unrelated to drug, 1 bone pain, 1 fatigue), and no grade 4 or higher toxicities reported. Final results of the study will be available in the fourth quarter of 2014.

Conclusions: Enobosarm demonstrates promise as a novel endocrine agent for AR+ MBC. The primary endpoint has been achieved, with 6/17 AR+ patients meeting statistical threshold for success (35% CBR at 6 months). Serum PSA appears to be a surrogate marker for AR activity associated with enobosarm administration. Based upon these favorable preliminary findings, a larger phase II study is anticipated.
Title: Role of pharmacogenetics in metastatic breast cancer (MBC) patients treated with exemestane as first-line hormonal therapy. An Italian multicentre study

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Body: Background: In a subset of MBC patients exemestane is not effective. Single Nucleotide Polymorphisms (SNPs) of genes involved in estrogen availability, in pharmacokinetics (PK) and pharmacodynamics (PD) of exemestane could be responsible for responsiveness. CYP19A1_Ex11+410A/C – rs4646 was found to correlate with improved overall survival in patients treated with letrozole(1) and anastrozole (2).

Methods: This multicentre study enrolled 423 patients with diagnosis of MBC or locally advanced-ER-positive breast cancer treated with exemestane as first-line hormonal therapy (prior adjuvant therapy and chemotherapy for metastatic setting were admitted). Objective of the study was to prospectively assess the predictive role of polymorphisms in aromatase gene, in particular CYP19A1_Ex11+410A/C – rs4646, and in genes involved in the PK and PD of exemestane on drug's activity (Response Rate - RR, time to progression - TTP). The correlation between SNPs and RR was assessed by the Fisher's exact test two-way. The CYP19A1_Ex11+410A/C – rs4646, CYP1B1*3_4326G/C (rs1056836) and other not pre-planned polymorphisms were analyzed. Moreover, in a subset of patients, PK and PD analysis were also performed.

Results: At present time we report the preliminary results on 196 patients. Among these patients, CYP19A1_Ex11+410A/C – rs4646 SNP shows no significant association with RR, whereas in patients carrying at least one variant allele of CYP1B1*3_4326G/C (rs1056836) polymorphism, a statistically significant association with a better response to exemestane was found (dominant model: OR =.436, 95% CI =.21 to .89, p = .029; Clinical benefit, dominant model: OR =.362, 95% CI =.17-0.75, p = .007, Fisher's Exact Test in accordance with the two-way). The same SNP seems, in the pharmacodynamic analysis, to be associated with the level of estrogen suppression (p =.0508 according to the Kruskal-Wallis ANOVA) and associated to better RR (p =.0287; Clinical B benefit: p =.0209 according to the Wilcoxon-Mann-Whitney test), providing a basis for phenotypic association. The overall results on the entire cohort of patients enrolled are under evaluation as well as the data concerning TTP, toxicity and the correlation with PR, HER2 and Ki67 tumor tissue expression.

Conclusions: A predictive role of the CYP1B1*3_4326G/C (rs1056836) polymorphism for RR to exemestane as first-line therapy has been found. Our study suggests that a simple genetic evaluation from peripheral blood, performed prior to therapy, may allow the identification of the patients who are more likely to be responsive to exemestane.
**Title:** Impact of visceral metastases on treatment patterns for hormone-receptor-positive (HR+)/human epidermal growth factor receptor-2-negative (HER2-) metastatic breast cancer (mBC)

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**Body:** Introduction:
Guidelines recommend reserving chemotherapy (CT) for HR+/HER2- mBC until either endocrine-based therapy (ET) is no longer effective or for patients with visceral crisis or need for rapid tumor control. The extent to which visceral metastases (VM), regardless of whether they are contributing to visceral crisis, impact real-world treatment patterns in mBC is not well understood. This retrospective analysis characterizes treatment patterns with ET and CT among HR+/HER2- mBC patients stratified by presence of VM.

Methods:
Postmenopausal women (age ≥50 years) diagnosed with HR+/HER2- mBC were identified from the MarketScan® database (2002Q3-2012Q2). Patients who initiated ET without CT for mBC were followed until transition to CT, discontinuation of ET (>90 days without evidence of ET), or end of data or insurance eligibility. Upon initiation of each line of therapy, patient characteristics including age, number of metastatic sites, and presence of VM were compared between those receiving ET and those receiving CT. The number of lines of ET received before CT, and the total duration of ET were described for patients with and without VM.

Results:
Of the 19,120 patients who initiated treatment for mBC, 5,418 received 2nd-line treatment and 1,471 received 3rd-line treatment. In each of these 3 lines, the corresponding numbers of patients receiving CT were 7,575 (40%), 2,397 (44%) and 650 (44%). Among the patients who received CT in each line, the majority did so without evidence of VM: 5,821 (77%), 1,511 (63%) and 435 (67%), in the 1st, 2nd and 3rd lines, respectively. Within each line, the average patient receiving CT was approximately 3 to 5 years younger and had 0.02-0.29 more metastatic sites, compared with the average patient receiving ET. Among the 11,545 patients who initiated 1st-line ET, 9,315 (81%) did not have evidence of VM. Patients with versus without VM upon initiation of 1st-line ET received similar average numbers of lines of ET (1.32 versus 1.37 lines). However, the median time prior to transition to CT, discontinuation of ET, or loss to follow-up was shorter for patients with VM compared to those without VM (5 months versus 10 months). In addition, patients with VM appeared to have shorter durations of ET at each line compared with patients without VM (Table).

Conclusions:
Although the present analysis could not distinguish visceral crisis from the broader group of patients with visceral metastases, it is notable that a large majority of patients receiving CT did so without evidence of VM. This indicates an unmet need during the study period (2002-2012) for effective disease control among patients without VM for whom CT was not a preferred option.

### Table. Durations (months) of Endocrine Therapy

<table>
<thead>
<tr>
<th>Line of ET / patient group</th>
<th>One Line</th>
<th>Two lines</th>
<th>≥ Three lines</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>VM</td>
<td>Without VM</td>
<td>VM</td>
</tr>
<tr>
<td>All Patients, median (IQR)</td>
<td>3 (1-8)</td>
<td>6 (2-16)</td>
<td>11 (6-23)</td>
</tr>
<tr>
<td>First line</td>
<td>3 (1-8)</td>
<td>6 (2-16)</td>
<td>6 (3-15)</td>
</tr>
<tr>
<td>Second line</td>
<td>-</td>
<td>-</td>
<td>3 (1-6)</td>
</tr>
<tr>
<td>Third line</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

VM = visceral metastases, IQR = interquartile range
A phase III study of fulvestrant 500 mg versus 250 mg in postmenopausal Chinese women with advanced breast cancer and disease progression following failure on prior antiestrogen or aromatase inhibitor therapy: Supporting superior clinical benefit for the 500 mg dose

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Body: Background: In the international Phase III COmparisoN of Faslodex In Recurrent or Metastatic breast cancer (CONFIRM) study, fulvestrant 500 mg was associated with significantly longer progression-free survival (PFS) over the 250 mg dose (hazard ratio [HR] 0.80; 95% confidence interval [CI] 0.68, 0.94; p=0.006) in postmenopausal women with advanced breast cancer (ABC) following failure on prior endocrine therapy. There were no clinically meaningful differences between the treatment groups in terms of the incidence or severity of adverse events. The present study was designed to compare the efficacy and safety of fulvestrant 500 mg versus 250 mg in a Chinese population for registration purposes.

Methods: This was a Phase III randomized, double-blind study in a Chinese population (ClinicalTrials.gov: NCT01300351). Postmenopausal women with estrogen receptor positive (ER+) ABC following failure on prior endocrine (antiestrogen [AO] or aromatase inhibitor [AI]) therapy were randomized 1:1 to fulvestrant 500 mg or 250 mg. Patients (pts) were stratified by post-AO/post-AI status and enrollment of post-AI pts was capped at 45%. Primary study endpoint was PFS. Consistency with the global CONFIRM study was to be concluded if the HR for the treatment comparison of PFS was <1 (full analysis set; stratified log-rank test); the study was not powered to detect significant differences between the treatment groups. Secondary endpoints included pharmacokinetics, ORR, CBR, DoR, DoCB, safety and tolerability.

Results: 221 pts were randomized to fulvestrant 500 mg (n=111) or fulvestrant 250 mg (n=110). 121 pts were in the post-AO subgroup and 100 pts were in the post-AI subgroup. Demographic and baseline characteristics were balanced between fulvestrant 500 mg and fulvestrant 250 mg and comparable with those in the global CONFIRM study. 98% (119/121) in the post-AO subgroup and 92% (92/100) in the post-AI subgroup had adjuvant endocrine therapy, while only 12% (14/121) in the post-AO subgroup and 51% (51/100) in the post-AI subgroup used salvage endocrine therapy. At the time of the primary analysis, 152 progression events (69%) had occurred (post-AO 59% [71/121]; post-AI 81% [81/100]). Median PFS was 8.0 months (m) in the fulvestrant 500 mg group vs 4.0 m in the 250 mg group (HR 0.75; 95% CI 0.54, 1.03; p=0.078); the predefined criterion for consistency with the global CONFIRM study was met. In a predefined subgroup analysis of PFS, the HR for fulvestrant 500 mg vs 250 mg was <1 in both post-AO (median PFS 8.1 m vs 5.6 m; HR 0.86; 95% CI 0.54, 1.37) and post-AI (median PFS 5.8 m vs 2.9 m; HR 0.65; 95% CI 0.42, 1.03) subgroups. Secondary endpoints favored fulvestrant 500 mg over 250 mg, with the exception of median DoR. Safety and tolerability profiles were consistent with the known safety profile of fulvestrant.

Conclusions: Data from the present study support the superior clinical benefit of fulvestrant 500 mg vs 250 mg demonstrated in the global CONFIRM study, in postmenopausal Chinese women with ER+ ABC. Hazard ratios favoring fulvestrant 500 mg were observed in both the post-AO and post-AI settings.
Body: **Background:**
Guidelines recommend maximizing the duration of effective treatment that can be achieved without chemotherapy for HR+/HER2- mBC, with the goal of avoiding toxicity and preserving quality of life. A target of three lines of ET is suggested, with CT reserved for patients requiring rapid control of symptomatic disease or who no longer benefit from ET. This study describes treatment patterns for HR+/HER2- mBC over the past decade.

**Methods:**
Postmenopausal women (age \(\geq 50\)) with HR+/HER2- mBC were identified from the MarketScan® databases (2002Q3-2012Q2). Patients whose first treatment after mBC diagnosis included ET without CT were followed until first use of CT, discontinuation of ET (>90 days without evidence of ET), or end of data or insurance eligibility. The distribution of agents used prior to CT was assessed for up to 3 lines of therapy. Median treatment durations were calculated by agent and by line among patients who received at least 3 lines of treatment that included ET without CT.

**Results:**
Of 19,120 HR+/HER2- mBC patients, 11,545 (60%) initiated a 1st-line therapy for mBC that included ET without CT. The remaining 40% initiated with CT. Of the 11,545 patients who initiated ET, the majority received the non-steroidal aromatase inhibitors (NSAIs) anastrozole (38%) and letrozole (27%) in the 1st-line. Among these patients, 3,021 (26%) patients received 2nd-line ET without CT, with the majority receiving exemestane (20%), anastrozole (18%), letrozole (18%) or fulvestrant (18%). A total of 821 patients, comprising 7% of the patients who received 1st-line ET, received at least 3 consecutive lines of ET without CT. The majority of these patients received exemestane (20%), tamoxifen (19%) or fulvestrant (19%) in the 3rd line. Among these 821 patients, the median treatment durations were 9, 6, and 3 months in the 1st, 2nd, and 3rd lines, respectively. Median durations for each ET agent were generally lower in later versus earlier treatment lines (Table). At the time of data cutoff for this study, limited data were available for everolimus treatment (n=21).

**Conclusions:**
The large majority of patients did not receive the guideline-recommended target of 3 lines of ET. Additionally, the shorter durations of therapy observed within each subsequent line of ET suggest that there may be diminishing returns for sequential use of ET monotherapies for HR+/HER2- mBC. These findings indicate an unmet need for more effective alternatives to available ET monotherapies after the initial ET discontinuation during the study period (2002-2012).

**Table. ET treatment duration (months) among the 821 patients who received at 3 three consecutive lines of ET without CT**

<table>
<thead>
<tr>
<th>Treatments/line of ET*</th>
<th>Line 1</th>
<th>Line 2</th>
<th>Line 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>Median</td>
<td>N (%)</td>
</tr>
<tr>
<td>Anastrozole</td>
<td>339 (41.3)</td>
<td>9</td>
<td>135 (16.4)</td>
</tr>
<tr>
<td>Letrozole</td>
<td>243 (29.6)</td>
<td>10</td>
<td>165 (20.1)</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>114 (13.9)</td>
<td>9</td>
<td>140 (17.1)</td>
</tr>
<tr>
<td>Exemestane</td>
<td>42 (5.1)</td>
<td>7</td>
<td>186 (22.7)</td>
</tr>
<tr>
<td>Fulvestrant</td>
<td>37 (4.5)</td>
<td>7</td>
<td>98 (11.9)</td>
</tr>
<tr>
<td>Megestrol</td>
<td>23 (2.8)</td>
<td>1</td>
<td>80 (9.7)</td>
</tr>
</tbody>
</table>

*Treatment duration of other ETs (i.e., toremefene, raloxifene and combination therapy) with less than 2% of users are not reported.
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Average Grade: 5.40

Title: First report of clinicopathological analysis in neoadjuvant treatment phase in NEOS: A randomized study of adjuvant endocrine therapy with or without chemotherapy for postmenopausal breast cancer patients who responded to neoadjuvant letrozole

Takehiko Sakai, Hiroji Iwata, Yoshiie Hasegawa, Rikiya Nakamura, Hiromitsu Akabane, Shoichiro Ohtani, Masahiro Kashiwaba, Naruto Taira, Tatsuya Toyama, Yutaka Yamamoto, Tomomi Fujisawa, Norikazu Masuda, Takuhiro Yamaguchi, Hirofumi Mukai and Yasuo Ohashi.

Body: Background: Whether adjuvant chemotherapy is required for patients with intermediate-risk endocrine-responsive postmenopausal breast cancer remains unknown. The New primary Endocrine-therapy Origination Study (NEOS: N-SAS BC06 study: UMIN 000001090) was a randomized controlled trial to verify the necessity of adjuvant chemotherapy in node-, ER+, and HER2- postmenopausal breast cancer patients who responded to neoadjuvant endocrine therapy. The primary registration and primary treatment have finished. This report evaluated clinical responses and radiological findings in the neoadjuvant LET treatment phase of 6 months.

Methods: Patients meeting eligibility criteria received LET preoperatively in weeks 24-28 after primary enrollment. Patients evaluated as complete response (CR), partial response (PR) or stable disease (SD) by each investigators underwent secondary enrollment and will be divided at random into two arms, an arm given LET for 4.5-5 years after chemotherapy and another arm given only LET for 4.5-5 years. Patients evaluated as progressive disease during LET treatment will receive discretionary treatment. The primary endpoint was disease-free survival and secondary endpoints were overall survival, clinical response rate in neoadjuvant treatment phase, pathological response, breast-conserving surgery rate, DFS/OS in subgroups according to clinical response, safety, HRQOL, and cost-effectiveness.

Results: Between May 2008 and June 2013, 905 patients entered primary registration. We excluded 42 patients without confirmed data. The 863 included patients' characteristics at baseline are: median age: 63 years old, median Body Mass Index (BMI): 23.90, T1c: 37%, T2: 63%, and PgR+: 79%, and 74% of patients had planned breast-conserving surgery (BCS). The clinical responses were evaluated with calipers, ultrasound and MRI (or CT) at the baseline and end of treatment before surgery. Clinical response rates were 2, 48, 46 and 4% in CR, PR, SD and PD, respectively. Excluding those who could not enter secondary registration according to the protocol, 83 (56.1%) of 148 patients with planned total mastectomy were converted to BCS according to final radiological evaluations before surgery. The correlation between the tumor size on MRI (r=0.87) after neoadjuvant LET and the pathological invasive tumor size was stronger than with other modalities (r=0.68, 0.57, 0.33 by CT, US and calipers, respectively). On univariate analysis, a small tumor size (T1c rather than T2), PgR+, HER2:2+ and a high BMI at the baseline were significantly correlated with the clinical response. On multivariate analysis, PgR+ (HR: 1.13, 95%CI: 1.01-1.27, p=0.032) and a small tumor size (HR: 1.11, 95%CI: 1.03-1.17, p=0.003) were significant independent predictors of the clinical response. Conclusion: This is the first clinical report of neoadjuvant hormone therapy for early breast cancer. Neoadjuvant LET therapy improved BCS rates. MRI was useful for predicting the residual pathological invasive tumor size. PgR+ and a small tumor size at the baseline were significant independent predictors of the clinical response.
Title: Neoadjuvant letrozole and lapatinib is feasible in Asian postmenopausal women with estrogen receptor (ER) and human epidermal growth factor receptor-2 (HER-2) positive breast cancer [Neo-All-In]: First efficacy and safety report

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Body: Purpose Neo-ALL-In (NCT 01275859) is a single center, prospective study aimed to evaluate the recruitment feasibility, efficacy and safety profiles as well as biologic features of neoadjuvant letrozole plus lapatinib in postmenopausal women with ER and HER2 positive breast cancer.

Methods Postmenopausal women with stage IIA to IIIB ER and HER-2 positive breast cancer were eligible. Patients received combination therapy of letrozole 2.5 mg orally daily plus lapatinib 1,500 mg orally daily for 18-21 weeks before surgery. Clinical responses were assessed by clinical palpation, ultrasonography (US), mammography and/or MRI. Tissue and/or blood samples were collected for analysis of biomarkers at three time points (baseline, day 15, and before surgery). Baseline Fluorine-18 Fluorodeoxyglucose (18F-FDG) and Fluorine-18 Fluoroestradiol (18F-FES) PET-CT imagings were obtained.

Results Among twenty-four patients enrolled, 22 patients underwent surgery while 1 patient is currently on neoadjuvant therapy and the other patient is waiting for surgery. Among 22 patients completed surgery, 16 patients (72.7%) completed planned neoadjuvant letrozole and lapatinib, whereas 3 patients (13.6%) prematurely terminated the treatment and proceeded to surgery due to minimal clinical response or progression. Except grade 3 liver toxicities revealed in 3 patients (13.6%), which resulted in sequential dose reduction and discontinuation, adverse events were mainly grades 1 to 2 (Skin, 83.3%; GI, 77.3%), and these were generally tolerable with excellent compliance. Overall clinical response rates including complete and partial response was 72.7% (n=16), and pathologic complete response in breast (pCR; ypT0-is) was 4.5% (n=1).

Clinical and pathologic responses of 22 patients by assessment modalities

<table>
<thead>
<tr>
<th>Clinical response</th>
<th>Total (N=22, %)</th>
<th>Clinical palpation</th>
<th>US</th>
<th>Mammography</th>
<th>MR</th>
<th>pathologic response in breast (ypT0-is)</th>
<th>pathologic response in breast and lymph nodes (ypT0-is N0)</th>
<th>Overall response</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR</td>
<td>1 (4.5)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>1 (4.5)</td>
<td>1 (4.5)</td>
<td>0 (0.0)</td>
<td>1 (4.5)</td>
<td></td>
</tr>
<tr>
<td>PR</td>
<td>17 (77.3)</td>
<td>12 (54.5)</td>
<td>9 (40.9)</td>
<td>9 (40.9)</td>
<td>11 (50.0)</td>
<td>15 (68.2)</td>
<td>15 (68.2)</td>
<td></td>
</tr>
<tr>
<td>No change</td>
<td>3 (13.6)</td>
<td>9 (40.9)</td>
<td>13 (59.1)</td>
<td>5 (22.7)</td>
<td>9 (40.9)</td>
<td>7 (31.8)</td>
<td>4 (18.2)</td>
<td></td>
</tr>
<tr>
<td>PD</td>
<td>1 (4.5)</td>
<td>1 (4.5)</td>
<td>1 (4.5)</td>
<td>0 (0.0)</td>
<td>1 (4.5)</td>
<td>0 (0.0)</td>
<td>2 (9.1)</td>
<td></td>
</tr>
<tr>
<td>Not available/evaluable</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>6 (27.3)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td></td>
</tr>
</tbody>
</table>

CR, complete response; PR, partial response; PD, progressive disease; US, ultrasonography

In analyses of biomarkers thus far, 81.8% of patients showed stationary expression of HER-2, 54.5% of patients showed decrease in Ki-67 expression, and 27.3% of patients showed increase in ER expression from baseline to surgery by immunohistochemistry (IHC) staining. Decreased expression of ER after surgery by IHC staining was significantly correlated with poor clinical response (p=0.004). However, no significant differences in baseline SUVmax in FDG-PET were found between responders and non-responders (8.8 VS 10.7, p=0.53).
Conclusion This chemo-free combination neoadjuvant therapy was feasible, with comparable efficacy outcomes and manageable toxicities profiles. Updated data on $^{18}$F-FES PET-CT and biomarkers will be provided.
Title: Association between ischemic cardiac events and targeting of the internal mammary nodal region with adjuvant radiation for breast cancer

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Body: Background: Cardiac toxicity has been well documented following adjuvant breast cancer radiation therapy (RT) using outdated treatment methods. Limited information exists, however, regarding cardiac outcomes following contemporary RT fields and techniques. Inclusion of the internal mammary nodal (IMN) field for appropriately selected patients with breast cancer coincided with our department’s transition to computed-tomography (CT) based planning. The objectives of this study, therefore, were to assess the risk of ischemic cardiac events following IMN irradiation and to assess the risks following CT-based versus two-dimensional (2D) planning.

Methods: All patients treated with adjuvant RT for breast cancer and without history of additional chest RT from January 1, 1984 – December 31, 2007 in our department were assessed. CT planning for breast cancer began for select patients in 1997 and was used for all patients as of 2001. The inclusion of the IMN region was determined by review of our clinical database. Ischemic cardiac endpoints were defined as myocardial infarction, coronary artery bypass grafting procedure, angioplasty/stent placement, and/or diagnosis of coronary artery disease. A text-based and diagnosis code-based search was used to flag possible endpoints which were then confirmed by manual chart review. Hypertension (HTN), diabetes (DM), hyperlipidemia (HLD), and anthracycline use were identified by a diagnosis code-based search.

Results: We identified 2,126 patients who received adjuvant RT. Median follow-up was 9.6 years. 311 (14.6%) patients had IMNs targeted and 1,813 (85.3%) did not (data not available for 2 patients). RT to the IMNs was not associated with a higher risk of ischemic cardiac events (HR: 0.88, P = 0.731). 1,072 (50.4%) patients had CT planning, 1,003 (47.2%) had 2D planning, and no information was available for 51 (2.4%) patients. Overall, there were 56 (5.6%) ischemic events in the 2D cohort and 27 (2.5%) in the CT cohort. After truncating follow-up to 10 years to account for differential potential follow-up, there were 28 (2.8%) and 23 (2.2%) ischemic cardiac events, respectively. The table lists the association of patient and treatment characteristics and risk of ischemic cardiac events. HTN, HLD, and DM were each associated with a significantly increased risk of ischemic cardiac events.

Conclusions: After reviewing all patients treated in our department for breast cancer from 1984 – 2007, we were unable to find an association between IMN irradiation and ischemic cardiac events. CT-based planning has been shown to allow accurate targeting of the IMNs. These data suggest that even with inclusion of the IMNs, CT-based planning minimizes dose to the heart and coronary arteries thereby decreasing the risk of ischemic cardiac toxicity. These results will be followed with time.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hazard Ratio</th>
<th>95% Confidence Interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RT to the IMNs</td>
<td>0.88</td>
<td>0.42-1.83</td>
<td>0.731</td>
</tr>
<tr>
<td>2D vs. CT planning</td>
<td>1.14</td>
<td>0.68-1.91</td>
<td>0.613</td>
</tr>
<tr>
<td>Left vs. right</td>
<td>1.21</td>
<td>0.79-1.85</td>
<td>0.372</td>
</tr>
<tr>
<td>Anthracycline use</td>
<td>0.75</td>
<td>0.44-1.26</td>
<td>0.270</td>
</tr>
<tr>
<td>HTN</td>
<td>5.61</td>
<td>2.06-15.30</td>
<td>0.001</td>
</tr>
<tr>
<td>HLD</td>
<td>1.90</td>
<td>1.25-2.90</td>
<td>0.003</td>
</tr>
<tr>
<td>DM</td>
<td>3.11</td>
<td>1.99-4.86</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Title: Utilization of hypofractionated radiation therapy for early stage breast cancer in women over 50 years of age

Malolan S Rajagopalan¹, Craig Lehockey¹, John C Flickinger¹, Dwight E Heron¹, Paniti Sukumvanich², Joseph L Kelley², Gretchen M Ahrendt³ and Sushil Beriwal¹. ¹University of Pittsburgh Cancer Institute, Pittsburgh, PA; ²University of Pittsburgh Cancer Institute, Pittsburgh, PA and ³University of Pittsburgh Cancer Institute, Pittsburgh, PA.

Body: Purpose: Hypofractionated whole breast irradiation (HF-WBI) following breast conserving surgery for early stage breast cancer is now recommended for women over 50 years of age by the American Society for Radiation Oncology (ASTRO). Herein, we explored the rate of HF-WBI utilization and its associated patterns of care using the National Cancer Data Base (NCDB), a comprehensive oncology outcomes database which captures 70% of all newly diagnosed cancer patients in the US.

Methods: We utilized NCDB to identify women aged ≥50 diagnosed with T1N0/T1Nx invasive breast cancer or Tis (DCIS) who underwent breast conserving surgery and adjuvant whole breast external beam radiation therapy. HF-WBI was defined when a definitive radiation dose (≥40 Gy) was delivered in ≤23 fractions. We evaluated factors associated with the use of HF-WBI through exploratory univariate and multivariable analyses.

Results: A total of 311,071 patients from 1998-2011 met inclusion criteria. The commonest HF-WBI regimen was 42.56 Gy in 16 fractions. The rate of HF-WBI utilization increased over time from 0.4% in 1998 to 16.5% in 2011 (p<0.001). HF-WBI was delivered more often for women of advancing age. In 2011, HF-WBI was delivered in 10.0%, 14.2%, 24.2% and 35.6% of women in their 50’s, 60’s, 70’s and 80+ (p<0.001). Multivariable analysis correlated significantly increased use of HF-WBI with (in order of association): later year of diagnosis, advancing age (decade), treatment in academic center, regional location in US, lower grade of disease, white race, residence in a higher income area (p<0.001), greater comorbidity score (p<0.03), presence of invasive cancer (p<0.01), right-sided disease (p<0.01), and greater distance from reporting facility (p<0.001).

Factors Associated with Increased Use of HF-WBI

<table>
<thead>
<tr>
<th>Association with HF-WBI</th>
<th>Odds Ratio of HF-WBI Use (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Year of Diagnosis (2011 vs. prior to 2009)</td>
<td>7.14 (6.85-7.52)</td>
</tr>
<tr>
<td>Age (Decade, 80+ years)</td>
<td>6.45 (6.06-6.90)</td>
</tr>
<tr>
<td>Facility Type: Academic</td>
<td>4.00 (3.69-4.33)</td>
</tr>
<tr>
<td>Location (Mountain Region)</td>
<td>1.82 (1.68-1.97)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>1.40 (1.33-1.47)</td>
</tr>
<tr>
<td>Race: White</td>
<td>1.38 (1.25-1.53)</td>
</tr>
<tr>
<td>Income Quartile 4 (&gt;-$46K/year)</td>
<td>1.30 (1.21-1.40)</td>
</tr>
<tr>
<td>Comorbidity Index (High, 2)</td>
<td>1.15 (1.02-1.31)</td>
</tr>
<tr>
<td>Invasive Cancer</td>
<td>1.09 (1.04-1.14)</td>
</tr>
<tr>
<td>Laterality (Right Side)</td>
<td>1.05 (1.02-1.09)</td>
</tr>
<tr>
<td>Distance from Treatment Facility</td>
<td>1.002 per mile (1.002-1.002)</td>
</tr>
</tbody>
</table>

Conclusions: This comprehensive patterns of care study of HF-WBI in women ≥50 years old with early stage breast cancer from a national database identified a substantial increase in the utilization of this technique with time. Advancing patient age and treatment at academic facility were also strong predictors of delivery of HF-WBI. Disparities in utilization of HF-WBI and overall employment of this technique, even in later years, remain suboptimal. Strategies to identify and break barriers to the adoption of HF-WBI should be explored.
Title: Long term outcomes in patients with phyllodes tumor of the breast: The UT MD Anderson experience

Bobbi A Porche¹, Pamela K Allen², Simona Shaitelman², B Ashleigh Guadagnolo², Wendy A Woodward², Constance Albarracin², Abenaa M Brewster², Kelly K Hunt² and Welela Tereffe². ¹UT Medical School at Houston, Houston, TX and ²MD Anderson Cancer Center, Houston, TX.

Body: Purpose: Phyllodes tumor is a relatively rare disease of the breast, and the risk factors for local versus distant failure are unclear. The goal of this study was to identify factors influencing local, distant, and cause-specific survival outcomes in patients with phyllodes tumors, and to assess the impact of local radiotherapy (RT).

Methods: We retrospectively reviewed the records of 387 patients recorded in the MD Anderson tumor registry as having "cystosarcoma phyllodes" or "phyllodes". After excluding patients with recurrent disease at presentation to the institution, concurrent invasive ductal carcinoma, absence of confirmation of phyllodes tumor, or inadequate follow up after surgery, 229 patients diagnosed from 1964-2011 were evaluable. The median tumor size was 4 cm (range, 0.7-28 cm); histology was benign in 29%, indeterminate in 7%, and malignant in 41% (23% unknown). Stromal overgrowth was present in 18% (n=40). Local therapy consisted of breast-conserving surgery (n=184) or mastectomy (n=43); 15% (n=34) also received local RT. Chemotherapy was administered in 9% (n=21). We used Kaplan-Meier analyses and Cox proportional hazards models to estimate the associations between patient/tumor characteristics and treatment on local control (LC), distant metastasis-free survival (DMFS), and cause specific survival (CSS).

Results: At a median follow-up of 76 months (range, 1-485 months), the actuarial 5-yr LC for the entire cohort was 75%; 5-yr DMFS was 78%; and 5-yr CSS was 86%. Factors influencing LC included receipt of RT (5-yr LC 97% versus 72%, p=.005) and age over 50 (85% versus 72%, p=.044). Neither malignant histology nor stromal overgrowth increased the risk of local failure. Factors influencing DMFS included malignant histology (5-yr DMFS 65% versus 91%, p=.001) and stromal overgrowth (50% versus 84%, p<.001). Factors influencing CSS included malignant histology (5-yr CSS 77% versus 100%, p=.002) and stromal overgrowth (58% versus 92%, p<.001). Patients with stromal overgrowth or malignant histology were much more likely to receive RT (p<.001 for both factors). RT improved 5-yr LC for both breast conserved and mastectomy patients, but did not improve DMFS or CSS. In multivariable models, the following associations were noted (HR=hazard ratio; CI= 95% confidence interval): LC – RT (HR 0.13, CI .03-.55); DMFS – malignant histology (HR 2.99, CI 1.41-6.34); CSS – malignant histology (HR 4.18, CI 1.43-12.24).

Conclusions: Adjuvant RT after surgery improves local control in phyllodes tumor; however, it does not improve DMFS or CSS. Stromal overgrowth and malignant histology are associated with worse DMFS and CSS, but do not impact local control. Therefore malignant histology and stromal overgrowth should not be deciding factors in the use of local RT, as escalated local therapy in patients with these tumor characteristics does not improve DMFS or CSS. Other strategies should be considered for patients with phyllodes tumor who are at high risk of distant failure, including systemic therapy.
The adoption of hypofractionated whole breast irradiation for early-stage breast cancer: A national cancer data base analysis

Elyn H Wang¹, Sarah S Mougalian², Pamela R Soulos³, Charles E Rutter⁴, Suzanne B Evans⁴, Bruce G Haffty⁵, Cary P Gross⁶ and James B Yu⁴. ¹Yale University School of Medicine, New Haven, CT; ²New Haven, CT; ³Cancer Outcomes, Public Policy, and Effectiveness Research Center at Yale, New Haven, CT; ⁴Department of Therapeutic Radiology, New Haven, CT; ⁵Rutgers Cancer Institute of New Jersey and Robert Wood Johnson Medical School, New Brunswick, NJ and ⁶Department of Internal Medicine, New Haven, CT.

Body: Purpose
Hypofractionated whole breast radiation therapy (hypofractionation) for early-stage breast cancer is a treatment innovation that is both supported by high quality randomized trial evidence and clinical guidelines, and is more cost effective and convenient than conventional fractionation. However, whether hypofractionation has been adopted nationally, and what factors are related to its adoption, are unknown.

Methods
We performed a retrospective study of breast cancer patients in the National Cancer Data Base from 2004-2011 who were treated with radiation therapy and met eligibility criteria for hypofractionation. We used logistic regression to identify factors associated with receipt of hypofractionation (vs. conventional fractionation).

Results
We identified 13,271 (11.7%) and 99,996 (88.3%) women with early-stage breast cancer who were treated with hypofractionation and conventional fractionation, respectively. The use of hypofractionation increased significantly, with 5.4% of patients receiving it in 2004 compared with 22.8% in 2011 (P<0.001 for trend). Patients living >50 miles from the cancer reporting facility had increased odds of receiving hypofractionation (OR 1.57 [95% CI 1.44-1.72], p<.001).

Adoption of hypofractionation was associated with treatment at an academic center (p<0.001) and living in an area with high median income (p<0.001). Hypofractionation was less likely to be used in patients with high risk disease, such as increased tumor size (p<0.001) or poorly differentiated histologic grade (p<0.001).

Adjusted odds ratios for receipt of conventional fractionation vs hypofractionation

<table>
<thead>
<tr>
<th>Feature (Reference)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Facility Type (Academic)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Community</td>
<td>0.38 (0.35-0.42)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Comprehensive Community</td>
<td>0.51 (0.48-0.53)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other</td>
<td>0.64 (0.52-0.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Facility Location (South)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>0.93 (0.88-0.99)</td>
<td>0.02</td>
</tr>
<tr>
<td>Midwest</td>
<td>1.04 (1.00-1.11)</td>
<td>0.07</td>
</tr>
<tr>
<td>West</td>
<td>1.69 (1.59-1.78)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Age in yrs (50-59)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60-69</td>
<td>1.22 (1.16-1.29)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>70-79</td>
<td>1.77 (1.66-1.89)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;=80</td>
<td>3.12 (2.88-3.38)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Race (White)</td>
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<tr>
<td>Black</td>
<td>0.96 (0.89-1.05)</td>
<td>0.41</td>
</tr>
<tr>
<td>Category</td>
<td>Odds Ratio (95% CI)</td>
<td>P value</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>-------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>White Hispanic</td>
<td>1.23 (1.10-1.36)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Other</td>
<td>1.23 (1.13-1.34)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Primary Payor (Private insurance)</td>
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<tr>
<td>No insurance</td>
<td>1.16 (0.96-1.41)</td>
<td>0.11</td>
</tr>
<tr>
<td>Medicaid</td>
<td>0.97 (0.86-1.10)</td>
<td>0.71</td>
</tr>
<tr>
<td>Medicare</td>
<td>1.05 (1.00-1.11)</td>
<td>0.05</td>
</tr>
<tr>
<td>Other government</td>
<td>0.81 (0.63-1.05)</td>
<td>0.12</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.22 (1.03-1.44)</td>
<td>0.02</td>
</tr>
<tr>
<td>Median Income Quartile (&lt;$30 000)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30 000 - 35 000</td>
<td>0.95 (0.87-1.04)</td>
<td>0.30</td>
</tr>
<tr>
<td>35 000 - 45 999</td>
<td>0.97 (0.90-1.06)</td>
<td>0.54</td>
</tr>
<tr>
<td>&gt;= 46 000</td>
<td>1.25 (1.16-1.35)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.04 (0.86-1.25)</td>
<td>0.70</td>
</tr>
<tr>
<td>Urbanization (Metro)</td>
<td></td>
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<tr>
<td>Urban</td>
<td>0.88 (0.83-0.95)</td>
<td>0.001</td>
</tr>
<tr>
<td>Rural</td>
<td>1.10 (0.99-1.22)</td>
<td>0.07</td>
</tr>
<tr>
<td>Distance from reporting facility (&lt;50 miles)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;=50 miles</td>
<td>1.57 (1.44-1.72)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Unknown</td>
<td>1.13 (0.91-1.40)</td>
<td>0.27</td>
</tr>
<tr>
<td>Year of Diagnosis (2004)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2005</td>
<td>1.03 (0.92-1.14)</td>
<td>0.65</td>
</tr>
<tr>
<td>2006</td>
<td>1.11 (1.00-1.24)</td>
<td>0.05</td>
</tr>
<tr>
<td>2007</td>
<td>1.31 (1.18-1.45)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2008</td>
<td>1.99 (1.80-2.19)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2009</td>
<td>2.88 (2.62-3.15)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2010</td>
<td>4.10 (3.62-4.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>2011</td>
<td>5.48 (4.83-6.21)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Grade (Well-differentiated)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intermediate</td>
<td>0.94 (0.89-0.97)</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Poor</td>
<td>0.86 (0.80-0.91)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>1.03 (0.5-1.63)</td>
<td>0.89</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.90 (0.82-0.99)</td>
<td>0.04</td>
</tr>
<tr>
<td>Tumor Size (&lt;10 mm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-29 mm</td>
<td>0.90 (0.87-0.94)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>30-49 mm</td>
<td>0.71 (0.62-0.80)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>&gt;=50 mm</td>
<td>0.94 (0.67-1.32)</td>
<td>0.76</td>
</tr>
<tr>
<td>Unknown</td>
<td>0.63 (0.42-0.95)</td>
<td>0.03</td>
</tr>
</tbody>
</table>

Conclusions
The use of hypofractionation is rising and is associated with increased travel distance and treatment at an academic center. Further adoption of hypofractionation may be tempered by both clinical and non-clinical concerns.
Title: Long-term outcome of electron intraoperative boost and hypofractionated external beam radiotherapy after breast-conserving surgery in premenopausal women

Maria Cristina Leonardi¹, Anna Morra¹, Cristiana Fodor¹, Luigi Santoro¹, Samantha Dicuonzo¹, Roberta Lazzari¹, Veronica Dell'acqua¹, Elena Rondi¹, Federica Cattani¹, Floriana Pansini¹, Alberto Luini¹ and Roberto Orecchia¹². ¹European Institute of Oncology, Milan, Italy and ²University of Milan, Milan, Italy.

Body: Purpose: To report long-term clinical results on breast cancer patients treated with an electron intraoperative boost followed by hypofractionated external beam radiotherapy (HEBRT) to the whole breast inside a clinical protocol.

Materials and Methods: Between June 2004 and December 2008, 357 premenopausal women with a diagnosis of early-stage breast cancer were given breast conservative surgery (quadrantectomy and sentinel node biopsy +/- axillary dissection). During surgery, an electron intraoperative boost of 12 Gy was administered to the tumour bed. Within 4 weeks after having received the IORT boost, the whole breast was irradiated with hypofractionated scheme. HEBRT consisted of 13 daily fractions of 2.85 Gy up to a total dose of 37.05 Gy, with 3-D conformal technique. Median age was 42 years (24-48). Pathologic stages were distributed as follow: stage 0 4 (1.1%), I 192 (53.8%), stage IIA 108 (30.3%), stage IIB 28 (7.8%), stage IIIA 18 (5%), stage IIIC 6 (1.7%), CR 1 (0.3%).

The biomolecular classification included 33 triple negative patients.

All patients but one received adjuvant systemic treatment, according to tumor features. Chemotherapy +/- endocrinotherapy was offered to 149 patients, while endocrinotherapy alone was given to 197 patients. In all cases, adjuvant chemotherapy commenced after the completion of HEBRT. Follow-up was carried out on a regular basis by breast surgeons and radiation oncologists.

Results: Median follow-up was 83.1 months (range 9.1-118.9). 340 patients were alive and 317 were free of any disease (89%). Three patients (0.8%) had nodal regional recurrence, 12 patients (3.4%) had local recurrence, 26 patients (7.3%) had distant metastasis. 13 patients (3.6%) developed contralateral breast cancer. 12 patients (3.4%) had a new primary tumor in other site. 15 patients (4.2%) died due to progression of disease, while two died of other causes not related to the breast cancer.

Conclusions: This shortened scheme including intraoperative anticipated boost and hypofractionated whole breast radiotherapy showed a good local control at 5 years follow-up. The patients' compliance is very high and in patients who are in need of chemotherapy, the time gap between surgery and radiotherapy treatment is avoided. As follow-up length increases, a greater number of patients will achieve the minimum 5-year period to receive the first long-term evaluation and the strength of the results on efficacy is bound to increase.
Title: Lobular histology and partial breast irradiation: To what extent is it a cautionary parameter?

Maria Cristina Leonardi¹, Patrick Maisonneuve¹, Anna Morra¹, Nicole Rotmensz¹, Samantha Dicuonzo¹, Roberta Lazzari¹, Veronica Dell'acqua¹, Federica Cattani¹, Mauro Mastropasqua¹, Alberto Luini¹ and Roberto Orecchia¹,². ¹European Institute of Oncology, Milan, Italy and ²University of Milan, Milan, Italy.

Body: Aim: The high likelihood of multicentricity and multifocality linked to lobular histology (ILC) has called into question the partial breast irradiation. Patients with ILC are categorized in the cautionary group according to ASTRO guidelines. We looked into the population treated with intraoperative radiotherapy with electrons (ELIOT) as full dose to investigate the impact of lobular compared to ductal histology (IDC) in terms of local recurrence.

Materials and methods: From 1999 to 2007, 2220 patients were treated with breast conserving surgery and intraoperative radiotherapy with electrons (21 Gy at 90% isodose) as sole treatment. The study population includes both patients treated outside and inside the clinical randomized phase III trial ELIOT. Out of 2220 patients, 255 (11.5%) presented ILC. We compared this group with patients having IDC, treated in the same period. The rate of local recurrences has been analyzed, with a minimum follow-up of 5 years. The role of patients and tumor features on local control has been evaluated.

Results: Compared to IDC group, patients with ILC were older (≥ 70, 20% vs. 11%), with no lymph node involvement (pN0, 79% vs. 70%), low grading (G1-2, 86% vs. 72%), absence of vascular invasion (81% vs. 98%), higher hormonal receptor status (ER+, 97% vs. 88%), HER2 negativity (96% vs. 89%), low proliferative index (Ki-67, 23% vs. 44%). No differences were noticed with regard to the technical parameters of ELIOT. On the whole, the incidence of local relapse was 4.5% at 5 years (0.9/100 per year). Within the first 5 years of follow-up, no differences were observed between lobular and ductal histology, but after 5 years the difference became significant (p 0.05). Higher risk of local relapse was also observed in old patients (over 60) with ILC.

Regarding the biomolecular classification, lobular carcinomas belonging to Luminal A subtype had a more aggressive local behavior than Luminal A ductal carcinomas, showing a significantly higher rate of local relapse (p 0.0021).

Conclusion: In spite of a favorable tumor profile, a greater incidence of local relapse occurred in tumors with ILC compared to ductal histology. When considering the anagraphic, histological and biomolecular variables, the old age (>60), the proliferative index and the luminal A subtype turned out to be significant. Interestingly, the incidence of local relapse becomes statistically significant 5 years after the treatment. This event points out the importance of an adequate follow-up length, especially in lobular histology for which other reports on whole breast irradiation in literature describe similar biological behavior.
Title: Simultaneous integrated boost incorporated into a hypofractionated regimen using tomoDirect: Acute toxicity assessment

Maria Cristina Leonardi¹, Anna Morra¹, Federica Cattani¹, Luigi Santoro¹, Samantha Dicuonzo¹, Raffaella Cambria¹, Rosa Luraschi¹, Alessia Bazani¹, Roberta Lazzari¹, Veronica Dell'Acqua¹ and Roberto Orecchia¹,². ¹European Institute of Oncology, Milan, Italy and ²University of Milan, Milan, Italy.

Body: Aim: We report on acute toxicity, which is the secondary endpoint of a phase II clinical trial specifically addressed to assess the chronic toxicity of a hypofractionated scheme including a simultaneous integrated boost, with intensity modulated radiotherapy.

Materials and methods: From 2/2012 to 12/2013 194 patients with early breast cancer entered a phase II study on hypofractionated radiotherapy including a boost dose to the tumor bed. All patients were operated on conservatively. The whole breast and the tumor bed are planned to receive a dose of 45 Gy and 50 Gy, respectively, in 20 fractions, over 4 weeks. Treatment plans are generated using the TomoDirect modality, which is available on Tomotherapy Hi-Art System (Madison, WI). Acute toxicity was evaluated according to the RTOG acute toxicity scale, up to 6 months after the treatment. Afterwards, chronic toxicity is evaluated using LENT/SOMA scale.

Results: 95% of the volume of the breast and boost PTVs received 99% and 100% of the planned dose, as median values, respectively and 0.1% (median value) of the entire breast volume received 100% of the boost dose. The median maximum dose to the breast and to the boost PTVs was 113% and 103.3%, respectively. At the end of treatment, the acute toxicity, was distributed as follows.

As far as erythema was concerned, at the end of treatment, 58% of the patients experienced grade 1 erythema, which dropped to 23% one month later. Grade 2 erythema affected 37% of the cases, and after one month, it decreased to 2%. Only 1 patient (0.5%) complained of Grade 3 erythema at the end of the treatment, which rapidly disappeared afterwards. With regard to breast oedema, at the end of treatment grade 1 was observed in 16% of the cases, for whom it tended to remain stable after one month, while grade 2 oedema was noted in 4% of the cases, decreasing to 1.5% on the first month follow-up visit. Regarding desquamation, dry desquamation (grade 1) was observed in less than 10% of cases at the end of treatment, but it tended to increase to 17% one month later. Patchy moist desquamation (grade 2) was present in 1.9% of the patients at the end of radiotherapy, and in 1% of them, one month afterwards. Confluent desquamation (grade 3) was noted only in 1 patient (0.5%), who was receiving concomitant chemotherapy with cyclophosphamide, methotrexate and fluoruracil: it was still present 1 month after the radiotherapy completion, as the patient continued to be on chemotherapy. No significantly different side effects were observed between the whole breast and the boost area. No patients experienced any lung and cardiac symptoms.

Conclusion: The clinical results of this SIB hypofractionated scheme showed low acute toxicity. In spite of the high dose per fraction, with the tumor bed receiving an even higher dose per fraction, acute toxicity was within the limit acknowledged by literature for conventional fractionation. This non-rotational treatment option allows us to deliver treatment with a traditional tangent-like dose, without spreading low doses to the adjacent structures. Chronic toxicity will be assessed after 2 years. Therefore, a longer follow-up is needed to assess the effective tolerance to the SIB schedule.
Title: Tangential fields (TgF) breast radiotherapy (RT): Prospective evaluation of the dose distribution in the sentinel lymph node area (SLNa) as determined intra operatively by clip placement

Yazid Belkacemi¹, Veronique Bigorie², Quiong Pan¹, Romain Bosc², Ryan Bouaita¹, Frederic Pigneur³, Elias Assaf⁴, Hakima Badaoui⁵, Emmanuel Itti⁶ and Elie Calitchi¹. ¹APHP, Henri Mondor Breast Center, UPEC; ²APHP, Henri Mondor Breast Center, UPEC; ³APHP, Henri Mondor Breast Center, UPEC; ⁴APHP, Henri Mondor Breast Center; ⁵APHP, Henri Mondor Breast Center and ⁶APHP, Henri Mondor Breast Center, UPEC, Creteil, France.

Body: Purpose:
The coverage of axilla contents by radiation therapy (RT) is an important issue in the new era of minimal axillary surgery based on sentinel lymph node biopsy (SLNB). Data from recent trials demonstrated equivalent survival in breast cancer (BC) patients with 1-2 positive SLNs with or without axillary lymph node dissection (ALND). In a similar context, AMAROS trial showed that complete RT coverage of the axilla is a better option than ALND regarding the risk of arm lymphedema. Our recent data showed that axillary levels are underdosed when only tangential fields (TgFs) are used (Belkacemi et al, Ann Oncol 2013). We aimed to evaluate the dose distribution in the SLNa defined intra operatively by clips placement. This could be a important for patients with SLN involvement who have neither ALND nor RT to the axilla.

Materials/Methods:
Twenty-five patients have been prospectively included in this study. They had clips placement in the SLNa during the SLNB procedure. Breast dose was ranged between 40 to 50Gy in 15 to 25 fractions. Additional boost to the tumor bed of 10 or 16Gy was delivered in 21 patients. Level I-III and organs at risk were contoured using the RTOG contouring atlas. The SLNa was defined as 1.5 cm in diameter around the clips. Dose-volume-histograms were analyzed regarding the volumes receiving 95% or 50% of the prescribed dose. Percentages overlap between TgFs and SLNa volume were analyzed to define 3 groups: 100% overlap ("suitable group" with SLNa completely included in the TgF), > 50% overlap ("partially suitable group" with SLNa partially included in the TgF) and 0-49% overlap or completely outside the TgFs ("unsuitable group").

Results:
The mean dose delivered to levels I, II, III and SLN area were 25, 5, 2 and 33Gy respectively. The volume covered by the 95%-isodose were respectively 2%, 0%, 0% and 4%. The average dose delivered to level I, II, III and SLN area were higher using High TgFs vs STgFs (38 vs 22Gy, p=0.004; 11 vs 3Gy, p=0.019; 5 vs 2Gy, p=0.003; 43 vs 31Gy, p=0.02), respectively. HTgFs covered better 50% of all axilla levels. Boost delivery and initial tumor site did not influence axilla coverage by the TgFs. The SNLa was totally or partially covered in 48% and 28% of patients, respectively. The mean dose delivered to 95% of the SNLa was only 22Gy using STgFs and 33Gy with the HTgFs. Using the STgFs, the SNLa was either totally (n=8/20) or partially (n=6/20) covered by > 50% of dose. Average dose was 46, 34 and 8Gy, respectively in the 3 groups. HTgFs allowed a complete coverage of the SLNa in all patients.

Conclusion:
In patients undergoing breast conservative therapy, TgFs provide a limited coverage of the SLNa. STgFs allowed total coverage of this area in less than half of the patients. Thus, SLNa should be delineated in patients who have only SLNB procedure. Some of these patients with nodal involvement without additional ALND could benefit from HTgFs irradiation or a better-personalized nodal RT using a dedicated nodal RT technique such that reported in AMAROS trial. The last allow better coverage of the axilla contents than TgFs.
Title: Patterns of practice of regional node irradiation in the sentinel node biopsy era: Results of the nodal radiotherapy (NORA) survey. On behalf of the EORTC Breast Working Party of the Radiation Oncology Group (ROG)

Yazid Belkacemi, Orit Kaidar-Person, Philippe Poortmans, Mahmut Ozsahin, Maria-Clara Valli, Nicola Russel, Ian Kunkler, Julie Hermans, Abraham Kuten, Geertjan van Tienhoven and Helen Westenberg. 1 APHP, Henri Mondor Breast Center, UPEC, Creteil, France; 2 Rambam, Haifa, Israel; 3 Institute Verbeeten, Tilburg, Netherlands; 4 CHUV, Lausanne, Switzerland; 5 Oncology Institute, Southern Switzerland, Switzerland; 6 The Dutch Cancer Institute, Amsterdam, Noord-Holland, Netherlands; 7 Edinburgh Cancer Centre, University of Edinburgh, Edinburgh, United Kingdom; 8 EORTC Breast Working Party of the Radiation Oncology Group (ROG), EORTC, Brussels, Belgium; 9 Italian Hospital and Rambam Campus, Haifa, Israel; 10 Academisch Medisch Centrum, Amsterdam, Noord-Holland, Netherlands and 11 Radiotherapeutisch Samenwerkingsverband Arnhem-Nijmegen (Radian), Locatie Arnhems Radiotherapeutisch Instituut (ARTI), Arnhem, Netherlands.

Body: Objective:
Predicting the outcome of breast cancer (BC) patients based on sentinel lymph node (SLN) status without axillary lymph node dissection (ALND) is a matter of debate, especially when it comes to the definition of regional nodal irradiation (RNI). This is even unclear in the framework of primary systemic therapy (PST). The aim of the NORA (NOdal RAdiotherapy) Survey was to examine the patterns of RNI practiced by European Radiation Oncology centers.

Methods:
A web questionnaire was distributed to EORTC centers, and responses were received during the period between July 2013-January 2014.

Results:
A total of 81 European and 3 non-European answers were analyzed. While 3D planning is performed in 81 (96%) of the centers for breast irradiation, nodal areas are delineated in only 51 (61%) of the centers. After breast conserving surgery (BCS), only 14 (17%) centers declared to treat systematically the internal mammary chain (IMC), when supraclavicular node RT (SCN-RT) is indicated. Extra-capsular extension (ECE) is the main factor impacting decision-making regarding IMC and axillary nodal RT (ALN-RT). Only half of the centers advocate SCN-RT for intermediate risk patients (1-3N+). For macro-metastatic SLN involvement, there was a significant impact of ECE on decision-making independently from the number of positive LNs. In the PST setting, only 49 (58%) centers take into account the histologic fibrotic changes of the axillary LNs in post-PST ypN0 patients with unknown pre-PST status. In ypN0 patients with inner and central BC, 32 (39%) and 23 (27%) centers centers indicate SCN-RT and IMC-RT, respectively. SCN-RT is delivered by 30, 44, 58, 67 centers in patients with ypN(i+), ypN(mi), 1-2N+ and > 3-4N+ disease, respectively. ALN-RT is indicated by in 25% of the centers in patients with ypN(mi) or 1-2N+. Older age > 70y was not considered as a limited factor for RNI.

Discussion:
The term of RNI is not uniformly defined in the literature. In the historic studies, RNI was dedicated to wide RT including whole lymphatic pathways of the breast. ALN and IMC RT indications have declined with the time during the last decades because of the risk of morbidity. However this practice is challenged by the recent RT trials that reported and increase of overall and distant metastatic survival with RNI and IMC delivery in high-risk N0 and inner-central tumors. In addition, AMAROS trial showed equivalence between axillary RT and ALND in SLN (mi+) patients. Taking these data altogether in the context of increased avoidance of ALND, future challenges should concern the multidisciplinary patients’ selection for RNI and better personalization of the lymphatic pathways RT according to the risk of locoregional and distant recurrences.

Conclusions:
The NORA Survey is unique by evaluating the impact of SLN status and PST on RNI decision-making. IMC RT is not frequently coupled to SCN-RT. ECE is the main factor impacting decision-making for RNI. Only 58% of the centers take fibrotic changes in the LNs into consideration in decision-making for RNI indications. Age is not considered as a limiting factor for prescribing RNI in Europe.
2014 San Antonio Breast Cancer Symposium

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Average Grade: 4.25

Title: Low utilization of hypofractionated radiotherapy for the treatment of early-stage breast cancer in the US

Yvonne M Mowery¹, Rachel A Greenup¹, Kevin Houck¹, Manisha Palta¹, Janet K Horton¹, Eun-Sil S Hwang¹, Julie A Sosa¹ and Rachel C Blitzblau¹. ¹Duke University Medical Center, Durham, NC.

Body: Background: Large randomized controlled trials have shown that hypofractionated whole breast irradiation (HF-WBI) is not inferior to or more toxic than conventionally fractionated whole breast irradiation (CF-WBI) for the treatment of early-stage breast cancer. Royal Marsden data were published in 2006 (10-yr), the Ontario trial was reported in 2002 (5-yr) and 2010 (10-yr), and the UK START trials were published in 2006 (5-yr) and 2013 (10-yr). We utilized the National Cancer Data Base (NCDB) to evaluate patterns of radiotherapy fractionation for early-stage, node-negative breast cancer in the U.S. We hypothesized that HF-WBI use would increase over time in response to emerging data supporting its use in this population.

Methods: We conducted a retrospective, population-based cohort study of women >18 years diagnosed with T1-2N0 invasive breast carcinoma and treated with breast-conserving surgery between 2004 and 2011. Radiotherapy was categorized as accelerated partial breast irradiation (APBI; 38-40 Gy/1-10 fractions), HF-WBI (40-56 Gy/15-24 fractions) or CF-WBI (50-66 Gy/25-40 fractions). Patients treated with alternate fractionation were excluded. Patterns of breast radiotherapy fractionation were compared using the chi-square test. Multivariable logistic regression was performed for patients diagnosed in 2011, the year with the highest levels of HF-WBI utilization.

Results: 217,789 patients in the NCDB met inclusion criteria. HF-WBI use increased over time, rising from 2.1% among eligible patients in 2004 to 15.1% in 2011, while APBI use remained low at <2%. Utilization of HF-WBI was significantly higher in academic centers than comprehensive community cancer centers and community-based cancer centers (p <0.0001). This pattern persisted when controlling for T-stage, hormone receptor/HER2-status, diagnosis year, and patient age >30 years. Table 1 shows frequency of HF-WBI use over time by center type. On multivariate analysis of patients diagnosed in 2011, the following factors were associated with higher use of HF-WBI: treatment at an academic center, older patient age, hormone receptor positivity, pT1 tumor size, and rural residence (Table 2).

Conclusions: Utilization of HF-WBI in the US is rising, but remains low overall despite level I evidence showing its non-inferiority to CF-WBI. Given the advantages of HF-WBI in terms of patient convenience and potential healthcare system costs, further research is indicated to explore disparities in HF-WBI utilization in the US and to guide education of breast cancer providers.

Table 1: % HF-WBI use

<table>
<thead>
<tr>
<th>Year</th>
<th>Community Cancer Center</th>
<th>Comprehensive Community Cancer Center</th>
<th>Academic Center</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>0.9%</td>
<td>1.7%</td>
<td>3.8%</td>
</tr>
<tr>
<td>2005</td>
<td>1.6%</td>
<td>2.1%</td>
<td>3.8%</td>
</tr>
<tr>
<td>2006</td>
<td>1.2%</td>
<td>2.0%</td>
<td>4.6%</td>
</tr>
<tr>
<td>2007</td>
<td>1.5%</td>
<td>2.8%</td>
<td>7.1%</td>
</tr>
<tr>
<td>2008</td>
<td>2.9%</td>
<td>5.3%</td>
<td>10.8%</td>
</tr>
<tr>
<td>2009</td>
<td>3.9%</td>
<td>8.5%</td>
<td>14.7%</td>
</tr>
<tr>
<td>2010</td>
<td>5.8%</td>
<td>10.7%</td>
<td>16.4%</td>
</tr>
<tr>
<td>2011</td>
<td>9.6%</td>
<td>13.9%</td>
<td>20.5%</td>
</tr>
</tbody>
</table>

Table 2. Multivariate analysis, year 2011 (n=5568)
<table>
<thead>
<tr>
<th></th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Academic Center vs. Community Cancer Center</strong></td>
<td>3.06</td>
<td>2.32</td>
</tr>
<tr>
<td><strong>Academic Center vs. Comprehensive Community Cancer Center</strong></td>
<td>1.78</td>
<td>1.53</td>
</tr>
<tr>
<td>Patient age, 50-90 vs. 18-49</td>
<td>2.37</td>
<td>1.86</td>
</tr>
<tr>
<td>T2 vs. T1</td>
<td>0.54</td>
<td>0.46</td>
</tr>
<tr>
<td>HER2+ vs. Hormone Receptor +/HER2-</td>
<td>0.75</td>
<td>0.59</td>
</tr>
<tr>
<td>ER-/PR-/HER2- vs. Hormone Receptor +/HER2-</td>
<td>0.66</td>
<td>0.52</td>
</tr>
<tr>
<td>Rural vs. urban</td>
<td>2.68</td>
<td>1.69</td>
</tr>
</tbody>
</table>
Recurrence rates, stratified by age and histology, for accelerated partial breast irradiation using strut-based brachytherapy: A report on 1005 patients

Catheryn Yashar, Deanna Attai, Ernest Butler, John P Einck, Steven E Finkelstein, Ben Han, Robert L Hong, Lydia T Komarnicky, Maureen Lyden, Sudha B Mahalingam, Constantine A Mantz, Nadim Nasr, Stephen S Nigh, Jondavid Pollock, Jay E Reiff, Daniel Scanderbeg, Margaret R Snyder, Jon Strasser and Robert Kuske.

Background: APBI is an integral part of many clinical practices, but the majority of data that exists is in the elderly and invasive patient groups. In order to understand APBI performance in other subgroups, we present a subset analysis by histology (DCIS & invasive) and age (<50, ≥50 to <60 and ≥60 yrs).

Methods: The SAVI Collaborative Research Group database has accrued APBI patients at 14 institutions treated with strut-based brachytherapy. All patients received monotherapy (34 Gy in 10 fractions), prescribed in the standard method. Patients were grouped into three age ranges, as well as by histology. Crude recurrence rates (ipsilateral, contralateral and distant) were analyzed and reported. Two subjects had ipsilateral recurrence, without localization (i.e., TR/MM vs ipsilateral elsewhere) and those were classified as TR/MM for the analysis. Patients were treated between 2007 & 2011.

Results: 1005 patients (1008 breasts) were included in this analyses (690 invasive, 285 DCIS, 30 unspecified). Median age was 64 yrs for all subjects, 65 yrs and 62 yrs for invasive and DCIS subjects, respectively. Nearly all patients were post-menopausal (89%), had tumor size <2.0 cm (88%), no positive nodes (92%), had negative margins (>2mm; 94%), were ER+ (90%) and received no chemotherapy (94%). The rates for these variables were almost identical for the invasive and DCIS subgroups (except for no chemotherapy (invasive=92%; DCIS=99%)). In contrast, hormone therapy was delivered to 53% of invasive and 41% of DCIS subjects. Median follow up (FU) was 38 and 34 mos for invasive and DCIS subject groups, respectively. Median FU was 40 and 29 mos for invasive (n=49) and DCIS (n=26) subjects of age <50 yrs, respectively. For subjects 50 to 60 yrs of age, median FU was 40 and 36 mos for invasive (n=119) and DCIS (n=74). Median FU was 37 and 34 mos for invasive (n=343) and DCIS (n=116) subjects >60 yrs, respectively.

For the entire cohort (=1008 breasts) there were 20 ipsilateral breast (only) tumor recurrences (2.0% IBTR failure rate) of which 13 were TR/MM, which was equivalent to the contralateral tumor rate (N=13). There were 3 distant failures in the entire cohort. For patients in the <50 yr group, there were 3 IBTR (3.26%) all of which were TR/MM (1 invasive subject, 2 DCIS subjects). There were 2 contralateral failures in the DCIS group and none in the invasive group of this age group. For patients in the 50-60 yr group, there were 8 IBTR (3.31%) 5 of which were TR/MM (3 invasive subjects, 2 DCIS subjects). There was 1 contralateral failure in the DCIS group and none in the invasive group. In the >60 yr group, there were 9 IBTR (1.34%) 5 of which were TR/MM (4 invasive subjects, 1 DCIS subject). There were 2 contralateral failures in the DCIS group and 7 in the invasive group.

Conclusions: In this large cohort of patients treated with strut-based brachytherapy, the crude rate of IBTR was low (1.3-3.3%) and not significantly different in the 3 age groupings analyzed. At 3 yrs median follow up, the absolute difference in IBTR was only 2% in women over age 60 versus 50-60 or younger than 50 years. Likewise, invasive and DCIS both had low IBTR, suggesting that guidelines may need to be revised for young age and histology criteria.
Title: Development of a photonumeric scale for acute radiation dermatitis in breast cancer patients

Dean Shumway¹, Eleanor M Walker², Nirav Kapadia³, Thy Thy Do¹, Kent Griffith¹, Mary Feng¹, Bonnie DePalma², Reshma Jagsi¹, Yolanda Helfrich¹, Erin Gillespie⁴, Alexandria Miller¹, Adam Liss¹ and Lori J. Pierce¹. ¹University of Michigan, Ann Arbor, MI; ²Henry Ford Hospital, Detroit, MI; ³Dartmouth-Hitchcock Medical Center, Lebanon, NH and ⁴University of California, San Diego, La Jolla, CA.

Body: Purpose
Scales for rating acute radiation dermatitis (ARD) are inconsistent and have not been validated despite decades of clinical use, making ARD difficult to report reliably. We sought to design a photonumeric scale to consistently describe ARD in breast cancer patients undergoing radiation (RT).

Methods
Patients undergoing RT for breast cancer were enrolled on a prospective study that included photographs and reporting of physician-rated erythema, hyperpigmentation, and CTCAE toxicity score at baseline and 2, 4, and 6 weeks after initiating RT. Erythema and hyperpigmentation were also quantified using a hand-held colorimetric device. Photographs were taken using a standardized protocol that included 3 views to fully assess the breast/chest wall, axilla, and inframammary fold. 209 photographs from 35 patients with white skin (Fitzpatrick skin types I-IV) and 369 photographs from 50 patients with skin of color (Fitzpatrick skin types V-VI) were clustered according to the apparent severity of ARD. Due to the prevalence of hyperpigmentation that obscured erythema in patients with skin of color, separate images were used to illustrate ARD in this population. Two photonumeric scales (for white skin and skin of color) were developed via an iterative process until group consensus was achieved. Four raters with experience in the evaluation of ARD in breast cancer patients used the photonumeric scale to independently score the entire collection of photographs, sequenced in random order. Intra- and inter-rater agreements were assessed using weighted kappa scores.

Results
Of the 35 patients with white skin, 20% experienced severe erythema, and 40% experienced dry or moist desquamation. Of the 50 patients with skin of color, 34% experience severe hyperpigmentation, and 48% experienced dry or moist desquamation. Using the photonumeric scales, we observed high intra-rater agreement for independent ratings of erythema or hyperpigmentation (70 to 89% agreement fraction, kappa 0.55 to 0.81) and desquamation (79 to 87% agreement fraction, kappa 0.52 to 0.64). Similarly, we observed moderate to high inter-rater agreement for independent ratings of erythema or hyperpigmentation (61 to 76% agreement fraction, kappa 0.40 to 0.62) and desquamation (69 to 84% agreement fraction, kappa 0.36 to 0.58). Quantitative measurements of erythema in white patients using colorimetry correlated strongly with photonumeric grade (correlation coefficient 0.76, p<0.001), as did physician-rated erythema at the point-of-care (p<0.001). Fitzpatrick score was not significantly associated with maximum photonumeric erythema grade (p = 0.14).

Conclusions
We report a new photonumeric scale with high intra- and inter-rater reliability for acute radiation dermatitis in breast cancer patients. To our knowledge, this is the first rigorously evaluated scale that is applicable to patients across the spectrum of skin pigmentation, including white skin and skin of color. The photonumeric scale will facilitate consistent reporting of acute radiation dermatitis in research and clinical settings using a simple, standardized instrument. Future work will include prospective real-time clinical validation with multiple raters and correlation with patient-reported outcomes.

Funded by a Munn Idea Grant (G011480).
Introduction:
Intra-operative radiotherapy (IORT) is increasingly being used for the treatment of low risk breast cancer. In the randomized TARGIT trial, late toxicity was not different between patients treated with IORT and external beam radiotherapy. Early toxicity of IORT has not been fully characterized.

Methods:
IORT with one dose of 20 Gy using the Intrabeam® device has been offered as an alternative to standard treatment as part of a single center prospective single arm trial. Patients over 60 years with clinically node negative, < 2 cm invasive duct carcinomas were eligible. Patients aged 50-60 years, patients with tumors 2-3.5 cm and patients with invasive lobular carcinomas were treated with IORT if they were not candidates for standard treatment.
For the present study information regarding complications occurring within the first year after surgery was analyzed. Patients treated between 2006 and 2012 were included in this analysis.

Results:
393 patients were treated from 2006 – 2012. 4 were lost to follow up. Median age was 70 years (55-90) and median clinical tumor size was 1.2 cm (5-30).
102 patients (26.2%) had a complication. Infections were most frequent. Misdiagnosis of radiation dermatitis as infection in some cases cannot be excluded. Clinically meaningful seromas occurred in 10.2% of patient and 8.2% had a wound dehiscence, possibly related to rupture of pre-existing seromas.

Table 1

<table>
<thead>
<tr>
<th>Complication type</th>
<th>N (%)</th>
<th>N with grade 3 (%)</th>
<th>N with grade 4 (%)</th>
<th>median time to recovery (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infection</td>
<td>42 (11)</td>
<td>3 (3.3)</td>
<td></td>
<td>0.62 (0.1 - 3.8)</td>
</tr>
<tr>
<td>Seroma</td>
<td>40 (10.2)</td>
<td></td>
<td>13.2 (0.3-50)</td>
<td></td>
</tr>
<tr>
<td>Wound dehiscence</td>
<td>33 (8.5)</td>
<td>2 (0.5)</td>
<td>5.8 (0.36-15.3)</td>
<td></td>
</tr>
<tr>
<td>Bleeding</td>
<td>11 (2.8)</td>
<td>3 (0.8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin necrosis</td>
<td>2 (0.5)</td>
<td></td>
<td>2 (0.5)</td>
<td>3.88 (3.45-4)</td>
</tr>
</tbody>
</table>

18 patients (4.6%) had a grade 3 complication. 2 patients (0.5%) had grade 4 radiation induced skin necrosis of small size and both resolved within 4 months. All other complications resolved completely (table 1). Risk factors for complications in this cohort will also be presented.

Conclusions:
Intra-operative radiotherapy was associated with a relatively high rate of clinically meaningful seromas and wound dehiscence and rare small size skin necrosis. Most complications were mild – moderate. 5% of patients experienced grade 3 or 4 complications, and all resolved completely. Further study of the factors predisposing to severe complications is warranted.
Title: Disease-free survival according to the use of postmastectomy radiation therapy after neoadjuvant chemotherapy

Himanshu Nagar¹, Dustin Boothe¹, Paula Ginter¹, Cristina Sison¹, Linda Vahdat¹, Sandra Shin¹, Michael Smith¹, KS Clifford Chao¹, Dattatreyudu Nori¹ and Mary Katherine Hayes¹. ¹Weill Cornell Medical College, New York, NY.

Body: Purpose: Determine predictors of recurrence for patients treated with neoadjuvant chemotherapy (NAC) and mastectomy according to the use of postmastectomy radiation therapy (PMRT).

Methods and Materials: An analysis of 161 clinically staged T1-T3/N0-N3 patients treated with NAC and mastectomy with and without PMRT at our institution from 2003 - 2010 was conducted. The Kaplan-Meier product limit method was used to estimate survival and time-to-recurrence rates and the log-rank test was used to compare groups. A Cox proportional hazard regression analysis was carried out for time-to-recurrence, RT, and their interaction in the model.

Results: The median follow-up period was 48 months and 18 patients developed a recurrence. The 5-year recurrence rate and overall survival was 16.1% (95% CI: 9.6%, 26.3%) and 93.6% (95% CI: 88.2%, 97.0%), respectively. Patients that underwent PMRT had a decreased risk of recurrence compared to patients who did not (HR=0.25, 95% CI: 0.097 to 0.661; p<0.005). The 5-year DFS rate for those who received PMRT was 91.3% (95% CI: 82.8% to 95.7%) and 64.8% (95% CI: 37.8% to 82.4%) for those that did not (p=0.0126). Among all clinicopathologic factors examined, pathologic T stage (ypT) and pathological N stage (ypN) significantly correlated with the risk of recurrence (p<0.05). Patients with any pathological nodal disease had an increased risk of recurrence compared to patients who were pathologically node negative (HR=7.196; 95% CI: 2.05 to 25.264; p<0.002).

Conclusion: Patients treated with NAC and mastectomy, but without PMRT had a higher risk recurrence with increasing ypT and ypN stages. PMRT may increase DFS.
Title: The utilization of hypofractionated breast radiotherapy in the United States between 2003 and 2011

Dezheng Huo¹, Yasmin Hasan¹ and Katharine Yao². ¹University of Chicago, Chicago, IL and ²NorthShore University HealthSystem, Evenston, IL.

Body: Background: Recently, several randomized trials have found that accelerated hypofractionated whole breast irradiation (HF-WBI) had equivalent local recurrence rates and disease-free survival, compared to conventional fractionated whole breast irradiation (CF-WBI). It is unknown if the pattern of care regarding HF-WBI has changed in the United States after the publication of these trials, given its administrative advantage.

Methods: Using data from the National Cancer Database, this study included 559,362 non-metastasic female breast cancer patients who received post-lumpectomy external beam radiotherapy to their breast or regional nodes. We examined the trend of HF-WBI use in the U.S. and factors related to its use using logistic regression models.

Results: Of patients receiving external beam radiotherapy, about 5% underwent HF-WBI. The most common HF-WBI dose schedule is 42.4-42.6 Gy in 16 fractions without or with boost radiation. There was a 20-fold increased trend in the use of HF-WBI, increasing from about 0.6% in 2003 to 13% in 2011 (p<0.0001). Among patients with pT1-2N0-1 disease, a subgroup for whom previous clinical trials of HF-WBI are mainly applicable, HF-WBI use increased from 0.6% in 2003 to 14% in 2011. Although not included in previous clinical trials, patients with ductal carcinoma in situ also experienced an increased use of HF-WBI, from 0.5% in 2003 to 12% in 2011. We found age was strongly correlated with HF-WBI use; the older a patient, the more likely she received HF-WBI. African Americans were less likely to use HF-WBI. Patients with low grade were more likely to receive HF-WBI than those with high grade and patients with negative nodes are more likely to received HF-WBI. Large volume, academic cancer centers are more likely to adopt HF-WBI than community cancer programs. Interestingly, patients who live at least 50 miles away from a cancer center are about 58% more likely to receive HF-WBI than patients living within 50 miles of a cancer center.

Conclusions: The utilization of hypofractionated radiotherapy significantly increased from 2003 to 2011 in the United States, which is presumably influenced by publication of several randomized trials. In 2011, HF-WBI accounted for more than 10% of all external beam radiotherapy for early breast cancer patients. Patient and facility factors impacted the patterns of HF-WBI use.
Title: Sustained acceptable cosmetic outcomes and local control following accelerated partial breast irradiation using CT-guided IMRT in the prone position: Results from a phase I/II feasibility study

Carmen Bergom¹, Phillip Prior¹, Kristofer Kainz¹, Natalya V Morrow¹, Ergun E Ahunbay¹, Alonzo Walker¹, X Allen Li¹, Tracy Kelly¹, Adam D Currey¹ and Julia White². ¹Medical College of Wisconsin, Milwaukee, WI and ²Ohio State University Comprehensive Cancer Center, Columbus, OH.

Body: Objective/Purpose
External beam accelerated partial breast irradiation (EB-aPBI) can have potential challenges in daily reproducibility, although it has broader potential use than aPBI using brachytherapy. Image-guide radiotherapy (IGRT) can improve daily reproducibility and allow smaller treatment margins. Our institution utilized IG-IMRT to administer EB-aPBI in the prone position in a Phase I/II study to increase dose homogeneity, conformality, normal tissue avoidance, and reliable targeting. Our preliminary results and toxicity were promising. Here we report final physician- and patient-reported cosmetic outcomes from this prospective trial.

Materials and Methods
Women with node-negative invasive breast cancer or DCIS, tumors less than 3.0 cm, a negative sentinel lymph node biopsy, and surgical clips demarcating the lumpectomy cavity underwent prone EB-aPBI using IG-IMRT on an IRB-approved phase I/II study. The lumpectomy PTV represented a 2.0 cm lumpectomy cavity expansion. 38.5 Gy was delivered in 10 fractions over 5 days, such that 95% of the prescribed dose covered greater than 99% of the PTV. Dose constraints for the whole breast, lungs and heart were met.

Results
Twenty patients were enrolled, with a median patient age of 61.5 and a mean tumor size of 1.0 cm. 35% of patients had DCIS. At a median follow-up of 18.9 months, 40% and 10% of patients had G1 and G2 fibrosis, respectively, and 95% of patients had good to excellent physician-assessed cosmesis. At a median follow-up of 60.0 months (range 54-79 months), physician-assessed cosmetic outcome was good to excellent in 80%, with 30% and 20% of patients experiencing G1 and G2 fibrosis. Patient-reported outcomes at one year yielded 90% of patients with good to excellent cosmetic outcomes. At 3 years, 75% of patients reported good to excellent cosmesis. Eighty-eight percent of patients were completely satisfied with the treatment and results, and 94% of patients would choose aPBI again. With one local recurrence, the actuarial five year rate of local control was 95%.

Conclusions
These data demonstrate that EB-aPBI in the prone position using IG-IMRT continues to yield acceptable cosmetic outcomes at longer term follow-up, and a very high percentage of patients would choose this treatment again. (Supported by Komen Grant: BCTR0504070).
Title: Evaluation of separation techniques and dosimetry of the inframammary fold during whole breast radiation therapy

Guy Jones¹, Peter Guion¹, Jason Cheng¹ and Aparna H Kesarwala¹. ¹National Cancer Institute, Bethesda, MD.

Body: Purpose/Objectives: The majority of patients receiving radiation therapy will experience some form of skin reaction, ranging from localized erythema to moist desquamation. Radiation dermatitis of the inframammary fold (IMF), the most common acute side effect of supine whole breast radiation therapy (WBRT), can be mitigated by separating the breast from the chest wall. The purpose of this analysis was an objective evaluation and comparison of practical and inexpensive separation techniques.

Materials/Methods: A breast phantom was created on a human torso phantom using tissue-equivalent bolus material. Four separation techniques were evaluated: 1) taping the inferior aspect of the breast to the shoulder or placing 2) 6" x 2" rolled bubble wrap, 3) a 6" x 1" x 1" styrofoam block, or a 4) 50 mL 2” diameter plastic conical tube in the IMF. For the control setup, the breast was not taped and nothing was placed in the IMF. Written and pictorial simulation instructions recorded by one radiation therapist were independently used by additional therapists to reproduce each setup, with medial and inferior measurements taken for comparison. Dosimetry of identical tangent 100 cGy WBRT fields was evaluated with thermoluminescent dosimeters (TLDs) placed at each of four locations: the ipsilateral IMF, the ipsilateral chest wall 5 cm inferior to the IMF, and 5 cm superior to the nipple of both the ipsilateral and contralateral breasts at the 12:00 position. Measurements and TLD data are reported as mean ± standard error of the mean of 3 separate TLD readings. One-way ANOVA and posthoc analyses were used to determine significance between groups with α for significance set at p ≤0.05.

Results: Compared to simulation, the average differences measured medially and inferiorly among the conical tube (10 ± 6 mm medial; 0.6 ± 0.2 mm inferior), styrofoam block (13 ± 8 mm medial; 1.2 ± 0.2 mm inferior), and bubble wrap (18 ± 4 mm medial; 0.6 ± 0.1 mm inferior) setups were not significant. The taping technique (0.2 ± 0.1 mm) was more reproducible inferiorly than the styrofoam block setup (p <0.01), but no different from the conical tube and bubble wrap setups. TLD readings demonstrated that the IMF received less of the prescribed dose with the taping (108 cGy ± 1.4%), styrofoam block (109 cGy ± 0.9%), or bubble wrap (110 cGy ± 0.9%) techniques compared to either the control (120 cGy ± 1.1%) or conical tube (121 cGy ± 0.4%) setups (each p <0.01). TLD readings from the three other locations were not significantly different between any of the setups.

Conclusions: The taping, styrofoam block, and bubble wrap setups were most effective in sparing dose to the IMF. The only difference in reproducibility was between the inferior aspects of the taping and styrofoam block setups, but taping had the disadvantage of being more invasive than the other setups. While this unique analysis did not reveal a single superior solution, the taping, styrofoam block, and bubble wrap separation techniques should be considered by radiation oncologists seeking practical, inexpensive, and easily-implemented solutions to mitigate IMF dermatitis in WBRT patients.
Title: Effects of statin use on skin toxicity during breast radiation therapy

Scott M Lieberman¹, Elizabeth Stone¹, Christopher Chen¹, Marlow Hernandez¹ and Leah Roberts². ¹Cleveland Clinic Florida, Weston, FL and ²Charles E Schmidt College of Medicine at FAU, Boca Raton, FL.

Body: Background: Recent San Antonio Breast Cancer Symposium abstracts have suggested that statin use confers a significant benefit in breast cancer risk reduction and specifically in the subset of inflammatory breast cancer. Additional studies evaluating the combination of statin use with various breast cancer treatments are currently on-going. The primary objective of this study was to determine the effects of statin use on acute skin toxicity during breast radiation therapy (RT). Methods: We performed a retrospective chart review of 325 patients diagnosed with breast cancer who underwent RT between 2004 and 2012. Of these, 227 patients were considered evaluable. Patients were excluded if the course of RT was fewer than 16 treatments. Patients were also excluded if follow-up at the end of RT was not obtainable. Of the evaluable patients, 46 were on statins at time of RT, while 181 were not. Patients were evaluated at two time points - midway through RT and at completion of RT. Skin reactions were measured using the RTOG grading system for acute radiation morbidity. Grade 1 reactions were erythema and slight skin atrophy. Grade 2 indicated follicular, faint or dull erythema/ epilation/dry desquamation/ decreased sweating. Grade 3 was tender or bright erythema, patchy moist desquamation/moderate edema. Lastly, Grade 4 was ulceration, hemorrhage, necrosis. Results: Our results showed a statistically significant increase in acute radiation skin toxicity in patients on statins at the time of radiation therapy. By the midway point of RT, 67% of the statin group had developed Grade 1-3 skin toxicity vs. only 42% of the control group. Odds ratio midway through RT was 2.75. By the completion of RT, 94% of the statin group compared with 67% of the control group had Grade 1-3 skin toxicity (p=0.005). Odds Ratio at completion of RT was 7.29. The statin group also progressed to more advanced stages of toxicity more rapidly than our control group. Grade 1 skin reactions were seen in 62% of our statin group vs. 41% of our control group midway through treatment (p=0.044). Grade 2-3 reactions were seen in almost 5% of patients in the statin group vs. 1% in the non-statin group midway through treatment. At the end of treatment, 48% of patients on statins had developed Grade 2-3 reactions when compared with 29% in the non-statin group (p=0.0199). These results still remained significant when controlled for chemotherapy use. Conclusion: Our study revealed that patients on statins developed acute skin toxicity at higher rates than our control group. These results were statistically significant at both the midway point as well as at the completion of RT. Study limitations include the small sample size and the retrospective nature of the study. Our data also raises the question of what effect statins may have on local recurrence rates as well as the potential effects of radiosensitization in various breast cancer cell lines. These results are hypothesis-generating and require larger retrospective analyses or prospective trials to verify this effect.
Title: Prospective study of proton radiotherapy for treatment of regional lymphatics in breast cancer

Julie A Bradley¹, Roi Dagan¹, Meng Wei Ho¹, Christopher G Morris¹, Zuofeng Li¹ and Nancy P Mendenhall¹. ¹University of Florida Proton Therapy Institute, Jacksonville, FL.

Body: Purpose: To compare dosimetric endpoints between proton therapy (PT) and conventional radiation and to determine the feasibility of PT for regional nodal irradiation (RNI) in women with breast cancer.

Methods: From May 2012 to February 2014, 18 women (stage IIA-IIIB) prospectively enrolled on a pilot study. Median age was 51.8 years (range, 42-73), with equal division between breast-conserving therapy (BCT) and mastectomy and right and left-sided cancers. Treatment targets (CTVs for breast/chest wall, supraclavicular, axillary, internal mammary nodes [IMNs]) and organs at risk were delineated on CT scans, and PT and conventional plans were developed. PT alone was used for 10 patients (9 post-mastectomy, 1 after BCT) and combined proton-photon in 8 (all BCT). Acute toxicity was prospectively recorded using CTCAE v4.0. A Wilcoxon signed-rank sum test compared the dose-volume parameters.

Results: Median followup was 10.6 months (range, 1.1-19.1). For all patients, the PT plan better met the dosimetric goals and was used for treatment. Breast/chest wall coverage was adequate (V47.5=96.6% for both plans). PT improved coverage of level II axilla (median D95, 48.9Gy [range, 44.8-51.4Gy] with PT vs 45.4Gy [range, 39.8-51Gy] with conventional; p=0.0005). Adequate coverage of IMNs was consistently achieved with PT (median D95, 48.9Gy with a range of 44.8-51.4Gy) but not with conventional (median D95, 45.4Gy with range of 39.8-51Gy; p=0.0005); PT reduced heart dose in all patients. Median ipsilateral lung V5 measured 35.3% for PT compared to 60.5% for conventional (p<0.0001). Ipsilateral lung V20 improved with PT (median, 21.6% vs 35.5%; p<0.0001).

Table 1: Cardiac dose in left-sided patients (comparison of medians of the cohort; range in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>Heart V5</th>
<th>Mean Heart Dose</th>
<th>Ventricle V5</th>
<th>Mean Ventricle Dose</th>
<th>Maximum LAD dose</th>
<th>Mean LAD dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proton Plan</strong></td>
<td>2.7% (0-12%)</td>
<td>0.6Gy (0-3.2Gy)</td>
<td>0.5% (0-15.6%)</td>
<td>0.1Gy (0-4Gy)</td>
<td>30.5 (13.4-42.6Gy)</td>
<td>1.7Gy (0.5-28.5Gy)</td>
</tr>
<tr>
<td><strong>Conventional Plan</strong></td>
<td>34% (7-60%)</td>
<td>5.9Gy (2-9.1Gy)</td>
<td>31.4% (8.7-79.8%)</td>
<td>5.4Gy (2.4-11Gy)</td>
<td>44.6Gy (35.1-54.7Gy)</td>
<td>27Gy (16.8-41.9Gy)</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.004</td>
<td>0.04</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Table 2. Cardiac dose in right-sided patients (comparison of medians of the cohort; range in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>Heart V5</th>
<th>Mean Heart Dose</th>
<th>Ventricle V5</th>
<th>Mean Ventricle Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Proton Plan</strong></td>
<td>0.3% (0-2.9%)</td>
<td>0.5Gy (0-0.8Gy)</td>
<td>3.3% (0-1%)</td>
<td>0.2Gy (0-0.5Gy)</td>
</tr>
<tr>
<td><strong>Conventional Plan</strong></td>
<td>13.2% (0.4-24.7%)</td>
<td>2.9Gy (1-5.1Gy)</td>
<td>3.3% (0-7.1%)</td>
<td>1.2Gy (0.8-1.7Gy)</td>
</tr>
<tr>
<td><strong>p-value</strong></td>
<td>0.004</td>
<td>0.004</td>
<td>0.008</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Grade 3 dermatitis developed in 4 patients (22%), which was the only grade 3 toxicity. All patients developed grade 2 dermatitis; other acute grade 2 toxicities included fatigue (n= 6) and esophagitis (n=5). No grade 4+ toxicities developed. Dermatitis resolved by 1 month after PT for all but 1 patient who developed cellulitis (grade 2).
Conclusion: PT for RNI after mastectomy or BCT significantly improves cardiac dose especially for left-sided patients and lung V5 and V20 in all patients without excessive acute toxicity. PT simultaneously improves target coverage for the IMN and level II axilla, which may positively impact long-term survival in breast cancer patients.
Title: Safety of eribulin mesylate and concomitant palliative radiotherapy for metastatic breast cancer: A single-center experience

Icro Meattini¹, Vieri Scotti¹, Carla De Luca Cardillo¹, Beatrice Detti¹, Vanessa Di Cataldo¹ and Lorenzo Livi¹.
¹Radiotherapy-Oncology Unit, Florence University Hospital, Florence, Italy.

Body: Background. Eribulin mesylate is a recently approved new therapeutic option for patients with metastatic breast cancer (BC). It is a structurally simplified synthetic analogue of halichondrin B that inhibits the growth phase of microtubule dynamics and sequesters tubulin into non-productive aggregates, inhibiting microtubule polymerization, and inducing irreversible mitotic block at G2-M phases and apoptosis.

Three phase II trials of eribulin in chemotherapy pretreated advanced BC patients were completed. In all these studies, eribulin showed a manageable tolerability profile, with most of the common drug-related adverse events being neutropenia, fatigue, alopecia, nausea, and anemia, according to the phase I trials findings. Eribulin was also associated with an overall low incidence of peripheral neuropathy.

When new chemotherapy options become available, one of the most important questions regarding the sequence of treatments is to address the safety of concomitant radiation treatments. Recently has been reported a clinical case treated with eribulin mesylate and whole-brain radiotherapy (RT) in a heavily pretreated patient with multiple visceral and bone metastases from triple negative breast cancer.

In our knowledge this is the first analysis focusing on the safety of eribulin in patients concomitantly treated with palliative RT.

Methods. All patients where heavily pretreated for metastatic breast cancer (≥4 previous chemotherapy lines), including anthracyclines and taxanes-based regimens. Patients received eribulin mesylate (1.4 mg/m2 i. v., on days 1 and 8, of a 21-day cycle).

Tumor response was assessed using the Response Evaluation Criteria in Solid Tumors (RECIST), every 8 weeks, or sooner if disease progression was suspected.

Safety was assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. Measures of bone-pain scores and analgesic consumption were evaluated at each visit, using a patient-rated scoring system.

Results. Concerning 25 consecutive treated patients, median performance status at baseline was 2 (range 1-3). A median of 4 cycles of eribulin (range 2-10) were administered. All patients received palliative bone RT during eribulin treatment. No suspension or delay in chemotherapy administration was necessary. 20/25 cases underwent spinal bone irradiation; 8 Gy in single fraction was given in 13/25 patients, 12 cases received 20 Gy in 5 fractions.

The overall response rate was 24% and the clinical benefit rate was 40%. Toxicity was manageable and in line with main published series; the most frequent grade 3 hematologic adverse events were neutropenia (56%), and anemia (20%).

Bone pain scores fell within a few weeks and remained below baseline value throughout the analysis. The mean pain score was 2 at baseline and 0.7 at the end of observation period. The mean analgesic score was 1.8 at baseline and 1.6 at the end of follow-up.

Conclusions. Our clinical practice experience supports the evidence that eribulin has clinical activity and is characterized by a manageable safety profile, also when combined to palliative RT. Concomitant treatment is feasible also in patients heavily pretreated, and with poor performance status.
Title: Comparison of cardiac dose between accelerated partial-breast irradiation and whole-breast irradiation in breast cancer patients

Kazuhiko Sato¹, Yoshio Mizuno¹, Hiromi Fuchikami¹, Naoko Takeda¹, Takahiro Shimo², Jun Kubota², Yuko Inoue³, Hiroshi Seto⁴ and Masahiro Kato². ¹Tokyo-West Tokushukai Hospital, Akishima, Tokyo, Japan; ²Tokyo-West Tokushukai Hospital, Akishima, Tokyo, Japan; ³Inoue Ladies Clinic, Tachikawa, Tokyo, Japan and ⁴Seto Hospital, Tokorozawa, Saitama, Japan.

Body: [Background] Breast-conserving surgery (BCS) followed by whole-breast irradiation (WBI) has now become the standard treatment for early-stage breast cancer. However, WBI is associated with an increased risk of coronary events, especially in patients with preexisting cardiac risk factors. In radiotherapy (RT), the highest dose is likely delivered to the left anterior descending artery (LAD), which is the typical site for ischemic heart disease. We initiated a prospective, observational study on accelerated partial-breast irradiation (APBI) using multicatheter brachytherapy after BCS. In this study, we compared the radiation dose to LAD between patients receiving APBI and those receiving WBI.

[Methods] The study participants included a cohort selected from consecutive patients who underwent BCS followed by RT since November 2007. In the WBI group, patients received 50 Gy in fractions of 2 Gy to the entire breast. APBI was initiated on the day of primary surgery in the form of multicatheter brachytherapy, at a dose of 32 Gy in 8 fractions. The planned target volume was defined as the estimated tumor volume plus a 20-mm margin. Dose distribution analysis was performed on the basis of postoperative CT using dose–volume histograms. LAD was outlined from its origin to each visible end using the planning CT images. First, the mean and maximal total doses to LAD were calculated. Second, the radiotherapeutic biologically effective dose of APBI was adjusted to that of WBI for comparisons between the two different RT schedules.

[Results] Of the 359 consecutive patients who underwent BCS followed by RT, we retrospectively reviewed 182 patients for radiation dose to LAD. The 82 patients receiving WBI were randomly selected; 42 patients had right breast cancer and 40 had left breast cancer. We selected 100 consecutive APBI patients with left breast cancer treated between September 2009 and December 2013 because the LAD dose is considered to be virtually zero in right breast cancer patients. In the WBI patients, the mean and maximal total LAD dose were significantly higher in left breast cancer patients (2.1 ± 0.8 and 8.2 ± 1.2 Gy, respectively) than in right breast cancer patients (0.4 ± 0.02 and 0.6 ± 0.03 Gy, respectively; p < 0.0001). Among the APBI patients, the total LAD doses were influenced by tumor location. The mean and maximal total LAD doses were significantly higher in patients with inner quadrants or central tumors (2.5 ± 0.2 and 4.4 ± 2.5 Gy, respectively) than in those with outer quadrant tumors (1.0 ± 0.1 and 2.1 ± 0.3 Gy, respectively; p < 0.0001). After adjustment for the total LAD dose, the mean and maximal total LAD doses were significantly decreased in APBI patients with outer quadrant tumors (1.1 ± 0.2 and 2.4 ± 0.4 Gy, respectively; p < 0.0001), but not in those with central and inner quadrant tumors (2.9 ± 0.3 and 5.4 ± 0.6 Gy, respectively).

[Conclusions] Our results show that APBI may decrease the risk of coronary artery disease, especially in patients with outer quadrant tumors in the left breast. Although APBI should be carefully interpreted until mature phase-III data are available, the risk of ipsilateral breast tumor recurrences and LAD dose must be considered together while administering RT after BCS.
Title: Clinical outcomes according to elective nodal irradiation and molecular subtypes in high risk N1 breast cancer patients

Jeong Il Yu¹, Won Park¹, Doo Ho Choi¹, Seung Jae Huh¹, Seok Jin Nam¹, Seok Won Kim¹, Jeong Eon Lee¹, Won Ho Kil¹, Young-Hyuck Im¹, Jin Seok Ahn¹, Yeon Hee Park¹ and Eun Yoon Cho¹. ¹Samsung Medical Center, Seoul, Korea.

Body: Objectives: To evaluate the clinical outcomes according to the application of an elective nodal irradiation (ENI) and molecular subtypes in high-risk pathologic N1 (pN1) breast cancer.

Methods: We performed a retrospective comparison study with high-risk pN1 patients who received curative resection followed by chemotherapy at Samsung Medical Center from January 2009 to June 2011. High risk was defined as having more than two of the following risk factors: ≥2 axillary lymph nodes (ALNs) or level II ALN metastasis, lymphovascular invasion and extracapsular extension. We compared clinical outcomes according to the application of ENI.

Results: Among 278 patients, 159 patients received ENI while 119 did not and their characteristics were displayed.

Table 1. Characteristics of 278 pathologic N1 breast cancer patients according to the usage of elective nodal irradiation (ENI).

<table>
<thead>
<tr>
<th>Variables</th>
<th>no ENI (n=119)</th>
<th>ENI (n=159)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>48</td>
<td>48</td>
<td>0.50</td>
</tr>
<tr>
<td>Range</td>
<td>27-80</td>
<td>27-71</td>
<td></td>
</tr>
<tr>
<td>Surgery (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRM</td>
<td>90 (75.6)</td>
<td>2 (1.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BCS</td>
<td>29 (24.4)</td>
<td>157 (98.7)</td>
<td></td>
</tr>
<tr>
<td>Pathology (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDC</td>
<td>116 (97.5)</td>
<td>153 (96.2)</td>
<td>0.74</td>
</tr>
<tr>
<td>Other</td>
<td>3 (2.5)</td>
<td>6 (3.8)</td>
<td></td>
</tr>
<tr>
<td>T stage (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>56 (47.1)</td>
<td>81 (50.9)</td>
<td>0.55</td>
</tr>
<tr>
<td>T2</td>
<td>63 (53.9)</td>
<td>78 (49.1)</td>
<td></td>
</tr>
<tr>
<td>Histologic grade (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>84 (70.6)</td>
<td>102 (64.2)</td>
<td>0.30</td>
</tr>
<tr>
<td>3</td>
<td>35 (29.4)</td>
<td>57 (35.8)</td>
<td></td>
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<tr>
<td>Nuclear grade (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>79 (66.4)</td>
<td>90 (56.6)</td>
<td>0.11</td>
</tr>
<tr>
<td>3</td>
<td>40 (33.6)</td>
<td>69 (43.4)</td>
<td></td>
</tr>
<tr>
<td>Dissected ALN number</td>
<td>Median</td>
<td></td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>19</td>
<td>17</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>2-61</td>
<td>5-48</td>
</tr>
<tr>
<td>Estrogen receptor (%)</td>
<td>Positive</td>
<td>92 (77.3)</td>
<td>124 (78.0)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>27 (22.7)</td>
<td>35 (22.0)</td>
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<tr>
<td>Progesterone receptor (%)</td>
<td>Positive</td>
<td>87 (73.1)</td>
<td>123 (77.4)</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>32 (26.9)</td>
<td>36 (22.6)</td>
</tr>
<tr>
<td>HER-2 amplification (%)</td>
<td>Positive</td>
<td>33 (27.7)</td>
<td>24 (15.1)</td>
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<tr>
<td></td>
<td>Negative</td>
<td>86 (72.3)</td>
<td>135 (84.9)</td>
</tr>
<tr>
<td>Hormonal therapy (%)</td>
<td>No</td>
<td>29 (24.4)</td>
<td>31 (21.9)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>90 (75.6)</td>
<td>128 (78.1)</td>
</tr>
<tr>
<td>Chemotherapy (%)</td>
<td>No</td>
<td>13 (10.9)</td>
<td>5 (3.1)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>106 (89.1)</td>
<td>154 (96.9)</td>
</tr>
<tr>
<td>Trastuzumab (%)</td>
<td>No</td>
<td>5 (15.2)</td>
<td>1 (4.2)</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>28 (84.8)</td>
<td>23 (95.8)</td>
</tr>
</tbody>
</table>
During follow-up, 21 patients (6 in ENI and 15 in no ENI) had recurrence, and loco-regional recurrence developed in 8 patients, 6 of whom had not received ENI.

Table 2. Patterns of recurrence according to ENI and molecular subtype

<table>
<thead>
<tr>
<th>Site</th>
<th>No ENI</th>
<th>ENI</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Recurrence (%)</td>
<td>n</td>
</tr>
<tr>
<td>Loco-regional-n</td>
<td>119</td>
<td>6 (5.0)</td>
<td>159</td>
</tr>
<tr>
<td>Luminal A</td>
<td>36</td>
<td>0 (0.0)</td>
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<tr>
<td>Luminal B</td>
<td>57</td>
<td>4 (7.0)</td>
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<tr>
<td>HER-2 enriched</td>
<td>12</td>
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<tr>
<td>Triple negative</td>
<td>14</td>
<td>2 (14.3)</td>
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<tr>
<td>Distnat-n</td>
<td>119</td>
<td>10 (6.5)</td>
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<tr>
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<td>36</td>
<td>2 (5.6)</td>
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<tr>
<td>Luminal B</td>
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<td>HER-2 enriched</td>
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<tr>
<td>Triple negative</td>
<td>14</td>
<td>4 (28.6)</td>
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In both univariate and multivariate analysis, ENI, adjuvant chemotherapy and endocrine therapy were the significant prognostic factors in recurrence-free survival (RFS). ENI showed higher RFS than no ENI when they were analyzed according to molecular subtypes, except HER-2 enriched. RFS was 96.9% at 3-years in luminal A patients with no ENI.

**Conclusions:** The application of ENI may improve RFS in patients with high-risk pN1 breast cancer, so ENI should be considered in those patients. Close observation without ENI might be an option in patients with luminal A who had received optimal systemic managements.
Title: Skin toxicities of obese African American breast cancer patients treated with hypofractionated radiation therapy

Arun G Paul¹, Amy Collins², Gregory Dyson² and Neha Amin³. ¹Detroit Medical Center, Detroit, MI; ²Barbara Ann Karmanos Cancer Center, Detroit, MI and ³Wayne State University, Detroit, MI.

Body: Objective: Hypofractionated radiation therapy (HFRT) for early stage breast cancer is an established treatment option with equivalent cancer outcomes and better cosmetic results than standard fractionation. There is limited published information about expected skin changes of African-American breast cancer patients (pts) undergoing HFRT. While HFRT is not recommended for women with large breasts, the use of prone position may allow for homogeneous HFRT plans. We prospectively monitored and reported on skin changes in African-American pts who received HFRT for their breast cancer.

Methods: A retrospective analysis at a single institution from 12/2012 to 08/2013 was performed to identify early stage breast cancer pts who underwent breast conservation surgery and received adjuvant whole-breast HFRT. An assessment form had been created to prospectively document weekly changes in radiation dermatitis (CTCAE V.4 Grade 0-4) and hyperpigmentation (none, faint, moderate, severe). Photographs of the treated area were collected before and at the end of treatment. Treatment planning guidelines were per RTOG 1005.

Results: There were 15 African-American pts with Tis-T2N0M0 breast cancer who were treated with HFRT to a dose of 4256 cGy in 16 fractions (266 cGy per fraction) followed by a lumpectomy cavity boost of 1000-1250 cGy (250cGy per fraction). There were 12 (80%) pts with right-breast cancer and 6 (40%) who were treated in the prone position. The median age was 61 (36-70). The median body mass index (BMI) for the pts treated in the prone position (42.2 [36-54]) was greater than the median BMI for pts treated in the supine position (29.7 [26-43]). The median breast volume of the prone pts (2335cc [2163-3369]) was more than twice the median volume of supine pts (920cc [231-1459]). The median separation distance for prone and supine pts were 25.1cm (17.5-31.2) and 22.5cm (17.5- 31.8), respectively. Radiation dermatitis: None (6%), Grade 1 (60%), Grade 2 (34%), Grade 3 (none). Only three pts had desquamation:1 pt had dry, and two pts had both dry and minimal moist desquamation in the infra-mammary fold. Hyperpigmentation: none (6.5%), faint (40%), moderate (47%), severe (6.5%). The areas of severe hyperpigmentation correlated to areas of desquamation. The median DMax, mean heart dose, and ipsilateral lung V20Gy were 107.2% (105.2 – 109.9), 60 cGy (29 – 544), and 8.25% (0.0 – 23), respectively.

Conclusion: African-American pts treated with HFRT experience minor skin toxicities with mostly Grade 1 dermatitis and moderate hyperpigmentation changes. Obese African-American pts can safely be treated with HFRT if they can be placed in the prone position. Some pts with a separation of > 25cm can be safely and effectively treated with HFRT.
Title: Phase II trial of hypofractionated post mastectomy radiation

Matthew M Poppe¹, Bruce G Haffty² and Atif J Khan². ¹Huntsman Cancer Hospital, University of Utah, Salt Lake City, UT and ²Rutgers Cancer Institute of New Jersey & Rutgers Robert Wood Johnson Medical School, New Brunswick, NJ.

Body: Purpose: The efficacy of irradiating the chest wall and draining lymph nodes after mastectomy in improving local regional control has been firmly established by multiple trials comparing mastectomy alone to mastectomy with postoperative radiation. The currently accepted standard of care involves 25 fractions of chest wall and nodal radiation plus an optional 5 fraction scar boost. Given recent publications with intact breast radiation demonstrating equivalence with shorter course radiation treatment, we sought to establish a short course of radiation for women post mastectomy that would be equivalent to conventional radiation in terms of local control and complications.

Methods: Starting in 2011, Stage II and III, unilateral breast cancer patients were prospectively consented to participate in our study at Rutgers Cancer Institute of New Jersey and the University of Utah. We designed a phase II non-inferiority trial, with plans to enroll 65 patients, allowing for a 18% loco-regional recurrence rate if the true rate is 10%. Patients were allowed to receive chemotherapy before or after adjuvant radiation with radiation starting 14-63 days after surgery or adjuvant chemotherapy. Patients underwent 3D simulation and were treated with ≥ 6MV photon radiation using 3D conformal tangents or intensity modulation. Regional nodal radiation was included when clinically appropriate with inclusion of internal mammary nodes at the treating provider’s discretion. The prescription dose was prescribed at 333cGy per day to the chest wall and regional nodes for 11 daily fractions followed by a recommend scar boost of 333cGy per day for 4 fractions. Chest wall PTV was keep within 90-115% of the prescribed dose, the maximum brachial plexus dose was keep below 3919cGy (107%) and no portion of the heart could receive >2Gy/day. The maximum dose of the boost field was kept to 120% of the boost prescription. Any breast reconstruction technique was allowed, per institutional standard.

Results: 48 patients have thus far been enrolled with a median follow up interval of 17 months. Looking at the 43 patients with > 4 months of follow-up, we have only seen a 4.7% grade 3 or greater acute or late toxicity rate, with one grade 3 wound complications, and one grade 3 fibrosis. There has been one chest wall recurrence, for a 2.3% local recurrence rate. Conclusion: Early results would appear to show that hypofractionated post mastectomy radiation appears to be safe and feasible. Acute toxicity is less than anticipated, however it is too early make any conclusions on efficacy or late effects, with only 48 out of 65 patients enrolled. We hope to use the results of this phase II trial to design a randomized prospective trial comparing hypofractionated post mastectomy radiation to standard fraction.
Title: Effect of margin width on local recurrence in invasive lobular carcinoma treated with multimodality therapy

Yasuaki Sagara, William T Barry, Ines Vaz-Luis, Fatih Aydogan, Jane E Brock, Eric P Winer, Mehra Golshan and Otto Metzger-Filho. Brigham and Women's Hospital, Dana-Farber Cancer Institute, Boston, MA and Dana-Farber Cancer Institute, Boston, MA.

Body: Background: Recent consensus guideline on margins for breast conserving surgery recommends the use of no ink on tumor as the standard for an adequate margin. Current recommendation extends to invasive lobular carcinoma (ILC), however the data in this subset is limited by small numbers. In the present analysis we sought to evaluate the influence of margin status on outcomes in ILC and mixed tumors.

Methods: We performed retrospective cohort study and reviewed 809 eligible patients diagnosed with ILC (337 with pure ILC; 472 with mixed ILC) with Stage I –III treated at Dana Farber/Brigham and Women’s Cancer Center (DFBWCC) between May 1997 and Dec 2007. Clinico-pathologic data was extracted following the Clinical Research Information Systems (CRIS) Database procedures and manually reviewed to confirm inclusion and details of margin status. Margin status was defined using the last ASCO/ASTRO/SSA consensus guidelines criteria. Analysis results were considered to be statistically significant when the two-tailed p-value was <0.05.

Results: Breast conservation was performed in 399 patients (49%). Margin status at the initial attempt for breast conservation was defined as follows: 180 (45%) negative, 64 (16%) positive, 71 (18%) ≤ 1mm margin, and 84 (21%) close margins (> 1 and < 3 mm). Following initial lumpectomy, 102 (25%) patients underwent additional surgery (96 re-excisions and 6 mastectomies) and residual invasive disease was found in 40 patients. Whole-breast radiation therapy was performed in 376 patients (96%). In multivariate models adjusted for classic clinico-pathologic factors, tumor size (HR= 1.8 95% CI 1.0 to 3.3, p=0.05), multifocality (HR= 2.0 95% CI 1.1 to 3.6, p= 0.02) and ILC subtype (HR= 2.0 95% CI 1.0 to 3.7, p=0.04) were correlated with positive margins, while year of diagnosis, age and pre-surgical MRI findings were not statistically significant.

With 72 months median follow-up, 12 ipsilateral breast cancers (3.1%), 5 other locoregional (1.2%) and 15 distant (3.8%) recurrences were observed after definitive breast conserving therapy. The incidence of locoregional recurrence (LRR) was 4.3% and similar for ILC and mixed ILC (p=0.76). In univariate analysis positive surgical margin was associated with LRR (HR=5.1, p= 0.03) and disease-free survival (DFS) (HR=8.9, p≤ .001), but due to limited number of cases and events this could not be adjusted for other clinico-pathologic prognostic factors in a multivariate model. Close surgical margins, margins within 1mm and multifocality were not associated with increased LRR or worse DFS. Re-excision did not impact on DFS for patients with close margin (p= 0.57) and within 1 mm margin (p= 0.85). By contrast, significant improvement of DFS following re-excision was observed in patients with positive margin (p= 0.01).

Conclusions: Following lumpectomy, local recurrence rates for ILC patients with close surgical margin and ≤ 1mm margin are low and equivalent to those in patients with negative margins. This study supports the validity of using no ink on tumor as the standard for an adequate margin for patients diagnosed with pure or mixed ILC treated with multimodality therapy.
Title: Multidisciplinary breast cancer care is associated with a higher rate of breast conservation in comparison with non-multidisciplinary care

Caitlin L Gomez1, Pin-Chieh Wang1, Nicole A Dawson1, Robyn L Dvorak1, Nova Foster1, Anne Hoyt1, Sara A Hurvitz1, Amy Kusske1, Charles Y Tseng1 and Susan A McCloskey1. 1University of California, Los Angeles, CA.

Body: Background
Mastectomy rates, both therapeutic and prophylactic, are on the rise in the United States. After recent implementation of a multidisciplinary breast clinic for newly diagnosed women at our institution, we sought to examine the impact of multidisciplinary care on surgical decision making.

Materials/Methods
Women with newly diagnosed breast cancer at our institution are referred to a multidisciplinary breast clinic where they are seen by a team of breast specialists (MDC) or to an individual practitioner (non-MDC) for initial consultation. We retrospectively analyzed rates of breast conserving surgery (BCS) and mastectomy among women with newly diagnosed breast cancer seen in either setting. For mastectomy cases, we designated the mastectomy as clinically indicated vs not clinically indicated based on National Comprehensive Cancer Network (NCCN) guidelines for breast conservation. T-test and chi-square were used to examine the comparability between MDC and non-MDC cohorts. Logistic regression was used to evaluate the overall prevalence of BCS among MDC and non-MDC cohorts. Stratification analysis was further conducted to examine BCS rates among women in each cohort receiving neoadjuvant chemotherapy vs up front surgery.

Results
A total of 341 consecutive patients were analyzed, including 202 MDC and 139 non-MDC patients seen in initial consultation between June 2012 and April 2014. The MDC and non-MDC cohorts were statistically equivalent in terms of age, tumor and nodal stage, histology, biomarker status, receipt of neoadjuvant chemotherapy, and proportion with genetic mutations. In the MDC cohort, 66% underwent BCS vs 42% in the non-MDC cohort (p<0.0001). Of those receiving neoadjuvant chemotherapy, 37% in the MDC cohort underwent BCS vs 12% in the non-MDC cohort (p=0.08). Of those proceeding to surgery without neoadjuvant therapy, 70% underwent BCS in the MDC cohort vs 46% in the non-MDC cohort (p<.0001). Among mastectomies performed in the MDC vs non-MDC cohorts, 77% and 41% respectively were clinically indicated (P<.0001). Rates of unnecessary contralateral prophylactic mastectomy were comparable in both groups, 39% (p=0.99).

Conclusions
Breast cancer patients seen in an MDC setting at the time of initial diagnosis are significantly more likely than women seen in a non-MDC setting to undergo breast conservation. We hypothesize that the MDC model of breast cancer care, via facilitation of more informed medical decision making, may be a viable strategy to curtail rising mastectomy rates in the United States.
Title: Residual disease after breast conservation surgery: To excise or not to excise?

Catherine R Campo¹, Erika Reategui¹, Sarah P Cate¹, John Rescigno¹, Priyanka Mittar¹, Alyssa Gillego¹ and Susan K Boolbol¹. ¹Mount Sinai Beth Israel Medical Center, New York, NY.

Body: Background:
The definition of adequate margins after breast conservation surgery for invasive breast cancer has been a highly debated topic. A recent consensus statement by the SSO/ASTRO recommends re-excision only for positive margins, defined as tumor at ink. In light of this consensus statement, we studied the characteristics of patients undergoing breast conservation surgery with subsequent re-excision at our institution in order to examine factors predictive for residual disease (RD+) in the re-excision specimen, as well as a second re-excision.

Methods:
In this IRB approved retrospective chart review of our breast cancer cases from 1998-2013, we reviewed 828 patients who underwent breast conservation surgery with re-excision for invasive breast carcinoma. A close margin was defined as less than 2mm from the inked margins, and a positive margin was defined as tumor at ink. We analyzed various clinicopathologic features. RD+ was missing in 7 patients (0.8%), who were excluded from that analysis. Pearson chi-square was used to test significance in univariate analysis. Binary logistic regression was applied in multivariate analysis of factors significant at p<0.05.

Results:
Overall, 230 patients (28%) had RD+, and 103 patients required a second re-excision (12.4%) due to persistently positive or close margins (44% of RD+). Factors not significant for RD+ were: diagnosis era (before June 2007 vs. after), menstrual status, age < 40, race, excision volume, tumor size, nodal status, grade, histology, lymphovascular invasion, and hormone receptor status. For patients with only close margins, there was no difference in RD+ by margin width, as defined by <0.5mm, 0.5 to <1mm, 1mm to <2mm. Factors significant for RD+ were: disease that was mammographically occult or calcifications only (35%) vs. mass or architectural distortion (25%), p=0.003; positive margin vs. close margin (33% vs. 24%, p=0.004); margin positive or close for DCIS (41%) vs. both DCIS/invasive (30%) vs. invasive alone (17%), p<0.001; presence of DCIS (30% vs. 16%, p=0.001); extensive intraductal component (EIC) (42% vs. 24%, p<0.001); the number of positive or close margins (1: 16%, 2: 26%, 3+: 48%, p<0.001).

In multivariate analysis, the number of positive or close margins was significant, (p<0.001), as was a margin positive or close for DCIS (p<0.001). Factors significant in univariate analysis for a second re-excision were similar as those for RD+. In multivariate analysis, independent factors for second re-excision were: EIC (p=0.007), number of positive or close margins (p<0.001), and a margin positive or close for DCIS (p=0.001).

Conclusions:
Residual disease at re-excision for positive or close margins was present in approximately one-fourth of patients. The probability of residual disease was unrelated to margin positivity or margin width within 2mm. Consideration of re-excision should take into account the burden of intraductal disease in the specimen, its presence at or close to the margin, and the number of positive or close margins.
Title: Intraoperative ultrasound-guided breast conserving surgery for palpable and nonpalpable breast cancer

Guldeniz Karadeniz Cakmak, Ali U Emre, Oge Tascilar, Burak Bahadir and Selcuk Ozkan. 'Bulent Ecevit University School of Medicine, Zonguldak, Turkey and 'Bulent Ecevit University School of Medicine, Zonguldak, Turkey.

Body: Objective: Intraoperative ultrasound guided (IUG) breast conserving surgery (BCS) is being increasingly embraced by breast surgeons worldwide. Real-time sonographic localization provides tumor free margins with low excision volumes and decreases rate of reoperations. We aimed to compare the efficacy of IUG-BCS for palpable and nonpalpable breast cancer with respect to margin status, re-excision rate, tumor free tissue sacrifice and cost-time analysis. The relationship between intraoperative assessment of gross macroscopic and ultrasonographic margins and frozen section results, were also analyzed. Methods: Between 2011 and 2014, IUG-BCS were performed to 208 patients with the diagnosis of in situ or invasive carcinoma. Intraoperative localization protocol includes ultrasound visualization of the lesion, tumor margin determination, and image confirmation of specimen and tumor bed. Sonographic and macroscopic assessment of the surgical margins by surgeon was followed by frozen section analysis of each margin. Moreover, cavity shaved margins from tumor bed were also obtained for permanent section analysis.

Results: Of the 208 patients, 89 (42.8%) had palpable and 119 (57.2%) had nonpalpable tumors. The sensitivity of intraoperative ultrasound localization was 100% (208/208 cases). Patients were on average 55 years old (range, 25-92). There was no difference with respect to patient characteristics including age, menopausal status, personal-family history, oral contraceptive usage, body mass index and tumor localization. Mean tumor size was 1.14 cm for nonpalpable and 2.87 cm. for palpable tumors. Negative margins were achieved in 92.43 % of nonpalpable (110/119) and 91.01% (81/89) of palpable lesions at the initial procedure verified by frozen section analysis. The involved margins were correctly identified by the surgeon via specimen sonography in 95.4% of the cases (15/17). According to frozen section analysis of the 1248 ultrasonographically clear margins, re-excisions were required for 16 margins of palpable and 14 margins of nonpalpable tumors. The overall positive margin rate determined by frozen section analysis was 2.4% (30/1248), with the majority of these patients (12/17) proved to have significant degrees of pure DCIS or mixed invasive ductal with DCIS at final histopathologic evaluation. Three patients (2.5%) with nonpalpable and two patients (2.24%) with palpable tumors required a second operation for either determination of close margins or multifocality at cavity shaved margins, without residual cancer on pathological examination of the reoperative specimens. IUG-BCS resulted in smaller excision volumes in nonpalpable cancers due to their size, as expected. However, calculated resection ratio was found to be similar for both groups. A cost and time analysis determined nothing significant between groups.

Conclusion: IUG-BCS is an invaluable and effective modality for both palpable and nonpalpable breast cancer in obtaining clear surgical margins with optimum resection volumes and reducing re-operations. Furthermore, frozen section analysis of the specimen margins together with shaving cavity margins of the tumor bed for permanent analysis could be a feasible method for minimizing the requirement for reoperations.
**Title:** Intra-operative imaging of surgical margins in breast conserving surgery using localized tissue dielectric response

J Michael Dixon¹, Lorna Renshaw¹, Oliver Young¹, Dhananjay Kulkarni¹, Talha Saleem¹, Moshe Sarfaty², Ramaswamy Sreenivasam², Catherine Kusnick² and Jeremy Thomas¹. ¹Edinburgh Breast Unit, Edinburgh, Midlothian, United Kingdom and ²L S Biopath, Mountain View, CA.

**Body:**

**INTRODUCTION:**
Breast conserving surgery (BCS) removes the tumor with a surrounding margin of normal breast tissue, and to reduce local recurrence clear margins are needed. Involved margins identified by pathology several days later require a second operation. Between 20-40% of BCS patients require re-excision. There is a clear unmet clinical need for effective and efficacious intra-operative, real time assessment of breast tumor margins. The handheld, battery operated TOUCH Imaging device displays spatially resolved images of margins based on tissue spectral dielectric response. This novel imaging device enables surgeons to assess in real-time the status of the excised tissue margins.

**AIM:**
To determine the efficacy and safety of TOUCH Imaging device as used by surgeons during BCS and its health economics.

**METHOD:**
The 1st study of the TOUCH Imaging device was run in the USA with freshly excised breast tissue as part of BCS. The study was run in the pathology lab to determine the efficacy and safety of using the device. The efficacy was determined by surgeons interpretation of device generated margin images compared with permanent section pathology. A follow up study in the UK is currently underway. Surgeons were trained to use the device in the operating room on freshly excised tissue. The imaging was performed after the surgery was completed with no subsequent intervention. The margins were scored as clear or positive and the localization of the abnormality was marked using special ink for specific pathology attention. In the learning phase 18 BCS cases were performed. The main study is being run in two phases and is currently underway. Phase-1 aimed to determine the accuracy of the device when used by individual surgeons in real time had been completed. Analysis is on the way, and phase-2 will start following the confirmation of device accuracy and will involve using the device to direct intra-operatively which margins require further excision. Phase 2 will provide insight to the potential health economics of using the TOUCH Imaging device.

**RESULTS:**
Data from the first study in the USA on 50 BCS specimens, imaging 214 distinct margins are shown in the table. The sensitivity of the device is high; the false negative rate low, the specificity and false positive rates are satisfactory.

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<th>TOUCH Compared to Permanent Section Pathology</th>
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<td>Positive Pathology</td>
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<tr>
<td>Positive TOUCH</td>
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<tr>
<td>Negative TOUCH</td>
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<td>Sensitivity</td>
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<tr>
<td>Specificity</td>
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<td>False Positive Rate</td>
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<td>False negative Rate</td>
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</table>

The training study in the UK on 18 BCS specimen results showed 50% True Positive, 39% True Negative, 11% False Positive, 6% False Negative rates. There were 5 cases (28%) of positive margins < 1 mm and the device images identified 4 with 1 case (6%) being missed.
Results from the ongoing study will be presented.

CONCLUSIONS:
• The TOUCH Imaging device is safe and efficacious.
• It is easy for surgeons to use in the operating room.
• This device has the potential to reduce BCS re-operation rate.
**Body:** Background: A positive margin status after breast conserving surgery (BCS) is one of the strongest predictors of local recurrence of intraductal and invasive carcinoma. As much as 20-50% of all patients with BCS need to undergo a second operation in order to receive free margins. In this study we tested the clinical performance of Margin Probe (Dune Medical Devices), a novel device for intraoperative margin evaluation.

Methods: A prospective clinical trial was performed: The device was applied to 150 lumpectomy specimen from consecutive patients with BCS treated during the first three months in 2013. The re-excision rate was compared to the re-excision rate of a historical group of 156 patients treated with BCS during the first three months in 2012, without the application of the device. We analyzed whether Margin Probe is affected by tumor morphology, grading, size of the tumor, breast density, age, body-mass-index or the use of wire-marker.

Results: Due to the application of Margin Probe the re-excision rate decreased significantly by 51% from 39.7% to 14.6%. In the subgroup of intraductal carcinoma (DCIS) the re-excision rate was reduced about two thirds from 66.7% to 23.1%. In the subgroup of invasive lobular carcinomas the re-excision rate decreased from 37.0% to 19.0%. Margin Probe results are not affected by grading, tumor size, breast-density, age, body-mass-index or wire-marker application.

Conclusion: Margin Probe is an effective tool for detection of positive margins during BCS and significantly decreases the re-excision rate. It is not limited to invasive carcinoma but also detects involved margins in DCIS as well as in invasive lobular carcinoma. It does not interfere with any of the factors we examined.
Title: Translating the concept of intrinsic subtypes into an oncoplastic cohort of more than 1000 patients-predictors of recurrence and survival

Mahdi Rezai¹, Stephanie Kellersmann², Sarah Knispel², Rainer Kimmig¹ and Peter Kern¹². ¹Breast Center Düsseldorf, Düsseldorf, North-Rhine Westfalia, Germany and ²University Hospital of Essen, Essen, North-Rhine Westfalia, Germany.

Body: Introduction:
The concept of breast cancer experienced a paradigm shift by Sørlie T. et al. with intrinsic subtypes as prognostic classification of breast cancer [1]. We validated this concept in a large cohort study of oncoplastic surgery.

Patients and methods:
We analysed 1035 patients with oncoplastic surgery (2004-2009) and survival parameters related to histopathological approximated intrinsic subtypes.
Data were retrieved from customized questionnaires and patients charts. Survival data were determined from cancer registries.

Results:
A total of 944 patients with primary unilateral breast cancer, median age 58 years, were eligible for analysis. At a median follow-up of 5.3 years, LRR was 4.1%, with 5-year-OS of 94.5% and DFS of 90.9%. Stage distribution was as follows: T1a 3%, T1b 12 %, T1c 44,2 % and T2 was 22,1%. 70,4 % of patients were nodal-negative and nodal involvement was predominantly low. Intrinsic subtypes, not T-size, nodal-status, resection margin width nor histopathology, governed the prognosis of this cohort.
Triple-negative and Her2 non luminal breast cancer had the highest recurrence and the lowest survival rates compared to Luminal A: Recurrence TNBC 11,3 %, Her2pos non luminal 9,3 %, Luminal A 2,5 %; Overall survival: TNBC 91,3 %, Her2 non luminal 93,7 %; Luminal A: 96,3 %. Our data confirmed the intrinsic subtype concept on a large basis in oncoplastic surgery.

Body: Background
Breast cancer treatment is a multimodality treatment with different options for the individual patient, aiming to preserve the breast contour in the vast majority of the patients. Different treatment modalities can be used for this goal: primary breast conserving surgery (BCS), BCS after neoadjuvant therapy and immediate breast reconstruction after ablative surgery. Which treatment strategy is chosen may depend on patient preferences as well as patient age. The aim of the present analysis was to describe the rate of breast contour preserving procedures (BCPP) as related to patient age.

Material and methods
All invasive M0 breast cancer patients diagnosed and operated in the Netherlands between 2011 – September 2013 were selected from the national NABON Breast Cancer Audit. Primary BCS rates, BCS rates after neoadjuvant therapy, and rates of immediate reconstructions after ablative surgery were calculated for various age groups: <40, 40 to 50, 50 to 70 and ≥ 70 years.

Results
A total of 34,577 patients were identified. Sixty percent (20,583 patients) underwent BCS. Primary BCS rates increased with age: 47%, 60%, 69% in patients aged <40 years, 40 to 50 years and 50 to 70 years, respectively. In patients aged ≥ 70 the BCS rate was considerably lower (48%). Immediate reconstructions were performed in 16% of all mastectomy patients. An inverse relationship was observed with patient age; the rate of immediate reconstructions decreased from 38% in patients <40 years to 1% in patients ≥70 years of age. A similar decrease in neoadjuvant therapy was seen over the various age groups in patients treated with BCS from 21% to 4% in patients <40 and ≥70, respectively.

The table shows different treatment modalities. After combining these outcomes the rate of breast contour preserving surgery was similar in patients aged <70 (62-73%).

Table 1. Various treatment modalities for invasive breast cancer patients

<table>
<thead>
<tr>
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<th>&lt; 40 years</th>
<th>40 – 50 years</th>
<th>50 – 70 years</th>
<th>≥ 70 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary breast conserving surgery</td>
<td>47%</td>
<td>60%</td>
<td>69%</td>
<td>48%</td>
</tr>
<tr>
<td>Breast conserving surgery and neoadjuvant therapy</td>
<td>21%</td>
<td>14%</td>
<td>6%</td>
<td>4%</td>
</tr>
<tr>
<td>Immediate reconstruction with mastectomy</td>
<td>38%</td>
<td>29%</td>
<td>16%</td>
<td>1%</td>
</tr>
<tr>
<td>Breast contour preserving surgery*</td>
<td>62%</td>
<td>68%</td>
<td>73%</td>
<td>48%</td>
</tr>
</tbody>
</table>

* Proportion of patients with primary breast conserving surgery, breast conserving surgery after neoadjuvant treatment and immediate reconstruction combined with mastectomy.

Conclusions
Patient age affected both the BCS with or without neoadjuvant therapy and the percentage of immediate reconstructions after mastectomy. Overall, more similar percentages in preservation of the breast contour are achieved for various age groups compared to BCS alone. Therefore, combining different treatment modalities into breast contour preserving surgery provides a more complete overview of maintaining the breast contour related to various age groups than BCS alone.
Title: Magnetic sentinel node and occult lesion localization in breast cancer: Initial results of the MagSNOLL trial

Muneer Ahmed\textsuperscript{1,2}, Ashutosh Kothari\textsuperscript{2}, Hisham Hamed\textsuperscript{2}, Sumit Goyal\textsuperscript{3}, Tibor Kovacs\textsuperscript{2}, Anand David Purushotham\textsuperscript{1,2}, Bauke Anninga\textsuperscript{1,2}, Phillipa Young\textsuperscript{3}, Julie Scudder\textsuperscript{2}, Sarah McWilliams\textsuperscript{2}, Sarah Pinder\textsuperscript{1,2}, Quentin Pankhurst\textsuperscript{4}, Ian Monnypenny\textsuperscript{3} and Michael Douek\textsuperscript{1,2}. \textsuperscript{1}King's College London, London, United Kingdom; \textsuperscript{2}Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom; \textsuperscript{3}University of Wales at Llandough, Cardiff, United Kingdom and \textsuperscript{4}University College, London, United Kingdom.

Body: Trial registration:
ISRCTN: 68689512
MREC No: 13/LO/0636
UKCRN ID: 14979

INTRODUCTION:
One-third of breast cancers diagnosed are non-palpable. These breast cancers require localization-guided surgery and axillary staging using sentinel lymph node biopsy (SLNB). We present our experience of the first 20 patients undergoing a novel technique of magnetic-guided lesion localization and concurrent SLNB, avoiding the need for wire-guided localization and radioisotopes.

MATERIALS AND METHODS:
Co-localization of the primary tumour and sentinel lymph nodes (SLNs) using a novel magnetic technique was initially undertaken on a protocol-driven predefined minimum of 10 patients with palpable breast cancers to assess the feasibility of the magnetic tracer to safely localize at the point of injection and concurrently drain to the lymphatics. In order to confirm proof of principle, the subsequent 20 patients with non-palpable breast cancers were analysed. An ultrasound-guided intra-tumoral injection of magnetic tracer was performed (0.5 mL Sienna+, Endomagnetics Ltd, UK). Once successful lesion localization was confirmed (peak magnetometer counts retained at the site of injection), the technique was undertaken in patients with non-palpable breast cancers awaiting wide local excision and SLNB. All patients underwent SLNB with the magnetic and combined technique (radioisotope and Patent Blue Dye).

RESULTS:
A total of 33 patients were recruited and 1 patient excluded due to breach of the trial protocol. This left a remainder of 32 patients for consideration, of which 12 patients (1 bilateral) possessed palpable breast cancer and 20 patients non-palpable breast cancer. Peak magnetometer counts were retained at the site of injection in all palpable (n=13) and non-palpable (n=20) breast cancers. Re-excisions (second operations) for involved margins were performed in 2 patients with non-palpable breast cancers (10%). The mean volume of excised specimens was 49 cm\textsuperscript{3} (SD 30.6). The SLN identification rate for the magnetic technique alone was 84\% (28/33) overall and 85\% (17/20) for non-palpable lesions. For the combined technique (radioisotope and blue dye) the SLN identification rate was 97\% (32/33) overall and 100\% (20/20) for non-palpable lesions. When the magnetic technique is combined with blue dye, the SLN identification rate overall was 97\% (32/33) and for non-palpable breast cancers was 95\% (19/20). The mean number of sentinel nodes excised was 1.75 versus 2.05 for the magnetic and combined techniques respectively.

CONCLUSION: Magnetic lesion localization and concurrent SLNB is a feasible technique. Further optimisation and validation of this technique, in a larger trial, is required.
Title: Anticipating ACA's impact on breast cancer screening for medically underserved women reached through the Avon Breast Health Outreach Program: Understanding the Massachusetts experience

Lindsay Senter¹, Marvin R Aliaga¹, Yixin Hu², Kelly Morrison Opdyke¹, Kathryn Gates-Ferris¹ and Marc Hurlbert³. ¹Cicatelli Associates Inc (CAI), New York, NY; ²Mailman School of Public Health, Columbia University, New York, NY and ³Avon Foundation for Women, New York, NY.

Background
The Avon Breast Health Outreach Program (BHOP) supports community-based organizations to conduct education and outreach to link low-income and uninsured women to routine breast cancer screening and care. Through the Affordable Care Act (ACA), the number of uninsured individuals is expected to decrease as of 2014. While ACA implementation varies by state, BHOP grantees may benefit from understanding the health reform experience in Massachusetts (MA) in 2006 and its impact on the number and proportion of uninsured clients served over time.

Objective
Describe changes in client volume, health insurance status, and demographics following implementation of health reform in MA to anticipate upcoming impact of ACA on BHOP grantees.

Methods
Confidential client intake records of continuously funded BHOP grantees from 2004-2012 were analyzed; dataset included females aged 40-64, recruited for breast cancer screening. We compared records for 11,199 clients served by 4 ‘MA’ BHOP programs with 283,720 clients served by 52 ‘control’ agencies funded in the US during the same time period. We examined trends across years in agency-specific rates of health insurance coverage, and client volume adjusting for annual funding. Changes in key demographic characteristics over time were also analyzed.

Results
The proportion of uninsured MA clients decreased dramatically from 46.6% to 6.2% from 2004-2012, with the biggest decrease occurring in the 3 years following health reform (42.1% to 13.0% from 2006-2009); as compared to only a slight drop among controls from 2004-2012 (69.3% to 67.4%). After adjusting for changes in annual BHOP funding, MA experienced a 74% increase in client volume from 2006-2009, compared with a 15% increase among controls. From 2004-2012, the mean age of clients (53 years) remained stable and similar for MA and controls. The proportion of racial/ethnic minorities served increased considerably in MA, from 62.0% to 77.7%, versus a smaller increase of 60.1% to 63.0% in controls. Likewise, the proportion of clients born outside the US rose from 37.2% to 50.7% in MA, compared with 33.7% to 36.6% among controls. However, the proportion of low-income clients (annual household income <$25,000) decreased overall from 90.4% to 83.1% in MA, and from 83.1% to 79.1% for controls.

Conclusion
In BHOP, Massachusetts observed a marked decrease (most noticeably in the 3 years following health reform) in the proportion of uninsured clients served, while overall client volume and the proportion of racial/ethnic minorities and foreign-born women increased.

Discussion
Historically, many BHOP grantees have relied on funding from the CDC’s National Breast and Cervical Cancer Early Detection Program or charity funds to pay for screening services for uninsured clients. As more clients nationally gain access to health insurance, BHOP grantees need to be prepared for the changing landscape and potential increase in client volume, as experienced by MA. Additionally, grantees should plan to update systems that aid in health insurance eligibility determination, assist with enrollment, and strengthen provider partnerships and referral systems that accept multiple types of insurance.
2014 San Antonio Breast Cancer Symposium

Publication Number: P1-17-02
Average Grade: 4.40

Title: Adaptive and acquired mechanisms of resistance to PI3K inhibitors

Sarat Chandarlapaty, Maurizio Scaltriti, Marie Will, Zhiqiang Li, Ana Bosch-Campos, Sarit Schwartz, Vanessa Rodrik-Outmezguine, Michael Berger, Baselga Jose and Neal Rosen. 1Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Background: Activation of PI3K alpha is among the most frequent oncogenic events in breast transformation, commonly occurring through mutations in the p110 alpha subunit of PI3K or amplification of the HER2 receptor tyrosine kinase. Therapeutic targeting of activated PI3K signaling has been made feasible by the development of highly selective and potent ATP competitive inhibitors of p110 alpha some of which are in late stage clinical development. Unfortunately, clinical response to these compounds has not been seen for the majority of patients and when response occurs it is often short-lived. We have hypothesized that relief of feedback regulation of PI3K signaling constitutes a major mechanism for tumor cell adaptation to inhibitors of oncogenes such as PI3K.

Methods: To understand the basis for resistance to PI3K inhibitors we studied the consequences of acute and prolonged PI3K inhibition in patient samples as well as in cell line and murine models of breast cancer. We examined the effects of selective inhibition of PI3K alpha on upstream and parallel signaling pathway components utilizing several phosphoproteomic methods including mass spectrometry, antibody microarrays, and immunoblotting. We further investigated the consequences of PI3K inhibition upon the genome using next generation sequencing.

Results: In the context of tumors with high levels of RTK signaling such as HER2+ breast cancers, inhibitors of PI3K alpha led to only transient inhibition of PI3K/AKT signaling with reactivation of signaling closely coinciding in time with loss of feedback suppression of RTKs such as HER3 and IGF1R. This reactivation of AKT signaling could be blocked using inhibitors of the induced RTKs and this was associated with a marked increase in tumor cell death. A second mechanism of reactivation of PI3K signaling was observed in examining the genomes of tumors where inhibition of PI3K alpha was associated with acquired inactivating mutations in PTEN. Loss of PTEN in such tumors was associated with failure of the PI3K alpha inhibitor to block PI3K/AKT signaling but sensitivity to combined PI3K alpha plus PI3K beta inhibition in laboratory models. Finally, in the context of hormone dependent tumor models, inhibition of PI3K alpha was associated with an increase in estrogen receptor activation. This was observed as increases in ER protein expression, ER phosphorylation, and ER binding to established ER target promoters. Combined inhibition of ER and PI3K alpha was demonstrated to be synergistic in these models in vivo.

Conclusions: Feedback suppression of upstream and parallel signaling pathways poses a major limitation to the antitumor effects of single agent PI3K alpha inhibition. Combination approaches to potently inhibit PI3K alpha, PI3K beta, and either ER or HER3/IGF1R may prove more effective and durable in the clinic.
Title: Impact of a culturally syntonic door-to-door breast cancer early detection intervention

Carmen J Calfa¹, Julie G Wilkinson², Mindy M Williams³, James M Pann⁴, Angela Yehl⁵, Stephanie E Hoogenbergen⁶ and Andrea D Ivory⁶. ¹Memorial Cancer Institute, Hollywood, FL; ²ImmunoSite Technologies, Miramar, FL; ³Memorial Division of Breast Surgical Oncology, Hollywood, FL; ⁴Nova Southeastern University, North Miami Beach, FL; ⁵Nova Southeastern University, Fort Lauderdale, FL and ⁶Women's Breast Health Initiative Florida Affiliate, Miami Lakes, FL.

Body: Introduction. Disparities in the detection of breast cancer persist despite efforts to reach underserved populations and increase mammography utilization. Black and Hispanic women are more likely to be diagnosed at a later stage compared to White women. Lack of health insurance and limited awareness of breast cancer are significant predictors of screening behaviors; other reasons remain poorly understood and warrant further research. Our study presents highlights of the Women’s Breast Health Initiative Florida Affiliate (WBHI) intervention and key findings related to its impact.

Methods. The intervention targeted single-family home neighborhoods with high incidence of late-stage breast cancer, median income at 200% of the poverty level, and access to affordable health clinics. Neighborhood mobile mammography or transportation to local facilities was provided, followed by navigation to assure proper follow up. Eligible women were uninsured, ≥40 years of age and were qualified for an annual screening mammogram.

During 2011-2012

<table>
<thead>
<tr>
<th>Intervention</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Educational Materials</td>
<td>21,079</td>
</tr>
<tr>
<td>Volunteers*</td>
<td>2,025</td>
</tr>
<tr>
<td>Women Interviewed</td>
<td>5,441</td>
</tr>
<tr>
<td>Eligible Women</td>
<td>643</td>
</tr>
<tr>
<td>Appointments Scheduled</td>
<td>581</td>
</tr>
<tr>
<td>Screening Days</td>
<td>15</td>
</tr>
<tr>
<td>Screening Mammograms Completed</td>
<td>409</td>
</tr>
</tbody>
</table>

*culturally relevant & trained

Descriptive and inferential statistical methods were used to analyze participant data divided into 3 groups based on neighborhood racial demographics: “Black” or “Hispanic” data groups had ≥75% Black or Hispanic residents respectively, while the “Other” did not have a predominant race. Post-visit phone surveys of 1,871 participants were conducted within 30 days.

Results. The intervention significantly increased screening rates amongst eligible women from 22% to 64%, had a 0.24% breast cancer diagnosis rate and motivated 59% of the women to propagate awareness. Health insurance coverage rates did not differ significantly between the 3 groups. Notably, amongst the insured, those from “Hispanic” neighborhoods had the highest recent mammogram rate while, amongst the uninsured, those from “Black” neighborhoods had the highest. Neighborhood culture and prior mammogram history impacted the efficacy of the intervention. Among the “Hispanic” and “Other” groups, women with no prior mammograms were less receptive to receiving one compared to those who had prior mammograms. This phenomenon was not observed in the “Black” group. Women’s responses and perceived benefit to the educational package differed according to the neighborhood race and ethnicity:

<table>
<thead>
<tr>
<th></th>
<th>“Black”</th>
<th>“Hispanic”</th>
<th>“Other”</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benefit</td>
<td>70%</td>
<td>85%</td>
<td>88%</td>
</tr>
<tr>
<td>Spread the Word</td>
<td>54%</td>
<td>57%</td>
<td>65%</td>
</tr>
</tbody>
</table>
**Discussion.** WBHI reached the goal of increasing screening rates in this underserved population, thus showing that this type of intervention can be effective. Educational packages presented in a face-to-face format within a door-to-door context were found useful by most women. Our findings highlight disparities beyond a woman’s access to breast healthcare and education by showing that neighborhood culture impacts response to outreach intervention and breast healthcare. This generates the hypothesis that given equal access to care, disparities in using screening mammography will remain. Educational methods further tailored to racial and ethnic characteristics may play a significant role in closing disparity gaps.
Title: Association of BRCA1 mutations with impaired ovarian reserve: A plausible connection between infertility and breast/ovarian cancer risks

Sara B Giordano¹, Navdha Mittal², Kristin Smith² and Mary Ellen Pavone². ¹Medical University of South Carolina, Charleston, SC and ²Northwestern University, Chicago, IL.

Body: Purpose: Mutations in either the BRCA1 or BRCA2 gene are associated with breast and ovarian cancer susceptibility. Lifetime risk estimates for ovarian cancer in the general population indicate that 1.4 percent (14 out of 1,000) of women will be diagnosed with ovarian cancer compared with 15 to 40 percent (150-400 out of 1,000) who have a BRCA1 or BRCA2 mutation. For decades, scientists have been attempting to establish a link between the risk of developing cancer and infertility. One prospective study suggested an association with BRCA1 mutations and occult primary ovarian insufficiency. This study showed a novel association between low response to ovarian stimulation and BRCA1 mutations, suggesting a link between double-strand DNA break repair dysfunction, infertility, and breast/ovarian cancer risks.

If substantiated, this may place BRCA mutation positive women at higher risk for chemotherapy induced ovarian failure. We also know that reproductive factors including low parity and infertility may place women at increased risk for breast cancer. Taking into account that 1% of females suffer from primary ovarian insufficiency and that the underlying mechanism remains unknown most of the time, discovery of susceptibility gene may have implications for understanding the link between infertility and breast/ovarian cancer.

We hypothesize that mutations in the BRCA1 gene will adversely affect ovarian reserve, as measured by AMH levels.

Participants and Methods: Subset analysis of serum samples taken from Northwestern Ovarian Cancer Early Detection Program (NOCEDPP) of reproductive aged women who had testing for the BRCA mutation. Using an IRB approved protocol, the NOCEDPP database was searched for women 18-45 years old who had previous BRCA testing. These women must have also provided consent for their stored serum to be used for research purposes. We excluded those with a BRCA2 mutation, a previous history of cancer and/or cancer treatment, and those with a previous unilateral or bilateral oophorectomy or other ovarian surgery. Statistical analysis was done using parametric and nonparametric testing.

Results: A total of 125 met the criteria for our study. 66 women were BRCA1 mutation-positive and 59 were BRCA mutation-negative. The median age for BRCA1 mutation-positive women was 33.5 years while BRCA mutation-negative patients was 37 years (p<0.05). Body mass index, gravidity, parity, and duration of birth control were similar between the groups. Overall median AMH values were 2.6ng/mL in both groups. However, when broken into age groups, BRCA1 mutation-positive women aged 35-39 had a significantly lower AMH level when compared to BRCA mutation-negative women (median 3.6ng/mL vs 1.3ng/mL, p<0.05).

Conclusion: AMH values were significantly lower in BRCA1 mutation-positive women aged 35-39. Women with the BRCA1 mutation should be counseled regarding this potential decrease in ovarian reserve.
**Title:** Formative evaluation of interventions addressing culturally relevant needs of young breast cancer survivors

Rochelle Shoretz¹, Adina Fleischmann¹, Elana Silber¹, Mary Ann Hall² and Ashani Johnson-Turbes². ¹Sharsheret, Teaneck, NJ and ²ICF International, Atlanta, GA.

**Body:** Background. Young women diagnosed with breast cancer face challenges impacting their quality of life, psychosocial functioning, and reproductive health outcomes. Ashkenazi Jewish women experience a disproportionately high prevalence of genetic mutations in the BRCA1/2 genes linked to a higher incidence of hereditary breast and ovarian cancer, and have unique needs based on genetic risk and cultural factors. Sharsheret, a national not-for-profit organization with an expertise in supporting Jewish young breast cancer survivors (YBCS), developed two interventions, the Peer Support Network (PSN) and Genetics for Life® (GFL), to address the culturally specific needs of Jewish YBCS. In 2011, Sharsheret conducted a formative evaluation to strengthen its existing PSN and GFL interventions.

Methods. Four focus groups were conducted with 27 YBCS having participated in the PSN or GFL. The groups investigated the information and support needs of YBCS of Jewish descent and YBCS perceptions of PSN and GFL content, materials and delivery channels. Two groups (online and in-person) investigated the needs of YBCS, PSN and GFL program content, and PSN and GFL program delivery related to the psychosocial health of YBCS. Two groups (online and in-person) explored YBCS needs, PSN and GFL program content, and PSN and GFL program delivery related to the reproductive health of YBCS. Participants were recruited using in-person methods, email blasts, social media and via Sharsheret website postings. Qualitative data were analyzed using a notes-based thematic analysis.

Results. Across groups, respondents identified the primary information needs for YBCS included resources addressing genetics, side effects and consequences of treatment (e.g., effects on fertility, intimacy and premature menopause) rather than treatment itself. Respondents identified that their support needs included culturally relevant peer support and genetic resources at the time of diagnosis, treatment and after treatment, as well as support for partners, family members and friends. Participants preferred to receive information and support through diverse modalities, including health care providers, the internet, peer supporters, support groups, symposia and teleconferences and mass media. Respondents recommended outreach efforts for the PSN and GFL interventions in locations that attract young people, including pediatricians’ offices, schools, salons, and college campuses, and to clergy, school principals, local Jewish organizations and health care providers. Participants also suggested potential technology-based modifications to PSN and GFL intervention delivery, including online intake forms, a LiveChat option, video testimonials, interactive expert column, online pedigrees and family conference calls with a genetic counselor or clinical staff member.

Conclusion. YBCS identified the PSN and GFL interventions as critical resources attentive to their culturally specific information and support needs. Focus group participants provided strategies to enhance intervention content and reach. Healthcare professionals serving YBCS of diverse backgrounds can utilize the data gleaned from this evaluation to shape culturally relevant interventions and materials to address the unique needs of YBCS.
**Body: Background**

Half of the patient treated with neoadjuvant chemotherapy (NAC) for a large operable breast cancer has no axillary lymph node involvement at the time of surgery. Sentinel lymph node detection (SLND), performed after NAC, must select patient who should be spared of an axillary lymph node dissection (ALND). The application of SLND for staging the axilla after NAC for patient who initially had a proven axillary lymph node involvement remains controversial because of a low detection rate (DR) and a high false negative rate (FNR).

**Objective**

The aim of GANEA 2 trial was to assess the DR and the FNR of SLND after NAC in the particular case of patients with a proven axillary lymph node involvement.

**Patients and Method**

GANEA 2 was validated by scientific and ethical national boards.

Inclusion criteria: FIGO stage T2-T3 infiltrating breast carcinoma, indication of NAC, surgery (radical or conservative) after NAC and signed consent form.

Exclusion criteria: inflammatory cancer, local relapse, previous surgical removal of the tumour, mental disorder, pregnancy or no contraceptive method, contra-indication to NAC, NAC interrupted due to progressive disease.

Design: Diagnosis and indication to plan a NAC, control of inclusion and exclusion criteria, consent form signature, axillary sonography with fine needle cytology before NAC to select patients with a proven lymph node involvement. After NAC patients underwent both SLND, with the combined technique Blue dye and radiolabeled colloid, and complementary ALND.

Pathological procedure: Pathological analysis, of sentinel and non sentinel nodes, carried out according to standard methods and classified according the last American Joint Committee staging system and Sataloff classification.

Studied parameters were detection rate, false negative rate and Sataloff grading on tumor and lymph nodes. We evaluated particularly the likelihood that the FNR in patients with one or more SLN examined was greater than 10%.

Patients with no lymph node involvement before NAC underwent only a SLND with an ALND only in the case of SLN macro-metastasis with a rigorous follow up. They are not part of this abstract.

**Results**

From July 2010, to February 2014, 242 patients from 19 institutions were enrolled, with a proven axillary lymph node involvement before NAC. After NAC, 1/3 had metastasis free axillary lymph node (80/142).

Detection rate was 83.1% (201/242). Half of the patients with a detection failure had an involved ALND. The false negative rate was 14.2% in the whole series but 24.5% in the case of only 1 SLN resected, and 8% in case of more than 1 SLN resected. In case of involved SLN, half of the patients had involved ALND. Considering the node Sataloff scoring, 18 of the 20 false negative cases were grade C or D (n=15 grade C, metastatic disease and therapeutic effect; n = 3 grade D, metastasis and no therapeutic effect).

**Conclusion**

Among patients treated by NAC for a large operable breast cancer with proven involved lymph node before NAC, who had only 1
SLN examined, the false negative rate was 24.5%. SLND with the combined technique, provides a FNR of less than 10% only in the case of 2 or more SLN resected.
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-01-02
Average Grade: 5.40

Title: Methods impacting the false negative rate of sentinel lymph node surgery in patients presenting with node positive breast cancer (T0-T4,N1-2) who receive neoadjuvant chemotherapy – Results from a prospective trial – ACOSOG Z1071 (Alliance)


1Mayo Clinic, Rochester, MN; 2Alliance Statistics and Data Center, Mayo Clinic, Rochester, MN; 3University of Texas MD Anderson Cancer Center, Houston, TX; 4Alliance Statistics and Data Center, Duke University, Durham, NC; 5Magee-Womens Surgical Associates, Pittsburgh, PA; 6University of Wisconsin Hospital and Clinics, Madison, WI and 7Columbia University Medical Center, New York, NY.

Body: Background: The American College of Surgeons Oncology Group (ACOSOG) Z1071 trial (Alliance) reported a false negative rate (FNR) of 12.6% with sentinel lymph node (SLN) surgery after neoadjuvant chemotherapy in women presenting with node-positive breast cancer. Proposed methods to decrease the FNR include clip placement in the positive node at initial diagnosis with confirmation of resection of the clipped node at surgery and inclusion of residual metastatic cells identified by immunohistochemistry (IHC) for cytokeratins and disease measuring <0.2mm on H&E in the definition of node positivity after chemotherapy. Herein we evaluate the impact of these methods on FNR.

Methods: Z1071 was a prospective multi-institutional trial in which women with clinical T0-4,N1-2,M0 breast cancer underwent both SLN surgery and axillary dissection (ALND) after neoadjuvant chemotherapy. The primary endpoint defined nodal metastasis as disease >0.2mm on H&E. Slides were submitted for central IHC staining when H&E-negative, unless performed locally. For this analysis, we expanded the definition of node positivity to include metastases less than 0.2mm on H&E and metastatic deposits identified by IHC.

In 171 cases, a clip was placed in the node at initial biopsy and in these cases the SLNs and ALND were evaluated by x-ray or pathology to document clip location. FNR in the clip cohort is reported using the primary endpoint definition of node positive disease (>0.2mm on H&E).

Results: In the 113 cases where the clip was found in the SLN the FNR was 6.4% (1.8-15.5%). In the 29 cases where the clip was found in the ALND specimen, the FNR was 22.2% (6.4-47.6%).

IHC was available on 470 of 525 patients with cN1 disease and 2 SLNs resected. Using the definition of H&E metastasis >0.2mm, the FNR in these patients was 11.3% (34/301, 8.0-15.4%) which decreased to 8.7% (27/311, 5.6-11.8%) when including any disease <0.2mm. SLNs from 16 patients had disease <0.2mm in size. Seven patients previously classified as false negative SLN changed to true positive with identification of disease in the SLN. Nine patients changed from node-negative to node-positive with the only disease being <0.2mm disease found in the SLN. Nodal pathologic complete response rate changed from 36.0% to 33.8% with the inclusion of metastases <0.2mm.

<table>
<thead>
<tr>
<th>Node positive definition</th>
<th>N</th>
<th>Residual disease identified in SLNs or ALND</th>
<th>FNR (%)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLN metastases &gt;0.2mm by H&amp;E</td>
<td>470</td>
<td>301 (64.0%)</td>
<td>11.3</td>
<td>8.0-15.4</td>
</tr>
<tr>
<td>SLN metastasis any size on IHC or H&amp;E</td>
<td>470</td>
<td>311 (66.2%)</td>
<td>8.7</td>
<td>5.6-11.8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Clip location</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Clip in SLN</td>
<td>114</td>
<td>62 (54.4%)</td>
<td>6.4</td>
<td>1.8-15.5</td>
</tr>
<tr>
<td>Clip in ALND</td>
<td>29</td>
<td>18 (62.1%)</td>
<td>22.2</td>
<td>6.4-47.6</td>
</tr>
<tr>
<td>Clip location unknown</td>
<td>29</td>
<td>20 (69.0%)</td>
<td>14.3</td>
<td>3.0-36.3</td>
</tr>
<tr>
<td>Clip not placed</td>
<td>354</td>
<td>207 (58.5%)</td>
<td>13.5</td>
<td>9.1-18.9</td>
</tr>
</tbody>
</table>

Conclusion: Placement of a clip at initial diagnosis of node positive disease with identification of the clip during the SLN surgery
reduces the FNR. Use of IHC with inclusion of metastases <0.2mm in the definition of residual nodal disease in women with node positive breast cancer after chemotherapy also improves the accuracy of SLN surgery. Use of one or both of these methods should be considered when performing SLN in this setting.
Title: Sentinel node procedure before or after neoadjuvant chemotherapy in clinically node negative or positive patients; results from 3 phase III studies of the Dutch breast cancer trialists’ group (BOOG)

Vivianne C Tjan-Heijnen¹, Birgitte E Vriens¹, Maureen J Aarts¹, Judith R Kroep², Cock J van de Velde², Gerrit-Jan Liefers², Ayoub Charehbili², Petronella G Peer³ and Maaike de Boer¹. ¹Maastricht University Medical Centre, Maastricht, Netherlands; ²Leiden University Medical Center, Leiden, Netherlands and ³Radboud University Medical Center, Nijmegen, Netherlands.

Body: Background
In breast cancer patients treated with neoadjuvant systemic therapy, the timing of the sentinel node (SN) procedure in patients with clinically node-negative disease (cN0) at diagnosis and the role of the SN procedure after neoadjuvant therapy in patients with initially clinical node-positive disease (cN+) still remains to be elucidated.

Methods
Between February 2006 and May 2012, 657 patients were enrolled in three clinical trials on neoadjuvant systemic therapy under the auspices of the Dutch Breast Cancer Research Group (BOOG). In the INTENS and NEO-ZOTAC study, patients were treated with TAC or AC-T neo-adjuvant chemotherapy (doxorubicin (A), cyclophosphamide (C) and docetaxel (T)). In the TEAM IIa trial, patients were treated with neoadjuvant endocrine therapy (exemestane for 6 months). Timing of the SN procedure in cN0 disease mainly depends on local policy and period of inclusion. We compared the pN0 rate after SN procedure performed before versus after neoadjuvant systemic therapy, in patients with cN0 disease at initial diagnosis. Further, we assessed the SN negative and the false-negative sentinel node rate in patients with initially cN+ breast cancer and conversion to cN0 when treated with neoadjuvant systemic therapy. The false-negative rate was obtained by dividing the number of patients who were SN-negative but non-SN positive by the number of patients who had a positive SN or a positive non-SN.

Results
In total, 271 patients (n=93 INTENS, n=107 NEOZOTAC, n= 71 TEAM IIa) underwent a SN procedure (n=233 cN0; n=38 cN+). Of patients with cN0 breast cancer at diagnosis, 131 (56%) underwent the SN procedure before and 102 (44%) after neoadjuvant systemic therapy, with ypN0(sn) or ypN0(i+)(sn) in 90/131 (69%) and 69/102 (68%) patients, respectively. Patients with initially cN+ disease who converted to cN0 disease after systemic therapy had a negative SN in 29% (11/38) of cases. The false-negative rate was 20% (6 of 30). More detailed analyses will be presented at the meeting.

Conclusion
To our knowledge, this is the first study comparing the impact of the timing of the sentinel procedure in patients with cN0 disease. We showed, that irrespective of the timing of the SN procedure - before or after neoadjuvant systemic therapy - in patients with cN0 breast cancer at diagnosis, two-third of patients had a negative sentinel node. In patients who converted after neoadjuvant chemotherapy from cN+ to cN0 the false negative sentinel node rate was 20%.
Correlation of percutaneously biopsied axillary lymph nodes marked with black tattoo ink prior to neoadjuvant chemotherapy with sentinel lymph nodes in breast cancer patients

Nicole Choy1, Jafi Lipson1, Sunita Pal1, Debra Ikeda1, Long Trinh1, Kimberly Allison1, Michael Ozawa1, Amanda Wheeler1 and Irene Wapnir1. 1Stanford University School of Medicine, Stanford, CA.

Body: Introduction:
Sonographic evaluation of the axilla and percutaneous biopsy of abnormal lymph nodes with fine needle aspiration (FNA) or core needle biopsy (CNB) has become more common practice in patients with newly diagnosed breast cancer prior to neoadjuvant chemotherapy (NAC). Sentinel lymph node (SLN) biopsy is considered the gold standard for axillary staging in clinically node negative breast cancer patients. Currently, there is no clear correlation of sonographically detected abnormal lymph nodes and open surgical assessment. We conducted an exploratory pilot study which marked suspicious axillary lymph nodes with black tattoo ink at the time of percutaneous needle biopsy prior to NAC. Black nodes visualized during axillary surgery were evaluated in comparison to SLNs.

Methods:
Breast cancer patients with clinical and/or sonographically suspicious axillary lymph nodes prior to NAC were included in the study. Following FNA or CNB biopsy of node, 0.1 to 0.5 ml of a sterile, highly purified, biocompatible fine carbon suspension (Spot™) was injected into the cortex of the lymph node and adjacent soft tissue. A total of 12 patients were injected with black ink prior to NAC. Intraoperative presence of black pigment was assessed and correlation between sentinel and tattooed nodes were evaluated.

Results:
Nine patients had a positive percutaneous lymph node biopsy prior to NAC. The average number of days that elapsed between injection and to surgery was 130 days. A successful SLN procedure was performed in all patients. A black tattooed node was identified in all patients and correlated to a SLN. 7 patients were down-staged in the axilla and 6 patients did not go onto completion axillary dissection. One patient with a negative SLN had a completion axillary dissection, but no additional positive lymph nodes were found. Four patients with positive SLN had a completion axillary dissection (1 of whom was a false negative percutaneous biopsy). In all four patients, the positive sentinel node contained visible black ink. There was one patient who had an additional positive sentinel node, which was not black. Two axillary dissections contained additional positive nodes.

Conclusion:
Black ink tattooing with sterile black ink (Spot™), successfully marked suspicious lymph nodes prior to NAC. These correlated to a SLN. In node positive patients with a partial response in the axillary lymph nodes following neoadjuvant chemotherapy, previously marked, black-inked node proved to be the persistent positive node. Tattooing of lymph nodes at the time of percutaneous biopsy may improve the accuracy of surgical axillary staging by aiding in the intra-operative identification of previously biopsied nodes.
Title: In patients with breast cancer, pre-operative sentinel node biopsy using intradermal microbubbles and contrast enhanced ultrasound predicts volume of axillary metastases

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Body: Introduction
In patients with early breast cancer, there is a trend towards conservative axillary surgery but there are concerns that patients with high volume axillary metastases may receive sub-optimal axillary treatment leaving them vulnerable to local recurrence. Determining the metastatic status of sentinel lymph nodes (SLN) in the pre-operative/diagnostic period would allow focused surgical planning and facilitate decisions regarding adjuvant therapy early in the patient pathway.

Methods
654 patients with primary invasive breast cancer and a normal grey-scale axillary ultrasound were included in the analysis. Pre-operatively, patients received periareolar intra-dermal injection of microbubble contrast agent, breast lymphatics were visualised by ultrasound and followed to identify axillary SLN. Contrast-pulse sequencing and grey-scale ultrasound modalities were used to image SLN. Sentinel lymph nodes were then subjected to core biopsy. Patients subsequently underwent tumour excision and axillary node clearance (ANC) or surgical sentinel node biopsy (SNB) using blue-dye and isotope +/- completion ANC.

Results
Sentinel lymph nodes were clearly visualised in 605 patients and successfully (B2-B5) core biopsied in 555. The test identified 53% of all SLN metastases with 100% specificity. The negative predictive value was 88%. The prevalence of axillary lymph node metastases was 23%. Given a benign (B2) SLN biopsy result, the post-test probability that a patient had SLN metastases on subsequent surgical excision was 12%. Following ANC, 55% of patients with a malignant (B4/5) biopsy result were found to have high volume axillary disease (2 macro metastases or more) whereas 21% of patients with an initial benign (B2) biopsy result and metastatic cells found at SNB had high volume axillary disease identified after completion ANC (P value less than 0.001). In total, only 2% of patients with a benign (B2) SLN core biopsy result had high volume axillary metastases and half of these had either multifocal cancer or invasive lobular carcinoma.

Conclusions
SLN can be readily identified and biopsied in the breast clinic using intradermal microbubbles and CEUS. In patients with primary invasive breast cancer and a normal grey-scale axillary ultrasound, a benign (B2) SLN core biopsy result may be highly predictive of either no metastases or low volume metastatic disease in the ipsilateral axilla. This group of patients is therefore likely to benefit from axillary conservation and in certain cases, it may be appropriate to completely omit a surgical SNB.
Title: Reoperative sentinel lymph node biopsy for ipsilateral breast tumor recurrence: Impact of axillary surgery and radiation therapy on lymphatic drainage patterns

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Body: Background: While sentinel lymph node biopsy (SLNB) is a well-established method for patients with clinically node-negative primary breast cancer, reoperative sentinel lymph node biopsy (re-SLNB) for ipsilateral breast tumor recurrence (IBTR) still remains controversial. The aim of this study was to evaluate the location of reoperative sentinel lymph nodes (re-SLNs) and the factors related to aberrant drainage patterns after breast conserving surgery (BCS).

Methods: Between April 2005 and August 2013, we performed re-SLNB on 132 patients who developed IBTR with no metastatic lymph nodes in preoperative examination. Both injection of radioisotope (Tc-99m phytate) and preoperative lymphoscintigraphy were performed on 97 patients of them, who were eligible for this retrospective study. We divided these patients into two groups according to their previous axillary surgery: 55 patients with just SLNB or no axillary surgery to the group S, and 42 patients with previous axillary lymph node dissection (ALND) to the group D. The patients in the group S were divided into two subgroups: 19 with adjuvant radiation therapy (RT) and 36 without RT. We compared visualization rates of lymph drainage patterns in these groups statistically by using Chi-square test. We evaluated the relationship between previous local treatments and lymphatic drainage patterns.

Results: Number of cases in which lymphatic drainage was visualized were 52 (94.5%) in the group S and 33 (78.6%) in the group D (P < 0.001). Re-SLNs were observed at ipsilateral axilla in 47 patients (90%) in the group S and 19 patients (58%) in the group D (P < 0.001), at internal mammary (IM) in 9 (17%) and 16(49%) (P < 0.01), and at contralateral axilla in 8 (15%) and 5(15%), respectively. Within the group S, lymphatic drainage was visualized in 34 (94.4%) of 36 patients without RT and 18 (94.7%) of 19 patients who had received RT after BCS. Re-SLNs were visualized at ipsilateral axilla in 34 (100%) and 13 (72%) (P < 0.01), at IM in 5 (15%) and 4 (22%), and at contralateral axilla in 1 (3%) and 7 (39%) (P < 0.001).

Re-SLNB was successfully performed in 62 (94%) of 66 patients whose lymphatic drainage was observed at ipsilateral axilla. Metastases for carcinoma in re-SLNs were found in 8 (13%) of them. At IM, re-SLNB was successful in 15 (60%) of 25, and 2 (13%) of them were positive for cancer. At contralateral axilla, re-SLNB was successful in 8 (62%) of 13 while no metastases were found among them.

Conclusion: Previous ALND affected the visualization rates of re-SLNs. In patients after previous ALND, SLN was more likely to be in IM. Adjuvant RT effected a change in lymphatic drainage patterns, but did not affect the visualization rate of re-SLN. At ipsilateral axilla, the rates of successful re-SLNB and metastases at re-SLNs were similar to SLNB for primary breast cancer. Metastasis to SLN of recurrent cancer is one of the most important information for management of IBTR. However, it was very difficult to predict localization of re-SLN because lymphatic drainage patterns could have been affected by previous treatments. Therefore, we recommend re-SLNB using radioisotope with lymphoscintigraphy in surgery for IBTR.
Title: Does axillary lymph node ratio still have a prognostic value in neoadjuvant setting?

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Body: Background: Axillary nodal status is one of the most important prognostic factor in breast cancer. The lymph node ratio has been reported as an independent prognostic factor because the dissected and involved lymph node could be different across the institutions along with surgical and pathologic procedure. Neoadjuvant chemotherapy has been preferred treatment method in locally advanced breast cancer for the purpose of reducing tumor mass in breast and/or axillary area. Neoadjuvant chemotherapy would downstage axillary nodal status and lower total number of excised lymph nodes compared with upfront surgery. Therefore, there has been emerged question whether the axillary nodal status and lymph node ratio following neoadjuvant chemotherapy could accurately predict the prognosis. To answer this question, we evaluated the axillary nodal status and lymph node ratio as a prognostic factor after neoadjuvant chemotherapy.

Methods: One hundred thirty two patients were eligible in this study between 2005 and 2011. The patients underwent breast surgery after 3 or 6 cycles of anthracycline with or without taxane based chemotherapy according to their axillary nodal status. The cut-off range of lymph node ratio were divided to low (≤0.20, n=45), intermediate (2<, ≤0.65, n=25) and high (<0.65, n=6). Clinical and pathologic factors including hormone status, tumor size, lymphovascular invasion and response to chemotherapy were evaluated and survival results were also investigated with nodal status.

Results: Mean age of total patients was 48 years. Mean follow up period was 40 months. The pathologic complete responses were observed 33% in breast, 25% in axillar, 17.4% in both areas, respectively. Tumor subtype was an independent factor for pathologic response rate. With multivariate analysis, clinical tumor size, pathologic nodal status and the presence of lymphovascular invasion were statistically significant to disease free survival in overall cohort. In node negative patients group (n=57), the hormone receptor status and molecular subtype were associated with the disease free survival using multivariate analysis. Whereas, pathologic tumor size, pathologic nodal status and lymph node ratio, especially lower cut-off limit 0.20, were significant risk factor for disease free survival in node positive (n=75) patients.

Conclusion: Neoadjuvant chemotherapy significantly affect axillary nodal status. Traditional nodal staging has been accepted as important prognostic factor. The response to neoadjuvant chemotherapy depends on tumor subtype. In this study, we proved that nodal ratio could be a candidate as prognostic factor in neoadjuvant setting and 0.20 is acceptable lower cut-off value of lymph node ratio.
Title: Application of the Z0011 criteria on Dutch breast cancer patients

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Body: Background
The American College of Surgeons Oncology Group reported a randomized controlled trial (Z0011-trial) among women with T1-2N0M0 breast cancer treated with breast conserving therapy. It showed that axillary lymph node dissection may be redundant in selected sentinel node positive patients. Though, it raises questions as to the general applicability of these results. Therefore, this study aims to examine the practice changing effect and the clinical relevance of the criteria described in the Z0011 trial, when applied to a Dutch population of patients diagnosed with invasive breast cancer.

Methods
This is a multicenter study including patients with T1-2N0-1M0 invasive breast cancer treated in either of the 10 different hospitals affiliated with the Dutch National Cancer Registry region South in the period between January 2007 and December 2012. All patients underwent a clinical examination, a mammography and sonographic examination of the breast and the ipsilateral axilla. Chi-square analyses or a Fisher’s exact test were used to assess differences in patient and tumor characteristics the Z0011 population and the Dutch cohort. A p-value of ≤0.05 was considered statistically significant.

Results
A total of 11,031 patients had invasive breast cancer, of whom 5368 patients were treated with breast conserving therapy. In 6.8% axillary status was determined by the ultrasound guided lymph node biopsy and in 93.1% axillary metastases were found through the sentinel node procedure after a negative or inconclusive ultrasound. When applying the Z0011 criteria to our study population of node positive patients, 5.7% of all breast cancer patients, equalling 11.8% of patients receiving breast conserving therapy, met the Z0011 criteria.

Conclusion
These results suggest that when applying the Z0011 criteria to all node positive patients, including patients with a positive sentinel node and patients with a positive ultrasound guided lymph node biopsy, only in 5.7% of all invasive breast cancer patients could be spared an ALND and its morbidity. This is a small practice changing effect. Nevertheless, it is worthwhile to consider implementing the findings of Z0011 trial in daily clinical practice.
Contrast enhanced pre-operative axillary staging in breast cancer: A systematic review and meta-analysis

Bauke Anninga¹, Mieke van Hemelrijck¹ and Michael Douek¹. ¹King’s College London, London, United Kingdom.

Background: The current method for identifying axillary metastasis in breast cancer is by performing a sentinel lymph node biopsy (SLNB) procedure. Although highly accurate, this invasive procedure is associated with several adverse events. Additionally, 70% of SLNBs are negative and thus expose many patients to an unnecessary invasive procedure without therapeutic benefit. Contrast enhanced imaging alternatives have the potential to replace SLNB. Several contrast agents have been evaluated of which gadolinium (Gd) and superparamagnetic iron oxide (SPIO) are most frequently documented. We conducted a systematic review and meta-analysis of the accuracy of contrast enhanced magnetic resonance imaging (MRI) techniques for pre-operative axillary staging in early stage, newly diagnosed breast cancer patients.

Materials and methods: We systematically searched the MEDLINE, Cochrane and EMBASE databases for prospective studies that evaluated MRI with contrast for axillary lymph node metastasis detection. Pooled sensitivity and specificity of contrast enhanced MRI were extracted from every study using histological diagnosis as the comparative gold standard. Per patient and per node analyses were undertaken. Statistical analyses were performed using OpenMetaAnalyst.

Results: Searching the databases resulted in a total of 211 abstracts of which 17 were considered for inclusion in the meta-analysis. Quantitative analyses on 14 full-texts and 1 abstract including a total of 493 (n = 11 studies) patients and 1373 (n = 7 studies) lymph nodes were performed. Six studies described the use of SPIO, five described the use of Gd, one study used gadopentetate dimeglumine and one gadofosveset. Overall weighted sensitivity and specificity of contrast enhanced MRI were 84% (95% CI: 77-89%) and 83% (95% CI: 70-91%) respectively, per patient. The sensitivity and specificity for detecting metastases on a lymph node basis were 80% (95% CI: 73-85%) and 96% (95% CI: 94-98%), respectively. In the studies evaluating SPIO enhancement, per patient sensitivity and specificity were 86% (95% CI: 75-93%) and 89% (95% CI: 82-94%), respectively. For studies evaluating Gd enhancement per patient sensitivity and specificity were 84% (95% CI: 74-93%) and 80% (95% CI: 60-92%), respectively.

Conclusion: Contrast enhanced MRI has the highest sensitivity and specificity when SPIO is used. Overall, it is less sensitive but more specific on a per node basis compared to a per patient analysis. Research is needed to optimise the field of view for nodal imaging in order to increase sensitivity and specificity for the detection of nodal metastases.
Title: Prospective evaluation of the reliability of the combined use of two models to predict non-sentinel lymph node status in breast cancer patients with metastatic sentinel lymph nodes: The MSKCC nomogram and the Tenon score – PHRC-NOTEGS study

Roman Rouzier\(^1\), Catherine Uzan\(^2\), Alexandra Rousseau\(^3\), Eugenie Guillot\(^1\), Sonia Zilberman\(^3\), Charles Meyer\(^4\), Pablo Estevez\(^1\), Pierre-François Dupre\(^5\), David Kere\(^6\), Virginie Doridot\(^7\), Gauthier D'halluin\(^8\), Xavier Fritel\(^9\), Nicolas Pouget\(^10\), Chafika Mazouni\(^2\), Tabassome Simon\(^3\) and Charles Coutant\(^10\). \(^1\)Institut Curie, Paris, France; \(^2\)Gustave Roussy, Villejuif, France; \(^3\)APHP, Paris, France; \(^4\)Hôpitaux Civils de Colmar, Colmar, France; \(^5\)CHU Brest, Brest, France; \(^6\)Institut Jean Godinot, Reims, France; \(^7\)Centre Médical République, Clermont-Ferrand, France; \(^8\)Centre Clinical Soyaux, Soyaux, France; \(^9\)CHU Poitiers, Poitiers, France and \(^10\)Centre Georges François Leclerc, Dijon, France.

Body: Background: Several mathematical models have been developed to predict non-SN status in patients with breast cancer with SN metastasis. The Memorial Sloan-Kettering Cancer Center nomogram and Tenon score outperform other methods in academic studies but their exportability at multiple geographic locations and practice settings has never been reported. The purpose of this study was to prospectively evaluate the combined use of the MSKCC nomogram (Memorial Sloan-Kettering Cancer Center) and Tenon score to select, in patients with metastatic sentinel lymph node (SN), those at low risk of metastatic non-SN in whom additional axillary lymph node dissection (ALND) could be avoided.

Material and methods: From January 2011 to July 2012, data on 3157 patients with breast cancer from 65 institutions (university affiliated, general, regional hospital, nonprofit private hospital and private practice) were prospectively recorded (NCT01509963). Selection criteria were patients aged over 18 years old with untreated invasive T1-2 breast cancer with an indication of SN procedure. The primary outcome measure was the false negative rate in patients with both a \(\leq 10\%\) probability of metastatic non-SN with the MSKCC nomogram and a Tenon score \(\leq 3.5\) (i.e. low risk): proportion of patients with metastatic non-SN in whom additional axillary lymph node dissection (ALND) was not mandatory. The hypothesis was a 5%±5% rate in this group of patients. Other patients were considered at high risk. Because of the results of the Z011 and IBCSG 23-01 trials, additional ALND was not mandatory in case of metastatic SN.

Results: Among the 2936 patients, at least one SN was metastatic (isolated tumor cells, micro- or macrometastasis) in 696 patients (23.7%). Among them, 178 did not have completion ALND. Among patients with completion ALND (n=518, 74.4%), 67 (13%), 437 (84%) and 14 (3%) patients were at low, high and undetermined combined risk while 47.5% were at low risk in patients without completion ALND (p<.001). This study did not meet its primary objective as the false negative rate in patients with low risk was 16.4% (11/67) [95% confidence interval: 8.5%-27.5%]. The false negative rate was significantly higher in patients in the high risk group: 33.9% (148/437) [95% confidence interval: 29.6%-38.4%] had non-SN metastasis (p=.004).

Analyzed individually, Tenon score and MSKCC nomogram had complementary performances (number of patients at low risk: 35.3% and 18.5%, false negative rates: 21.9% and 17%, concordance index: 0.63 and 0.65, respectively).

Conclusion: In this controlled prospective trial, metastatic SN patients with both a \(\leq 10\%\) probability of metastatic non-SN with the MSKCC nomogram and a Tenon score \(\leq 3.5\) had completion axillary dissection in 47% of cases: in these patients, the false negative rate was statistically over 5% and did not reach the primary endpoint. Further evaluation is warranted to determine the outcome of patients with and without axillary dissection.
Title: Impact of the ASOCOG Z0011 trial on a multi-institutional mixed practice setting

Swapnil D Kachare¹, Nasreen A Vohra¹, Kathryn M Verbanac¹, Timothy L Fitzgerald¹, Emmanuel E Zervos¹ and Jan H Wong¹.
¹East Carolina University, Greenville, NC.

Body: Background
The ASOCOG Z0011 trial is widely considered to be practice changing, but reports to date on the adoption of Z0011 are largely confined to tertiary academic institutions with breast cancer surgical specialty services. The aim of this study was to assess the impact of Z0011 on the care of breast cancer patients in a mixed academic/ non-academic practice setting.

Methods
All patients who underwent surgical treatment for breast cancer in the Vidant Healthcare System, a multi-county network, between the years January 1, 2009 and September 20, 2013 were identified. Patients with Stage IV disease and ductal carcinoma in situ were excluded.

Results
One thousand six hundred four patients met initial inclusion criteria, 661 in the pre-Z0011 period and 973 in the post-Z0011 period. The majority of patients were female (99%) and Caucasian (65%). Infiltrating ductal cancer (67%) was the most common tumor type. Tumors on average were 23mm in size with the majority being ER (74%), PR (63%) positive and Her2 (82%) negative. Most patients had node negative disease (73%). Table 1 summarizes the characteristics of the pre- and post-Z0011 study populations.

Patient and Tumor Characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pre-Z0011</th>
<th>Post-Z0011</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female (%)</td>
<td>656 (99.2)</td>
<td>968 (99.4)</td>
<td>0.86</td>
</tr>
<tr>
<td>Race (%)</td>
<td>0.49</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>448 (67.8)</td>
<td>642 (65.9)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>205 (31.0)</td>
<td>314 (32.2)</td>
<td></td>
</tr>
<tr>
<td>Mean size (mm)</td>
<td>19.9</td>
<td>22.3</td>
<td>0.06</td>
</tr>
<tr>
<td>Histology (%)</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltrating ductal cancer</td>
<td>434 (65.7)</td>
<td>663 (68.1)</td>
<td></td>
</tr>
<tr>
<td>Infiltrating lobular cancer</td>
<td>30 (4.5)</td>
<td>62 (6.4)</td>
<td></td>
</tr>
<tr>
<td>ER Positive (%)</td>
<td>484 (73.3)</td>
<td>722 (74.2)</td>
<td>0.92</td>
</tr>
<tr>
<td>PR Positive (%)</td>
<td>406 (61.5)</td>
<td>622 (63.9)</td>
<td>0.61</td>
</tr>
<tr>
<td>Her2 Negative (%)</td>
<td>304 (78.4)</td>
<td>897 (83.5)</td>
<td>0.01</td>
</tr>
<tr>
<td>Triple Negative Subtype (%)</td>
<td>83 (23.9)</td>
<td>168 (18.9)</td>
<td>0.07</td>
</tr>
<tr>
<td>Node Positive Status</td>
<td>175 (27.5)</td>
<td>926 (26.7)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

A total of 142 patients met Z0011 criteria, with T1-2 disease, underwent partial mastectomy and had a positive SNB. There were a similar number of patients who met Z0011 criteria in the pre- (n=63) and post-Z0011 (n=79) periods, p>0.05. In the post-Z0011 period, patients were more likely to undergo SNB alone (56 vs. 19%, p<0.001). Post-Z0011 the decrease in ALND was in those who had immediate ALND (34% vs. 71%), with no substantive change in the delayed group (10.1 vs. 9.5%), p<0.001. Patient who underwent SNB alone had smaller tumors (22 vs. 32mm, p<0.001), but similar histologic subtypes (p=0.60) and receptor status (p≥0.15) as compared to patients who underwent SNB with ALND. Over the entire study period patients in an academic practice
were less likely to undergo SNB alone (31 vs. 43%). In the post-Z0011 period, patients cared for in an academic practice had an increase in SNB alone from year 1 (2012) to year 2 (2013) (40% to 57%), while the non-academic practices had a decrease in SNB alone from year 1 to year 2, (63% to 56%).

**Conclusion**

In this mixed practice setting there was an overall decrease in ALND in T1/T2 patients with low metastatic axillary burden following publication of Z0011. Immediately post-Z0011 the non-academic practices more rapidly adopted SNB alone, however these differences were no longer apparent two years following Z0011. Continued longitudinal studies will further assess the variability in the incorporation of Z0011 among various surgical practices.
Title: Vascularized lymph node transfer for the treatment of upper extremity lymphedema in breast cancer

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Body: Background: It is estimated that approximately 6.8 million people suffer with lymphedema in the United States. These patients can experience pain, heaviness, restricted motion and recurring infections all leading to poor quality of life. Vascularized lymphnode transfer (VLNT) not only offers the possibility of reduction of symptoms but also significant improvement in quality of life.

Methods: We evaluated the efficacy of VLNT in upper extremity lymphedema in breast cancer patients. MRI lymphangiogram was utilized to determine extent of lymphedema, area of blockage and transition zones. Pre surgical evaluation consisted of circumferential and volumetric measurements, pain, heaviness and quality of life. Eligible patients underwent VLNT with placement of transferred lymphnodes to the axilla or upper arm. Reassessment occurred at 1, 3, 6, 9, 12 and 18 months after surgery.

Results: Preliminary results showed arm volumes decreased by 22% at 3 months and 52% at 6 months and almost 60% at 9 months. Quality of life scores improved from 4.9 at preop to 7.4 at 3 months and 8.6 at 6 months. Pain and heaviness scores improved from 4.22 and 4.94 at preop to 0.14 and 1 at 3 months and 1 and 1.5 at 6 months.

Conclusions: Vascularized lymph node transfer has transformed the outlook for patients afflicted with limb lymphedema after breast cancer therapy. It has led to limb volume reduction and improvement in pain and heaviness. Additionally, quality of life has improved for all patients.
Pre-operative axillary staging results in over-treatment in some breast cancer patients

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Body: Introduction
Following a diagnosis of breast cancer, pre-operative ultrasound staging of the axilla is recommended. Patients found to have metastatic disease on biopsy or FNA can proceed directly to axillary clearance (ALNC) at the time of their breast tumour excision. However, although ultrasound staging is a sensitive test to detect axillary disease, it does not differentiate between low and high volume nodal metastasis. Recent evidence suggests that, in selected patients with low volume axillary disease following SLNB, completion ALNC may be safely omitted. This has been reflected in the recently updated ASCO guidelines. In light of this current trend towards axillary conservation, some patients undergoing ALNC after positive axillary staging may be over-treated. The aims of this study is to establish the nodal burden of patients undergoing ALNC following positive axillary staging and to compare the nodal burden of those patients with those undergoing completion ALNC after a positive SLNB.

Methods
Data was collected prospectively over 12 months from nine hospitals within the North of England Cancer Network. Age, tumour characteristics, breast operation, axillary staging results, axillary operation(s) and nodal results were recorded.

Results
A total of 1010 patients with breast cancer underwent pre-operative axillary staging. 215 patients (21%) had an ALNC. Of these, 115 (53%) cases underwent a primary ALNC after positive staging. The remaining 100 (47%) patients had a completion ALNC following a positive SLNB. The nodal burden for patients undergoing ALNC is shown in the table below:

<table>
<thead>
<tr>
<th>Total number of positive nodes</th>
<th>ALNC after positive staging</th>
<th>ALNC after positive SLNB</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n patients</td>
<td>% patients</td>
</tr>
<tr>
<td>0</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>1</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>2</td>
<td>13</td>
<td>11</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>4</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>≥5</td>
<td>39</td>
<td>34</td>
</tr>
<tr>
<td>Totals</td>
<td>115</td>
<td>100</td>
</tr>
</tbody>
</table>

Analysis of the data revealed that tumour size was a significant predictor of low volume axillary disease (i.e. <3 nodes positive) [p=0.02, Mann-Whitney test].

Conclusion
In this study, 46% of patients undergoing ALNC after positive staging had low volume disease (2 or less positive nodes). In the context of recent evidence if these patients had undergone a SLNB following the positive pre-operative staging they may have avoided a completion ALNC.

This study demonstrates tumour size to be a significant predictor of low volume axillary disease and thus may be an important factor to consider alongside pre-operative staging when selecting patients who may be better managed by a SLNB rather than proceeding directly to ALNC.
Title: Noninvasive assessment of axillary lymph node metastases in breast cancer using EpCAM antibody

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Body: Background: Axillary lymph node metastasis is one of the most important prognostic determinants for patients with breast cancer. It’s a big progress from axillary lymph node dissection (ALND) to sentinel lymph node biopsy (SLNB), however, SLNB also has some limitations, including the detection failure rate, false negative rate, tracer allergies et al. Since about 70% of early breast cancer patients have negative SLNs, surgery maybe overtreatment for these patients and noninvasive assessment of axillary staging seems to be a better choice. It’s not easy to detect and mapping metastasis lymph nodes in vivo non-invasively because we don’t have specific cancer markers, but what we know is that most breast cancer cells come from epithelial origin and should have epithelial markers, and this kind of markers should not show up in lymph system. EpCam is a surface epithelial marker and expressed in 80-100% breast cancer patients. The aim of this study was to evaluate the value of EpCAM antibody in detecting breast cancer lymph node metastases non-invasively.

Methods: VX2 rabbit model which is an epithelial tumor model with lymph node metastasis was used in this experiment. Anti-EpCAM was labeled with a near infra-red (NIR) fluorescence imaging agent Cy7 and selected as metastasis tracer. New Zealand white rabbits were assigned to study group and control group at random. Twelve rabbits in the study group received VX2 tumor injection to bilateral mammary glands and 12 rabbits in the control group were tumor free. Twenty four cases of axillary lymph nodes metastasis were investigated in each group because of bilateral experiments. Anti-EpCAM/Cy7 was subcutaneously injected around the tumors in study group or in mammary gland in control group at a dosage of 0.1ml (10umol/L). After 30 hours of screening by a NIR fluorescence detection machine, all the axillary lymph nodes in both groups were removed for pathological examination and the fluorescence status of each node was recorded.

Result: The fluorescence signal could be detected 2 hours after injection in 24 cases in the control group and 23 cases in the study group. Thirty hours later, fluorescence disappeared in all cases in the control group and all the lymph nodes resected were pathological negative. While in study group, fluorescence signal could be detected continuously in 14 cases and 13 were proven to be pathological metastasis. The case failed to have fluorescence image from the beginning finally proved to be vascular invasion and cancerous node formation. The sensitivity, specificity rate of anti-EpCAM/Cy7 to detect axillary lymph node metastasis was 92.8% (13/14) and 90.0% (9/10).

Conclusion: The NIR dye labeled EpCAM antibody (anti-EpCAM/Cy7) has a high accuracy in evaluating the axillary lymph node status of breast cancer in the animal experiment. Our tiny preclinical experiment showed the value of using EpCAM antibody as a new method for noninvasive lymph node evaluation for breast cancer and potentially extended in a lot of other epithelial originated cancers.
Title: Role of 18 FDG PET/CT in axillary staging in early breast cancer

Cristina Noblia¹, Eugenia Azar¹, Dolores Mansilla¹, Amilcar Osorio², Eduardo Armanasco¹, Diana Montoya¹, Martin Ipiña¹, Gaston Berman¹, Eduardo Gonzalez¹, Christian Gonzalez², Gabriel Bruno², Patricia Parma², Carla Pulero¹ and Ana Alvarez¹. ¹Angel H. Roffo Institute, Buenos Aires, Caba, Argentina and ²Nuclear Diagnostic Center Foundation, Buenos Aires, Caba, Argentina.

Body: OBJECTIVE To evaluate the 18F-FDG PET/CT capacity for the detection of axillary metastases and compare results with sentinel lymph node biopsy (SLNB). MATERIAL AND METHODS: 99 female patients with clinical T1T2N0 breast cancer were included. Patients with recent breast or axillary surgery, T3T4 disease, ductal carcinoma in situ, inflammatory carcinoma, uncontrolled diabetes mellitus and pregnant or lactation patients were excluded. Pre-operative FDG PET-CT was performed 15 days before surgery, SLNB took place with the combined method (radioisotopes and patent blue). Pearson and Spearman correlation test were used to evaluate association of main variables with a significance level (p) of 0.05. RESULTS: Breast PET-CT results: 80 positive PET/CT. Negative PET/CT in 19 patients (4 invasive lobular carcinoma, 7 tumors <7mm) Sensitivity 81%, specificity 100%. 97 patients were operated (2 were stage IV with no surgery criteria). We found significant correlation of the FDG tumor uptake (SUV) with tumor size (p<0.0001), histological grade (p<0.009), nuclear grade (p<0.001), mitotic grade (p<0.007) and Ki 67 (p<0.0001). In all cases the correlation was positive. There was no correlation with hormonal receptors rate. Axillary PET CT results: positive PET/CT in 16 patients (16%). Of this 6 SNLB were negative (FPR= 9%). Specificity 91%, PET/CT were negative in 81 patients (84%), 17 had axillary metastases(5 micrometastases, 2 isolated tumor cells and 2 lobular carcinoma). Sensitivity:37%. FNR=63%. Correlation of histopathological axillary metastases was positive with breast tumor SUV (P<0.002) and tumor size (p<0.01), but was not significant with histological, nuclear and mitotic grade, Ki 67, hormonal receptor rate and molecular subtype CONCLUSION: 1- PET/CT does not provide benefits for axillary staging in initial stages due to its low spatial resolution (6-8 mm) 2 – A negative axillary by PET/CT does not replace the sentinel node technique. 3 – We can suspect the presence of axillary metastases when there is an intense FDG uptake in the breast tumor.
Title: Total tumoral load as a prediction tool of non-sentinel node metastases in patients with early breast cancer and positive sentinel lymph node assessed by OSNA

Martin Espinosa-Bravo¹, Francesc Pérez-Ceresuela¹, Sebastian Diaz-Botero¹, Vicente Peg² and Isabel T Rubio¹. ¹Breast Surgical Unit, Breast Cancer Center, Hospital Universitari Vall d'Hebron, Universitat Autònoma de Barcelona, Barcelona, Spain and ²Hospital Universitari Vall d'Hebron. Universitat Autònoma de Barcelona, Barcelona, Spain.

Body: Background: Total tumoral load (TTL) in the sentinel lymph nodes (SLN) assessed by the OSNA assay is a new variable that is able to predict the likelihood of more axillary metastasis. Compared with the number of positive SLNs, the TTL is independent of the number of metastatic SLNs and a better predictor of further nodal metastasis. Although establishing specific cutoff-points of the TTL can be questionable because they may change in the future and increasingly become more patient specific, we have seen that TTL <15,000 could be a good cut off by its high negative predictive value (NPV). Changes in practice have been occurred after the ACOSOG Z0011 trial and nowadays, patients with ≤ 2 positive SLNs may spare an axillary lymph node dissection (ALND) with no impact on the oncological outcome. In the Z0011, 27% of patients had additional non-SLN metastasis in the cALND. The objective of this study was to compare two methods of SLN metastatic burden, the TTL and the number of positive SLNs, for predicting non-SLN metastasis in patients with positive SLNs by OSNA.

Methods: This is a retrospective cohort study of 145 consecutive patients with cT1-T2 invasive breast cancer with ultrasonographically node-negative treated between April 2010 to April 2013, where there is at least one positive SLN assessed by OSNA. We design a prediction tool based on the TTL results determined by OSNA to calculate the likelihood of not finding more positive non-SLN (http://www.vhebron.net/es/calculadora-risc-metastasi-2). Our group have demonstrated that TTL=15,000 could be a good cut off by its high NPV and sensitivity, 85.5% and 76.7%, respectively.

Results: A total 325 SLNs were removed with a mean of 2.24. The type of SLN metastasis were macrometastasis in 85 patients (58.6%) and micrometastasis in 60 patients (41.4%). When considering patients with number of positive SLNs, of 109 patients with ≤2 positives SLNs and an cALND, 17 patients (22%) had non-SLN metastasis; and of 5 patients with ≥3 SLN, four (80%) had non-SLN metastasis. Taking the TTL results, of 51 patients with TTL <15,000 copies/µL with cALND (90.2% with one positive SLN), 7 patients (14%) had non-SLN metastasis, were a media of 2.2 extra non-SLN metastasis. Of 55 patients with TTL ≥15,000 copies/µL (with 1 or 2 positives SLNs) with cALND, 20 patients (36%) had non-SLN metastasis and there were a media of 2.5 non-SLN metastasis (range 1-8). Of 34 patients (62%) with one positive SLN with TTL ≥15,000 copies/µL, 12 patients (35%) had non-SLN metastasis; and of 21 patients with two positive SLN with TTL ≥15,000 copies/µL, eight patients (38%) had non-SLN metastasis.

Conclusion: The total tumor load obtained by OSNA predicts non-SLN metastasis independent of the number of positive SLNs. The assess of the TTL in our cohort of patients show that patients with one or two positives SLNs with high TTL had high likelihood of non-SLN metastasis greater than 27% of Z0011 criteria. Prospective studies are needed to determine the clinical impact of this variable in patients outcome in the management of patients with SLN assessed by OSNA.
**Title:** Preoperative axillary imaging with ultrasonography: Among the breast cancer patients with lymph node metastases, can we identify the patients who may omit axillary dissection?

Naoto Kondo\(^1\), Takashi Fujita\(^1\), Masataka Sawaki\(^1\), Masaya Hattori\(^1\), Akiyo Yoshimura\(^1\), Mari Ichikawa\(^1\), Junko Ishiguro\(^1\), Yayoi Adachi\(^1\), Haruru Kotani\(^1\), Tomoka Hisada\(^1\) and Hiroji Iwata\(^1\). \(^1\)Aichi Cancer Center Hospital, Nagoya, Aichi, Japan.

**Body:**

(\textit{Introduction}) ACOSOG Z11 and EORTC AMAROS showing little benefit to axillary dissection (ALND) for early stage breast cancers with limited nodal disease have led us to questioning the value of preoperative axillary imaging. It may not result in a benefit to the patients to perform ALND by diagnosing a few or small axillary lymph node (ALN) metastases preoperatively.

(\textit{Aim}) A purpose of this study is to determining the association between diagnostic method and metastatic number of ALN metastases, and to find the patients who can omit ALND safely even if with ALN metastases.

(\textit{Methods}) A database of consecutive primary breast cancer patients who underwent complete ALND at our institution in 2008-2011 was analyzed. After we excluded patients treated with neoadjuvant systemic therapy, a total of 390 patients were included. By diagnostic methods of ALN metastases, we classified them in four groups as follows. Group A (n=41) : suspicious ALNs on axillary ultrasound (AUS) and ultrasound-guided fine needle aspiration cytology (FNAC) positive, Group B (n=47) : only one abnormal ALN on AUS +/- FNAC, Group C(n=53) : multiple abnormal ALNs +/- FNAC, Group D (n=249) : negative ALNs on AUS but SLNB positive.

(\textit{Results}) The median number (range) of ALN metastases were 3(1-22) in GropuA, 2(0-12) in Group B, 7(1-37) in Group C, 1(1-17) in Group D. There were significant differences in number of metastases between Group A, B, C and Group D (\(p=.02\), \(p=.01\), \(p=.002\)). Patients with 3 or less positive ALNs were 24.5% (13/53) in Group C, whereas 61.0% (25/41) in Group A and 68.1% (32/47) in Group B (\(p=.04\), \(p=.02\)). We next evaluated the influence of patient- and tumour-related variables on the number of positive ALNs in Group A/B. Factors such as age, tumour size (<20mm vs >20mm), ER status (positive vs negative), HER2 status (positive vs negative), nuclear grade (1/2 vs 3), menstruation status (pre vs post menopausal) were examined. However there were no significant differences in all factors between the patients with 4 or more ALN metastases and patients with 3 or less ALN metastases. As a result of multivariable analysis, relative risk (95%CI, \(p\)-value) of age was 0.92 (0.546-1.347, 0.84), tumour size : 0.82 (0.511-1.418, 0.76), ER status : 1.06 (0.873-1.821, 0.76), HER2 status : 1.17 (0.853-2.390, 0.28), nuclear grade : 0.43 (0.420-1.22, 0.34), menstruation status : 0.85 (0.538-1.693, 0.89). (\textit{Conclusion}) In our contemporary series, patients diagnosed as ALN metastases preoperatively have significantly more involved nodes compared to SLNB positive patients regardless of the diagnostic method, suggesting that such patients should proceed to ALND. Preoperative axillary imaging are useful to identify node-positive breast cancer patients requiring ALND.
Title: Long-term follow-up of the node-negative breast cancer patients before treatment evaluated with sentinel node biopsy alone following neoadjuvant chemotherapy

Hiroko Nogi, Ken Uchida, Makiko Kamio, Kumiko Kato, Yasuo Toriumi and Hiroshi Takeyama. Jikei University School of Medicine, Tokyo, Minato-ku, Japan.

Body: BACKGROUND Sentinel node biopsy (SNB) for the node negative breast cancer is standard treatment as an accurate assessment of axillary lymph node status. The use of neoadjuvant chemotherapy (NAC) has increased during the past several years. At present, the use of SNB following NAC is controversial. Proponents of SNB after NAC prefer a single surgical procedure with potential for fewer axillary dissections.

OBJECTIVE Our objective was to examine SNB evaluation alone following NAC in patients with clinically node-negative breast cancer before treatment and to evaluate the axillary lymph node recurrence for patients undergoing NAC versus patients undergoing surgery first.

METHODS A total of 1176 patients with clinically node negative breast cancer underwent SNB from 2007 to 2013. Clinicopathologic and survival data were reviewed and comparisons made between patients receiving NAC and those undergoing surgery first. Lymphatic mapping was performed with both radioactive colloid and blue dye. Patients with negative SN for metastasis were followed without axillary lymph node dissection (ALND). Patients with metastases to a SN underwent ALND.

RESULTS Of the patients, 180 (15.3%) underwent SNB following NAC and 996 (84.7%) underwent surgery first. SN identification rates were 98.3% in the NAC group and 98.9% in the surgery first group. 152 (84.4%) patients in the NAC group and 877 patients (88.1%) had negative SNs. At median follow-up of 49.2 months, 1 patient (0.007%) in the NAC group and 3 patients (0.003%) in surgery first group had axillary lymph node recurrences. There were not any significant differences between the NAC group and the surgery first group.

CONCLUSION The SNB following NAC in patients with node-negative breast cancer has a low axillary recurrence rate and could be acceptable.
**2014 San Antonio Breast Cancer Symposium**

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**Average Grade:** 5.40

**Title:** The ratio and size of positive sentinel lymph nodes predicts the involvement of non-sentinel lymph nodes following completion axillary lymph node dissection

Rajiv V Dave¹, Muhhamed N Chauhan¹, Maria Ghaus¹, Sana Ahmed¹, Shiv Shapra¹, Joshua Marriott¹, Craig Sayers¹, Zbigniew Kryjak¹ and Deedar Ali¹. ¹Pinderfields Hospital, Wakefield, West Yorkshire, United Kingdom.

**Body:** Background: The role of Completion Axillary Lymph Node Dissection (CALND) following positive Sentinel Lymph Node Biopsy (SLNB) is being actively debated. The involvement of our unit in the POSNOC trial (which has a no-treatment arm), has prompted a review of our unit’s CALND results, in order to examine predictors of involvement of non-sentinel lymph nodes (n-SLN), to better inform our patients during recruitment.

Methods: We retrospectively analysed our experience of SLNB between July 2008 to 2013. A total of 1152 breast cancer patients underwent SLNB based on lymphoscintigraphy, intraoperative gamma probe detection, and blue dye mapping using 99mTc-nanocolloid and Patent Blue V injected peri-areola.

Results: Out of 1152 SLN biopsies performed, 224 were positive for metastatic disease. 203 patients were anaesthetically capable of progressing to CALND. On univariate analysis, involved n-SLN on CALND could not be predicted by age (<50 years; 16/77 vs ≥50 years; 93/125, p=0.272), size of tumour (<50mm; 44/193 and ≥50mm; 4/10, p=0.188), procedure (mastectomy; 24/93, WLE; 24/106, p=0.444), lymphovascular invasion (22/97 vs 26/106, p=0.812), number of positive SLN (≤2; 45/196, >2; 3/7, p=0.213), receptor status; ER (negative; 3/13, positive 45/187, p=0.619), PR (negative; 6/28, positive 41/169, p=0.478), Her2 (negative; 43/175, positive 5/25, p=0.414), triple negative (2/8 vs 46/192, p=0.612). There was a trend toward higher incidence of positive n-SLN with increasing grade (G0-2; 28/139 vs G3; 20/64, p=0.062) and extracapsular spread (14/41 vs 32/149, p=0.073), but these did not reach statistical significance. Positive n-SLN on CALND was however predicted by macrometastases in SLN (macrometastases; 39/141 vs micrometastases; 9/62, p=0.029) and ratio of positive nodes (<0.5; 18/109 vs ≥0.5; 30/94, p=0.008). There were 4/224 recurrences (3 distant metastases and 1 loco-regional), which were not predicted by any of the clinicopathological variables investigated. 3 patients who recurred only had one positive node on SLNB.

Conclusion
In our series of more than 200 SLNB, a ratio of >0.5 positive SLN yield and presence of macrometastases in positive SLN, were associated with positive n-SLN on CALND.
Title: Management of the axilla in breast cancer patients: Do we over treat patients with preoperative diagnosis of nodal metastasis?

Ioannis Michalakis¹, Jaroslaw Krupa¹ and Louisa Dunk². ¹Glenfield Hospital, Leicester, United Kingdom and ²Glenfield Hospital, UHL Trust, Leicester, United Kingdom.

Body: Introduction: Axillary lymph node status is the most significant single prognostic factor in breast cancer patients. Axillary ultrasound scan (AUS) followed by fine needle aspiration (FNA) is the gold standard modality in preoperative staging of the axilla and spares the patient a possible second operation. Sentinel lymph node biopsy (SLNB) accurately stages the axilla with low axillary recurrence rates and reduced morbidity. Increasing evidence suggests that surgical removal of the axillary lymph nodes (completion ALND) in early breast cancer with limited nodal disease yields no advantage in terms of either overall or disease-free survival. The purpose of this audit is to identify possible changes in management of the axilla in patients having preoperative diagnosis of nodal metastasis.

Methods: We reviewed the available data from breast cancer patients that had positive FNA after AUS (441) or positive nodes at a SLNB (324) in our unit from 2007 to 2013.

Results: 410 of the 441 (93%) patients with a preoperative diagnosis of nodal metastasis received completion ALND. 147 of the 410 (36%) patients had neoadjuvant chemotherapy and of these 34 (23%) had complete pathological response at the axilla. 101 of the 263 (38%) patients who did not receive neoadjuvant chemotherapy had 1 or 2 nodes involved and also had a T1 or T2 tumour.

Patients with positive axillary FNA and ALND

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>ALND without neoadjuvant chemotherapy:263</th>
<th>ALND after neoadjuvant chemotherapy:147</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median)</td>
<td>64</td>
<td>48</td>
</tr>
<tr>
<td>Tumor size (T)</td>
<td>T1:56 T2:190 T3:16</td>
<td></td>
</tr>
<tr>
<td>ER status positive</td>
<td>200 (76%)</td>
<td>96 (65%)</td>
</tr>
<tr>
<td>Her 2 status positive</td>
<td>49 (19%)</td>
<td>44 (30%)</td>
</tr>
<tr>
<td>Median nodes removed</td>
<td>20</td>
<td>17</td>
</tr>
<tr>
<td>Median positive nodes</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Patients with &lt;3 nodes positive</td>
<td>103 (39%)</td>
<td>72 (49%)</td>
</tr>
</tbody>
</table>

Total number of patients 410

161 of the 324 (47%) patients with positive SLNB had completion ALND. 67 of the 161 (42%) patients received chemotherapy prior the completion ALND. All the patients who had a positive SLNB and who did not receive completion ALND received radiotherapy to the axilla.

Patients with positive SLNB

<table>
<thead>
<tr>
<th>Number of patients</th>
<th>Radiotherapy to the axilla:163</th>
<th>With completion ALND after chemotherapy:67</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median)</td>
<td>64</td>
<td>50</td>
</tr>
<tr>
<td>Tumor size (T)</td>
<td>T1:83 T2:73 T3:5</td>
<td>T1:20 T2:39 T3:8</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>With completion ALND before chemotherapy:94</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G1:13 G2:60 G3:21</td>
</tr>
<tr>
<td></td>
<td>Group 1</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------</td>
</tr>
<tr>
<td>ER status positive</td>
<td>144 (88%)</td>
</tr>
<tr>
<td>Her 2 status positive</td>
<td>10 (6%)</td>
</tr>
<tr>
<td>Median nodes removed</td>
<td>16</td>
</tr>
<tr>
<td>Median positive nodes</td>
<td>1</td>
</tr>
<tr>
<td>Patients with &lt;3 nodes positive at the SLNB</td>
<td>150 (92%)</td>
</tr>
<tr>
<td>Patients with &lt;3 nodes positive in total after completion ALND</td>
<td>40 (60%)</td>
</tr>
</tbody>
</table>

Total number of patients 324

Conclusions: Breast cancer patients with a preoperative diagnosis of nodal metastasis are treated in a different way than the patients with positive SLNB. Axillary treatment may be improved if we could identify patients with a preoperative diagnosis of nodal metastasis that fulfil the criteria of the Z11 trial and so potentially save patients unnecessary axillary surgery.
Internal mammary sentinel lymph node biopsy with modified injection technique: High visualization rate and accurate staging

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¹Shandong Cancer Hospital&Institute, Jinan, Shandong, China.

Background: Although the 2009 AJCC incorporated the internal mammary sentinel lymph node biopsy (IM-SLNB) concept, there has been little change in surgical practice patterns due to the low internal mammary sentinel lymph nodes (IM-SLNs) visualization rate (VR) with the traditional radiotracer injection technique (average 13%, 0-37%). In this study, various injection techniques were evaluated in terms of the IM-SLNs VR in clinically axilla lymph nodes (ALNs) negative patients, and the impact of IM-SLNB on the diagnostic and prognostic value were analyzed both in clinically ALNs negative (NCT01642511) and positive patients (NCT01668914).

Methods: 340 patients with T1-2 invasive breast cancer were enrolled. Clinically ALNs negative patients (n=293) were divided into 3 groups according to the study period and radiotracer injection technique. Group A: traditional technique (peritumoral intraparenchymal injection) for the initial 58 cases; Group B: periareolar intraparenchymal injection under the ultrasonography guidance in the latter 235 cases, the injection sites were chosen at the 6 and 12 o’clock positions 0.5-1.0 cm from areola (about 2.0-4.0 cm from the nipple). Group B was then separated into 2 groups according to the radiotracer injection volume: Group B1, low volume (<0.5ml/point, n=41); Group B2, high volume (≥0.5ml/point, n=194). Clinically ALNs positive patients (n=47) were managed as group B2. IM-SLNB was performed for patients with IM-SLNs visualized on preoperative lymphoscintigraphy and/or detected by intraoperative gamma probe.

Results: Clinically ALNs-negative: The IM-SLNs VR was significantly higher by lymphoscintigraphy in Group B than that in Group A (63.0% vs. 13.8%, P<0.001), while the axillary VR was similar in these two groups (84.3% vs. 77.6%, P=0.227). The VR of sentinel lymph nodes was improved by the intraoperative gamma probe compared to lymphoscintigraphy: Group A (axilla: 77.6%→98.3%, P=0.001; internal mammary: 13.8%→15.5%, P=0.794) and Group B (axilla: 84.3%→98.7%, P<0.001; internal mammary: 63.0%→70.6%, P=0.078). Group B2 was found to have the highest IM-SLNs VR (74.2% vs. 53.7% Group B1, P=0.009). The successful rate of IM-SLNB was 95.8%, and arrived 100% after 20 cases learning curve. In the patients who underwent IM-SLNB, the IM-SLNs metastases rate was 8.7%, systemic treatment was changed only in 4.3%; however, radiotherapy treatment was changed in 8.7%. For the patients with upper inner quadrant tumor, systemic and radiotherapy treatment was changed in 8.8% and 20.6%.

Clinically ALNs-positive: IM-SLNB was performed in all patients with drainage to IM-SLNs (VR: 70.2%, 33/47); the successful rate was 100% (33/33). The IM-SLNs was positive in 21.2% patients, and the internal mammary radiotherapy could be guided with this IM-SLNB results.

Conclusions: The modified injection technique (periareolar intraparenchymal, high volume and ultrasonography guidance) significantly improved the IM-SLNs VR, making the routine IM-SLNB possible in daily practice. IM-SLNB could provide individual staging, prognosis, and decision-making of the internal mammary radiotherapy for breast cancer patients, especially in positive ALNs and high-risk negative ALNs patients.
Title: Validation of diagnostic procedure for metastatic lymph nodes in breast cancer using a semi-dry dot-blot method and novel anti-cytokeratin 18+19 antibodies

Ryota Otsubo1, Masahiro Oikawa1, Hiroshi Hirakawa2, Hiroshi Yano1 and Takeshi Nagayasu1. 1Nagasaki University Hospital, Nagasaki, Japan and 2Chiba Aiyuuukai Memorial Hospital, Chiba, Japan.

Body: Introduction: Sentinel lymph node (SLN) biopsy is a common diagnostic procedure for breast cancer. However, because of a shortage of pathology specialists in Japan and discordance between intra-operative and final pathological diagnoses of SLN metastasis, new diagnostic modalities are desperately required. We previously reported a novel method of detecting metastasis in SLNs by a semi-dry dot-blot (SDB) method with 93.3% sensitivity, 96.9% specificity and 96.6% accuracy. Here, we evaluated the efficacy of the SDB method using novel anti-cytokeratin (CK) 18+19 antibodies to diagnose lymph node metastases in breast cancer.

Materials and methods: We obtained 73 lymph nodes dissected from 43 patients with breast cancer from July 2013 to May 2014 at Nagasaki University Hospital and the Japanese Red Cross Nagasaki Genbaku Hospital, including 55 sentinel lymph nodes and 18 dissected axillary lymph nodes, which were sliced at 2-mm intervals and washed with phosphate-buffered saline. This lavage fluid was used to diagnose lymph node metastasis by the SDB method; whereas the washed lymph nodes were blindly diagnosed by pathologists using with hematoxylin and eosin (H&E) stain. Suspended cells in the lavage fluid were centrifuged; the cell pellet was lysed with lysis buffer to extract protein, which was then challenged and visualized with anti-cytokeratin 18 and 19 antibodies, each at 1 µg/ml and 0.1 µg/ml to distinguish between micrometastases or isolated tumor cells (ITC) and macrometastases; and with chromogen on a dot-blot membrane. Diagnoses based on the SDB method were compared with their H&E counterparts. When the SDB method and H&E-based examinations did not agree, we examined specimens immunohistochemically with anti-cytokeratin18+19 antibodies.

Results: Of the 73 lymph nodes, 25 were assessed as positive and 48 as negative by the permanent pathological examination with H&E. The SDB method made correct diagnoses in all positive cases and 42 of the 48 pathologically negative cases. We found four micrometastases and one ITC in the positive cases, which were difficult to diagnose as positive at the lower antibody concentration, but were clearly positive at the higher concentration. When the 6 discrepant cases were examined immunohistochemically, we found two cases of ITC and one of micrometastasis. Sensitivity, specificity and accuracy of the SDB method in detecting cancer cells were 100% (95% confidence interval [95% CI]: 100–100%), 93.8% (95% CI: 86–101%) and 95.9% (95% CI: 91–100%), respectively.

Conclusions: The SDB method using anti-CK18+19 antibodies is simple and accurate for diagnosing lymph node metastases; estimating metastatic amount may be possible with different antibody concentrations. We are producing an SDB kit that uses these antibodies.
Title: Sentinel lymph node biopsy using intraoperative indocyanine green fluorescence imaging navigated with preoperative computed tomography lymphography for early breast cancer

Hajime Abe¹, Keiichi Yamazaki¹, Masao Ogawa¹, Masayasu Kawasaki¹, Kohri Yoneda¹ and Masao Kameyama¹. ¹Bell Land General Hospital, Sakai, Japan.

Body: Background: Sentinel lymph nodes (SLN) biopsy has been established as a standard of care in the treatment of early breast cancer. The combination of the radioisotope and dye-staining methods is the most accurate way to identify sentinel lymph nodes. We had reported a novel technique of SLN identification using fluorescence imaging of indocyanine green (ICG) injection. However, if any lymphatic vessels are injured, further fluorescence navigation will be difficult because of ICG contamination in the surgical field in overweight or obese patients. In this study, a new diagnostic approach for imaging lymphatic drainage and identifying SLN using preoperative computed tomography-lymphography (CTLG) and an intraoperative ICG fluorescence method was investigated.

Patients and method: Between January 2013 and April 2014,105 breast cancer patients without clinical evidence of lymph node metastasis were treated. On the day before the operation, CTLG was performed using 64-row multidetector CT. We performed an intradermal injection in the periareolar area, using 4 ml of contrast agent with 0.5 ml of local anesthetic. The contrasted lymph flow and SLN were identified in reconstructed three-dimensional imaging. The SLN spot was indicated by CT laser light navigator system. During the operation, fluorescence images were obtained using the fluorescence imaging system, Photodynamic Eye (pde-neo, Hamamatsu Photonics Co., Japan). After ICG was injected intradermally in to the periareolar skin, lymphatic drainage was observed with fluorescence images. SLN biopsy was performed by referring to the marker preoperatively placed on the CTLG. Moreover, we classified enhancement of SLN as “whole” and "partial" by CTLG with visual patterns, and examined the relation with SLN metastasis.

Results: The median age of the 105 patients was 63 (range 33 – 87) years old. CTLG and fluorescence imaging was safely performed in all patients. This method was visually easy to identify the location of SLN on the axillary skin even in obese patients. CTLG could visualize lymphatic flow and accurately identify SLN in 99 (94%) of 105 patients, whereas fluorescence imaging identified successfully lymphatic flow and SLN in all patients. Lymphatic flows of CTLG were completely consistent with those of fluorescence imaging. The number of SLN identified by CTLG was significantly lower than that by fluorescence imaging (1.1 vs. 1.6, p<0.01). Fifteen patients (14%) were found to have lymph node metastases pathologically, and five of them had micrometastases of lymph node. In 6 patients without detected SLN by CTLG, only one patient had lymph node metastasis. In case of partial enhancement of SLN with CTLG, the rate of positive metastasis was significant higher compared to the cases of whole enhancement (p<0.01).

Enhancement of SLNs by CTLG

<table>
<thead>
<tr>
<th></th>
<th>Metastasis(+)</th>
<th>Metastasis(-)</th>
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<tr>
<td>Whole</td>
<td>4</td>
<td>59</td>
</tr>
<tr>
<td>Partial</td>
<td>10</td>
<td>26</td>
</tr>
<tr>
<td>Not identified</td>
<td>1</td>
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</table>

Conclusion: This new navigation method of CTLG and fluorescence imaging revealed more easy and effective to detect SLN intraoperatively than fluorescence imaging alone. In addition, the information from CTLG may be helpful for the prediction of SLN metastasis.
Title: Not all sentinel lymph nodes are equal – A predictive model for axillary burden in early breast cancer

Yirong Sim¹, Sue Zann Lim¹, Shaun S Tan¹, Alvona Z Loh¹, Cindy Lim², Preetha Madhukumar², Gay H Ho², Veronique KM Tan² and Kong Wee Ong³. ¹Singapore General Hospital, Singapore, Singapore; ²National Cancer Centre, Singapore, Singapore and ³National University of Singapore, Singapore, Singapore.

Body: Introduction
The Z11 trial demonstrates that an axillary lymph node dissection (ALND) can be avoided in patients with low axillary burden who undergo breast conserving surgery (BCS), combined with whole-breast radiotherapy and systemic therapy. Our group propose a standardisation of sentinel lymph node biopsy (SLNB), incorporating 2 novel sentinel nodal stations (SNS) which represent sequential echelons of SLN draining the breast – the intercostalbrachial nerve (ICB) and the medial pectoral neurovascular bundle (MP). By increasing the specificity and positive predictive value of SLNB, we aim to identify a subgroup of patients who undergo mastectomy who can be spared from ALND and its associated complications.

Materials and Methods
313 female patients who underwent sentinel lymph node biopsy for breast cancer from 2 February 2012 to 19 December 2013 were prospectively studied. 12 patients had bilateral breast cancers, and each laterality was counted as distinct cases, giving a total of 325 cases. 7 had breast surgery performed prior to the SLNB, and the rest had their oncological surgery performed at the same setting of the SLNB. The surgeries were performed by three surgeons in the National Cancer Centre Singapore, using a specific surgical technique to identify SLN at the 2 SNS. Relevant patient demographics, status of SNS and the rates of metastatic non-sentinel lymph nodes were collected and analysed.

Results
A total of 325 SLNBs using the ICB and MP SNS were identified from 169 simple mastectomies, 35 skin sparing mastectomies, and 129 breast conserving surgeries. The median age was 56 (range 27-89). The ICB SLN and MP SLN were identified in 313 (96.3%) and 258 (79.4%) cases respectively. In 249 (76.6%) cases were both ICB and MP nodes identified, of which 55 (16.9%) had metastatic involvement of the SLN. An axillary clearance was performed if at least one ICB or MP node was positive, and only 27 (49.1%) had further axillary involvement. More than 2 positive SLNs had 100% positive predictive value for further axillary LN metastases. There was a low sensitivity (29.6%) and high false negative rate (70.4%) for positive axillary nodes in patients with ≤2 positive SLNs. MP nodal status, however, was 85.7% specific (p<0.001) and 48.1% sensitive (p=0.649) and a positive predictive value of 76.5% for axillary nodal involvement. Logistic regression also shows that MP node status is significant for predicting axillary nodal status (OR 5.57, p=0.006).

Conclusion
Our study shows that MP node status is specific, and has a positive predictive value for further axillary LN metastases. Therefore, we propose that in all patients who undergo SLNB with their BCS or mastectomy, an axillary clearance should be performed if the MP node is positive, regardless of the number of positive SLNs.
Title: Role of age, menopausal status and biological tumor characteristics on sentinel lymph node metastasis in early breast cancer patients with favorable prognostic features: A retrospective, mono institutional study on 345 cases

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Body: Background: Sentinel lymph node biopsy (SLNB) is recognized as a standard procedure for women with early breast cancer. Recently some studies reported the futility of axillary lymph node dissection in patients (pts) who are SLN positive but bear favorable clinical and primary tumor biological characteristics. Since we believe that the choice of a proper axillary treatment in early breast cancer could be personalized and axillary SLNB could be avoided in specific subgroups of pts, we designed this study to identify key primary tumor characteristics and pts’ clinical features that could influence the indication to perform SLN biopsy.

Patients and methods: Retrospective analysis was carried out in women who had undergone surgery and SLNB for early breast cancer (pT1-2) from 2005 to 2013 at the Fatebenefratelli e Oftalmico Hospital in Milan, Italy. All SLNs were examined histologically in toto on seriated permanent sections. The association between SLN positivity and the following variables as age, menopausal status, tumor size, histological grading, presence of extensive "in situ" components and lymphovascular invasion (LVI), quantitative evaluation of Ki-67, HER2 expression, oestrogen and progesterone receptors, was assessed by means of univariate and multivariate logistic models.

Results: The records of 345 pts with early breast cancer who underwent surgery were retrieved. Mean age was 61 years and 79% pts were postmenopausal. 85% were treated with quadrantectomy and 66% had only one SLN removed. SLN metastasis was detected in 24% of cases. Tumor size was <2 cm in 76% of pts, and 86% were of luminal subtypes. Peritumoral LVI was detected in 32% of cases. At univariate analysis a statistically significant association was found between tumor size [odds ratio (OR) 1.05, confidence interval at 95% (95%CI) 1.01-1.08; p=0.005], histological grade (OR 1.50, 95%CI 1.04-2.16; p=0.029), presence of LVI (OR 3.81, 95%CI 2.27-6.37; p < .0001). At multivariate analysis only LVI confirmed to increase the risk of SLN positivity in the whole series (OR 3.26, 95%CI 1.89-5.64; p < .0001) as well as in subgroup of pts with luminal A and B subtype (OR 3.51, 95%CI 1.92-6.44; p < .0001). Negative and positive predictive values of LVI were 83.9% and 42.2%, respectively. Interestingly, in a pre-planned subgroup analysis according to menopausal status, an association between tumor dimension and SLN positivity was found in premenopausal women (OR 1.1, 95%CI 1.01-1.21; p < .034), while in postmenopausal pts LVI was associated with SLN positivity (OR 3.05, 95%CI 1.65-5.64; p < .0004).

Conclusions: Our data suggest that in a population with favorable early breast cancer (luminal subtypes, postmenopausal status and small tumour size) LVI increases the risk of SLN metastasis. These results are confirmed in luminal subgroup. In addition the absence of LVI has a significant negative predictive value. As far as menopausal status is concerned we found that the positivity of SLN is associated with tumor dimension in premenopausal and with LVI in postmenopausal women.
Title: In patients with micrometastatic in sentinel lymph node biopsies, involvement of the non-sentinel lymph nodes cannot be predicted by clinicopathological variables

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Body: Background: The Sentinel Lymph Node Biopsy (SLNB) procedure is recognised to be an accurate method of staging the axilla in patients with early stage breast cancer. There remains a debate as to whether patients with micrometastases should undergo completion axillary lymph node dissection (CALND). We aimed to assess the indicators for positive non-sentinel lymph nodes (n-SLN) following CALND.

Methods: We retrospectively analysed our experience of SLNB between July 2008 to July 2013. A total of 1152 breast cancer patients underwent SLNB based on lymphoscintigraphy, intra-operative gamma probe detection, and blue dye mapping using 99mTc-nanocolloid and Patent Blue V injected peri-areola. Statistical analysis was performed using Fisher’s exact and \( \chi^2 \) for categorical data.

Results: Out of 1152 SLNB biopsies performed, 224 (19.5%) were positive for metastatic disease; macrometastases in 150 (67%), micrometastases in 72 (32%) and ITC in 2. CALND was not performed in 20 cases (9 macrometastases, 10 micrometastases, and 1 ITC), largely due to concerns regarding fitness for anesthesia. Macrometastases on SNLB were more likely to predict positive n-SLN on ANC (macrometastases; 39/141(27.7%) vs micrometastases; 9/62 (14.5%), \( p=0.029 \)). On univariate analysis, positive n-SLN in CALND for patients with micrometastases on SLNB was not predicted by grade (G0-G2; 6/43, and G3; 3/19, \( p=0.565 \)), size of primary breast tumour (<40mm; 8/58, \( \geq 40 \text{mm} \); 1/4, \( p=0.475 \)), lymphovascular invasion (5/30 vs 4/31, \( p=0.503 \)), age (<50 years; 3/24 vs \( \geq 50 \text{ years} \); 6/38, \( p=0.496 \)), or number of positive SLNB (all patients had <2 positive nodes on SLNB). Recurrences were detected in 4 patients, of which 1 was in a patient with micrometastases on SLNB. Out of the 4 recurrences, 3 were distant (liver and bone) and one was locoregional, with new disease in the contralateral breast.

Conclusion
In our series, 14.5% (9/62) of patients with micrometastases had positive n-SLB on CALND, which was not predicted by any clinicopathological characteristics. We have recently changed our practice toward not routinely offering CALND in patients with micrometastases, in keeping with current vogue. However, it is important to inform our patients that 14.5% of patients with micrometastases on SLNB may have positive n-SLN.
Title: Prognosis of metastatic internal mammary sentinel nodes (IMSN) in breast cancer

Eli Avisar¹, Shai Libson² and Eduardo Perez¹. ¹University of Miami Miller School of Medicine, Miami, FL and ²Ben Gurion University, Beer Sheva, Israel.

Body: Introduction:
The presence of positive internal mammary nodes has historically been associated with a significant worse prognosis. Recent studies have also demonstrated a worse prognosis associated with drainage to the internal mammary nodes. Intense search for those nodes, biopsy, appropriate staging and modern treatment for positive IMSN might improve outcome. We sought to study the prognosis of pathologically positive IMSN at our institution.

Methods:
A retrospective analysis of a prospectively collected database including all breast cancer patients identified with an IMSN that was biopsied between 2005 and 2012 was performed. Demographics, histologic markers, patterns of recurrence as well as survival data were collected. Univariate and multivariate analysis were performed.

Results:
Thirteen of 82 patients with a biopsied IMSN were metastatic (16%). In 8 of those (62%), the biopsy resulted in a change in staging. In all cases of positive IMSN additional radiation to the internal mammary chain was added. Eighteen percent (18%) of positive nodes did not show drainage to the internal mammary basin during Lymphoscintigraphy. There was no statistical difference in regional and distant recurrences between the patients with positive IMSN and those with negative IMSN. Furthermore there was no difference in disease specific survival.

Conclusions:
Intense search for IMSN presence and biopsy of those nodes is associated with changes in staging and treatment for metastatic IMSN. In our study, pathologically positive IMSN were associated with a non-inferior prognosis than negative IMSN.
Title: Axillary involvement in lobular breast cancer

Pilar Zamora¹, Covadonga Marti¹, Ana Roman¹, Jose M Oliver¹, Javier de Santiago¹ and Jose I Sanchez-Mendez¹. ¹Hospital Universitario La Paz, Madrid, Spain.

Body: Purpose:
Since the beginning of the use of the technique of selective sentinel node biopsy (SLNB), multiple models have been proposed to predict both the probability of involvement of the sentinel lymph node (SLN), and, in case it is positive, the likelihood of non-sentinel lymph node (NSLN) involvement.

Some of the factors that increase the likelihood of SN involvement (multifocallity, hormonal receptors, larger sizeâ–¦) are frequently found in invasive lobular carcinoma.

Invasive lobular carcinoma (ILC) is the second most common histologic type of invasive mammary carcinoma, comprising 5%–15% of all invasive breast carcinomas. However, in recent decades an increase in the relative incidence of this variant has been reported in the literature. This has been linked to the employment of hormone replacement therapy, the use of assisted reproduction techniques (IVF) or greater availability of diagnostic tools such as breast ultrasound or MRI that allow their better detection.

The aim of this study is to determine the extent of axillary involvement in both SLN and NSLN in patients with ILC

Patients and Methods
369 cases of infiltrating carcinoma candidates for SLNB technique between April 2010 and April 2013 were reviewed retrospectively.

Patients must have a diagnosis of breast cancer of any histological type, with a screening ultrasound without clinical suspicion of axillary involvement.

Cases of intraductal carcinoma that underwent SLNB were excluded and even those that were performed prior to neoadjuvant treatment.

Data on age, histological type, positivity / negativity of the SLN, implementation or not of axillary lymphadenectomy and outcome of involvement of NSLN were collected.

Statistical calculations were performed with SPSS.

Results
Of the 369 selected patients, 291 (79.9%) had invasive ductal carcinoma (IDC), 55 (15.1%) ILC and 18 other histological types (mucinous, colloid, tubular, papillary or mixed). The median age was 58.6 years.

SLN could not be found in 5 patients. 225 (61.8%) had a negative SLN. In 139 (38.2%) patients, SLN was positive, 64 of these cases showed micrometastasis while 75 presented macrometastasis.

Among IDC, 108 (37.1%) of the cases had a positive SLN, 53 (49.1%) of them with macrometastases.

Among ILC, 27 (49.1%) of the cases presented positive SLN, in 20 of them (74.1%) with macrometastasis.

Differences in both the SLN involvement and the presence of micrometastases observed between IDC and ILC were statistically significant (p< 0.001). In those cases were a lymph node dissection was performed, the probability of finding NSLN affection was 18.8% in the case of IDC compared to 55% in patients with ILC (p< 0.001).

Conclusions
Axillary lymph node involvement in the case of candidates for SLNB is more frequent and more extensive in patients with ILC than in patients with IDC.
Title: A new nomogram to predict axillary metastasis in breast cancer patients without axillary surgery

Valery Rodionov¹,², Vlada Cometova¹,², Sergey Panchenko¹,², Sereda Idrisova¹,², Yurij Savinov and Maria Rodionova.¹ Ulyanovsk State University, Ulyanovsk, Russian Federation; ²Ulyanovsk Regional Clinical Cancer Center, Ulyanovsk, Russian Federation and ³N.N. Blokhin Russian Cancer Research Center, Moscow, Russian Federation.

Body: Background: Several studies have concentrated on finding a combination of predictive parameters to establish a mathematical model that can identify patients with no axillary metastasis for whom routine lymph node dissection could be safely avoided. We developed a new model of nomogram (the Ulyanovsk Cancer Center axillary lymph node metastasis nomogram, UCC-ALNM nomogram); it employs clinically and pathologically relevant variables and offers possible advantages over the others nomograms.

The purpose of the study: To assess the predictive power of UCC-ALNM nomogram.

Methods: A total of 530 breast cancer patients treated between 2008 and 2010 were used as the modeling group for validating the UCC-ALNM nomogram. Clinical and pathologic features of patients were assessed by multivariable logistic regression to predict the presence of axillary metastasis in breast cancer patients. The predictive accuracy of our nomogram was measured by calculating the area under the receiver-operating characteristic (ROC) curve (AUC). Clinical factors included into analysis were: patient’s age and localization of the primary tumor. Pathological factors evaluated were: traditional pathological criteria (primary tumor size, histological type, tumor grade, HR- and Her-2 status) and new total pathological index (Ulyanovsk prognostic index - UPI), introduced by pathologists of the Ulyanovsk Regional Cancer Center. UPI is total score of six main pathological criteria that characterize the malignancy of epithelial tumors: degree of cellular differentiation, cellular polymorphism, mitotic activity, growth pattern, lymphovascular invasion, stromal reaction.

Results: By the multivariate analysis, patient’s age (p=0.04), tumor size (p<0.001), UPI (p<0.001), PR (p<0.001) and Her2 status (p=0.02) were identified as independent predictors of axillary metastasis. The nomogram was then developed using the six variables associated with axillary metastasis: age, tumor size, PR, Her2, UPI.

The new model was accurate and discriminating with an AUC of 0.7510 when applied to the modeling group.

Conclusions: UPI is a new predictive factor of axillary metastasis in breast cancer patients. UCC-ALNM nomogram.
Title: Sentinel node mapping with fluorescein and comparison with methylene blue and technitium sulphur colloid in early breast cancer

Anurag Srivastava¹, Rajendra A Badwe², Amar Prem¹, Vani Parmar², Vuthaluru Seenu¹, Anita Dhar¹, Nita S Nair², Rohini Hawaldar² and Vaibhav Sanmali². ¹AIIMS, New Delhi, Delhi, India and ²Tata Memorial Hospital, Mumbai, Maharashtra, India.

Body: Background: Sentinel lymph node biopsy is currently standard of care in node negative early breast cancer. There are various tracers for detecting sentinel lymph nodes in breast cancer. Sentinel lymph node biopsy (SLNB) using Technetium tagged Sulphur colloid is a convenient and safe method to assess lymph node status. However, sulphur colloid is radioactive and its use needs gamma camera which is very costly and available in very few centers in India. Methylene blue dye is an economical alternative for sulphur colloid having identification rate similar to the isotope. However, it may react with hemoglobin forming meth-hemoglobin resulting in difficulty in pulse oximetry during operation and causes skin eruptions and necrosis. Hence, there is a need to identify a sensitive, inexpensive and safe dye for sentinel node biopsy. In this study we have investigated the effectiveness and safety of fluorescein in sentinel node biopsy in a cross-sectional analytical study compared to Methylene Blue and Technitium Sulphur colloid.

Methods: This trial was conducted at two centers : Tata Memorial Centre, Mumbai and All India Institute of Medical Sciences New Delhi in India. We examined 86 patients of early breast cancer with no palpable axillary nodes undergoing SLNB with three tracers (Sulphur colloid /Methylene blue / Sodium Fluorescein). Patients underwent complete axillary dissection after identification of sentinel node for validation of sentinel node biopsy. Hot nodes were identified using hand held gamma probe and fluorescent nodes were identified using Ultra Violet lamp. All nodes were examined with Haematoxylin and Eosin staining. Since this study is first with use of fluorescein for sentinel node biopsy in breast, we injected fluorescein at decreasing time interval from 12 hours to 5 minutes to ascertain the most appropriate time for injection of fluorescein in first 15 patients. The sentinel nodes were visualized only when fluorescein was injected 5 minutes before incision.

Results: Sentinel nodes could be identified in eighty out of eighty six patients by combined tracers. In 72 out of 86 patients hot nodes could be identified (83.7%) while in 54 out of 86 patients fluorescent nodes could be identified (62.8%). Blue nodes were identified in 64 out of 86 patients(74.4%). Fluorescein delineated the lymphatic pathway going from breast to axilla in most patients. No side effects of Fluoroscein were observed. Of 86 cases, histologically positive sentinel lymph node were found in 15 patients. False negative with combined three tracers was in 3/18 (16.6%) patients. False negative rate with individual tracers was sulphur colloid- 3/16 (18.8%), Fluorescein – 2/15(13.3%) and with Methylene blue – 2/17(11.8%).

Conclusions: Fluorescein can be used as a low cost and effective alternative in sentinel lymph node biopsy for carcinoma breast without the risk of radiation exposure. Moreover, excellent visualisation of lymphatic pathway during procedure can act as a guide to trace the sentinel node.
Body: Background:
Intra-operative sentinel node biopsy (SNB) is performed for clinically node negative invasive carcinoma (IC). Despite a negative result for metastasis from rapid frozen section (FS) diagnosis of intra-operative SNB, some cases may be diagnosed positive when permanent sections (PS) are analyzed (i.e., false negative). In order to reduce the false-negative rate, it is necessary to determine why macrometastases measuring greater than 2 mm cannot be accurately diagnosed using frozen specimens. The aim of this study was to compare the pathological characteristics of false-negative and positive cases of macrometastasis using FSs. We also reviewed whether there were differences in postoperative prognoses between positive and false-negative cases.

Methods:
We retrospectively reviewed 1632 intra-operative SNBs collected from 2008 to 2011 at St. Luke’s International Hospital, Tokyo, JAPAN. Of these, 980 patients had undergone surgery for the treatment of IC without neoadjuvant chemotherapy. Lymph nodes were sectioned every 2 mm and examined. For FSs, we performed hematoxylin and eosin (HE) staining, and for PSs, we used HE and cytokeratin (AE1/AE3) staining. Image J (NIH Image, Bethesda, MD, USA) was used for assessment of the metastatic area of lymph nodes. Micro- and macrometastases were defined as metastatic lesions measuring between 0.2 and 2 mm or measuring 2 mm or more, according to TMN classification.

Results:
Using FSs, we identified 104 patients (10.6%) who were positive for macrometastasis, 16 patients (1.6%) who were positive for micrometastasis, and 860 patients (87.8%) who were negative for metastasis. Of the negative cases, the result was changed to micrometastasis in 37 cases (4.3%) and to macrometastasis (false-negative cases) in 14 cases (1.6%). Ten of the 14 patients did not have additional axillary lymph nodes dissection. The mean age of the patients was 55.1 ± 3.1 years for false-negative cases and 52.3 ± 1.2 years for positive cases. In terms of histological features, there were statistically significant differences between false-negative and positive cases for invasive ductal carcinoma (IDC), invasive lobular carcinoma (ILC), and IDC plus ILC (p < 0.05). There were no statistically significant differences between false-negative and positive cases in terms of the total number of slices for lymph nodes (8.4 ± 1.6 vs. 9.7 ± 0.6, respectively; p = 0.41) but tendency in the number of slices for metastases (2.4 ± 1.2 vs. 4.8 ± 0.5, respectively; p = 0.07). The maximum area ratio of metastasis was significantly lower in false-negative cases than in positive cases (3.0% ± 0.1% vs. 20.0% ± 0.2%, respectively; p < 0.05). There was no significant difference in disease-free survival between false-negative and positive cases (p = 0.43).

Conclusions:
Although it is necessary to reduce false-negative cases, no difference in prognosis was detected in our study. False-negative cases had lower metastasis area ratios than positive cases diagnosed using rapid diagnosis of intra-operative SNBs, and ILC is known to be high in histological features. Therefore, using AE1/AE3 stain together with rapid diagnosis of intra-operative SNBs may lower the false-negative rate when a diagnosis includes ILC in pre-operation analysis.
Randomised controlled trial of stereotactic 11g vacuum-assisted or core biopsy for diagnosis and management of malignant microcalcification

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Body: Background
Vacuum-assisted biopsy (VAB) is claimed to improve diagnosis of malignant microcalcification (MM) and prevent underestimation of invasive component compared to stereotactic core biopsy (SCNB) 14g biopsies. VAB is also reported to reduce requirement for further surgical procedures versus SCNB.

Methods
In a randomised controlled trial, we compared both techniques in the firstline diagnosis of MM and subsequent surgical management in two UK Breast Screening Units. Patients with MM were identified and gave written informed consent to the study before randomisation 1:1 to SCNB or VAB. Exclusions included bleeding diathesis or significant comorbidity preventing surgery. SCNB was performed by advanced radiography practitioners and VAB by experienced radiologists under local anaesthesia. Quality of life was assessed using EORTC QLQ-BR23 questionnaire: Pre-biopsy, 2, 6, and 12 months post-randomisation. The primary objective compared the final pathological diagnosis with the initial biopsy (VAB or SCNB) and required 110 patients to be randomised to show a 25% improvement in diagnostic accuracy with VAB compared to SCNB with a 90% power.

Results
Three quarters of MM investigated was less than 15mm in size. Overall 787 women were investigated for eligibility. 476 did not meet the inclusion criteria, 82 declined to participate and 100 met exclusion criteria. In total, 129 women were randomised. Both groups were evenly matched for age 57.2 years (range 47-75) and lesion size but VAB took longer (p<0.001) and produced on average, 4 more cores (12 versus 8) (p<0.01). Three (4.7%) VAB developed bleeding causing procedure abandonment. The PPV for biopsy of MM was 30.2% and 1 patient withdrew before the procedure. There was an 84% diagnostic accuracy of SCNB which precluded any effect on surgical outcome.

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<tr>
<th>Analysis</th>
<th>VAB</th>
<th>Core SCNB</th>
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<tr>
<td>n</td>
<td>63</td>
<td>64</td>
<td></td>
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<tr>
<td>Accurate diagnosis</td>
<td>54 (86%)</td>
<td>54 (84%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Subsequent therapeutic porcedures - 1</td>
<td>9 (56%)</td>
<td>17 (71%)</td>
<td>0.50</td>
</tr>
<tr>
<td>Subsequent therapeutic procedures - 2</td>
<td>7 (44%)</td>
<td>7 (29%)</td>
<td>0.50</td>
</tr>
<tr>
<td>DCIS upgrade to invasive</td>
<td>12.5%</td>
<td>8.7%</td>
<td>0.86</td>
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</table>

Both groups had significant Quality of Life (QOL) falls in Global Health (p<0.001) and Social Functioning over time (p<0.04) but no overall difference between groups was seen. At 12 months, body image scores fell and fatigue increased in patients undergoing surgery.

Conclusion
SCNB has equal accuracy as VAB in the firstline diagnosis of malignant microcalcification. Devicor provided the equipment to Dr S.B. and Prof. N.B., to do this study.
Title: A review of the accuracy of wire localisation using digital breast tomosynthesis (DBT) in a prospective series of 81 patients

Simon DH Holt¹, Khaldoun MY Nadi¹, Amrita Gurung¹, Helen R Williams¹, Anita M Huws¹ and Yousef M Sharaiha¹. ¹Prince Philip Hospital, Llanelli, Carmarthenshire, United Kingdom.

Body: Introduction: Digital breast tomosynthesis allows identification of impalpable lesions in three-dimensions. We have devised a technique for wire placement using a gridded plate that allows localisation of such lesions for subsequent surgical excision. Method: 81 consecutive cases were studied prospectively between Jan 2012 and May 2014. The localisation is usually performed by the operating surgeon under local anaesthetic shortly before the patient is transferred to the operating theatres. Our surgeons consider the optimal position for the tip of the wire to be 1 cm beyond and 1 cm to the side of the lesion placed using only the CC or lateral projection. The patient is positioned with the aperture of a gridded plate over the lesion needing to be surgically removed. A single DBT is acquired and the volumes reviewed. The lesion is identified and the depth in the breast recorded (Z coordinate). The ideal position for the wire is then marked in that plane and the volume scrolled through to bring the calibrated plate into focus. This identifies the X and Y coordinates, which are then recorded. The wire is then placed freehand in the line of the beam and a further DBT performed to check the position of the wire without needing to reposition the patient. A specimen x-ray is performed immediately following excision to confirm the target lesion is included. The results are verified at the subsequent multidisciplinary team (MDT) meeting.

Results: Once the target is identified, the average time for the localisation procedure and check DBT was 4 minutes. This series included both diagnostic (N=24, 30%) and therapeutic excisions (N=57, 70%). Patients presented both from screening (N=64, 79%) and symptomatically (N=17, 21%). The age range of the patients was 42-79y (mean 59y). 54 (67%) cases were undergoing excision for calcifications, 15 (19%) for impalpable masses, 11 (13%) for radial scars or distortions and 1 for glandular asymmetry. The final diagnoses following excision were IDC 34 (42%), DCIS 29 (36%), benign 13 (16%) and 1 case each of ILC, spindle cell tumour, mucinous carcinoma, phyllodes and pleomorphic LCIS.

In all cases the target lesion was shown to be present in the specimen x-ray, confirmed on histology and ratified by the MDT.

Conclusions: Wire localisation using DBT is a quick and accurate method of localising impalpable lesions for surgical excision.
Title: Retrospective analysis of the accuracy of ultrasound-guided core needle biopsy in the diagnosis of breast invasive ductal carcinoma: Experience in Chinese population

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Body: Background: Percutaneous imaging-guided core needle biopsy is a reliable alternative to surgical biopsy for a histological diagnosis. Nowadays, core needle biopsy is considered to be the standard technique for histological diagnosis of breast lesions. However, the accuracy of ultrasound-guided core needle biopsy in predicting tumor grade, which is scored according to mitotic index, tubular differentiation, and nuclear atypia, is not well established. The aim of this study is to evaluate the concordance of histological diagnosis between ultrasound-guided core needle biopsies and subsequent excision specimens of breast invasive ductal carcinomas.

Methods: We retrospectively reviewed the medical records of 457 consecutive female breast invasive ductal carcinomas that were biopsied under sonographic guidance, using 14-gauge core needles exclusively, and that were subsequently excised surgically from June 2011 to May 2014. A minimum of four cores were taken per lesion. Tumor grade was assigned using the standard modified Scarff-Bloom-Richardson system. Core biopsy pathological diagnosis and grades were compared with final surgical excision specimens. The diagnostic coincidence rate and the agreement rate were expressed in percentages and in kappa statistics; the rates of overestimation and underestimation were also assessed. The correlation among tumor size (small, \( \leq 0.5 \) cm; medium, 0.6–1.9 cm; and large, \( \geq 2.0 \) cm), diagnostic coincidence rate, and agreement rate was also evaluated.

Results: Compared with the postoperative pathological diagnosis, the diagnostic coincidence rate of ultrasound-guided core needle biopsy was 94.75% (433/457). 24 cases were diagnosed with ductal carcinoma in situ or intraductal carcinoma by core needle biopsy. The overall agreement between core needle biopsy and surgical pathology grade was 67.28%. Agreement by biopsy grade was 73.33% (77/105) for grade 3, 70.97% (198/279) for grade 2, and 57.53% (42/73) for grade 1. Core needle biopsy underestimated 19.47% (89/457) and overestimated 10.07% (46/457) of the lesions. Small tumors were inclined to be more easily misdiagnosed as ductal carcinoma in situ or intraductal carcinoma, and large tumors were more likely to show underestimation rather than overestimation when discordant (\( p < 0.0001 \)).

Conclusion: Ultrasound-guided core needle biopsy accurately predicts high-grade breast tumors but is moderately accurate for lower-grade lesions. Large tumor size negatively impacts the accuracy of tumor grade found on biopsy and is associated with underestimation. Our finding indicates that ultrasound-guided core needle biopsy has important significance in estimating breast carcinoma grade and instructing clinical neoadjuvant chemotherapy.
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Average Grade: 6.00

Title: Are all small tumors low risk? Characterization of small invasive node negative breast cancers (BC) enrolled in the EORTC 10041/BIG 3-04 (MINDACT) trial

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Body: Background: Adjuvant systemic therapy for subcentimetric N0 BC is controversial. These tumors have good prognosis, but a subgroup has high risk of relapse and requires adjuvant therapy. The best tool to identify this subgroup is unknown. We clinically and biologically characterize pT1abN0M0 tumors from patients enrolled in MINDACT. Patients and Methods: All MINDACT pts with pT1abN0 tumors were included. Descriptive statistical analysis included age, menopausal status, centrally assessed tumor grade, Ki67, ER, PR and HER-2 status. Cut-off value for high Ki67 was 20%. IHC subtypes are per 2013 St. Gallen consensus/ ESMO guidelines. Genomic tests (Mammaprint, Targetprint, BluePrint) were performed and their concordance rate with central pathology evaluated. We report clinical (modified version of adjuvant online!) and genomic risk assessment (MammaPrint) for these patients. Survival data will be reported after MINDACT primary analysis. Results: 826 patients with T1abN0 tumors were enrolled in MINDACT, [12.3% of all patients (6694) and 16% of all N0 tumors]; 310 (35%) patients were ≥ 60 years and 537 (65%) were postmenopausal. Most (710; 86%) samples were tested by central pathology. Tumors were mainly ductal (579; 81.5%), ER+ (650; 91.5%), and PR+ (593; 83.5%); 44 (6.2%) were HER-2+; 520 (73.2%) had a low Ki67 (& 24.5%) had high Ki67 labeling index; 80 (11.3%) were grade 3 & 328 (46.2%) grade 2 tumors. According to Targetprint, 761/826 (92.1%) tumors were ER+; 624/826 (75.5%) PR+; 45/826 (5.4%) HER2+. Concordance between Targetprint and central pathology was higher for ER (kappa=0.90) and lower for PR and HER2 (kappa=0.60 and 0.73 resp). Molecular subtype classification by IHC surrogates showed 420/710 (59.2%) Luminal A tumors; 189/710 (26.6%) Luminal B; 36/710 (5.1%) Luminal B/HER2+; 8/710 tumors (1.1%) HER-2+ and 37/710 (5.2%) TNBC. Using BluePrint 739/826 (89.5%) were Luminal; 29/826 (3.5%) HER-2+ and 58/826 (7.0%) basal. Combining BluePrint and Mammaprint 609/826 (73.7%) tumors were Luminal A & 129/826 (15.6%) Luminal B. Agreement between Mammaprint and Ki67 for distinction of luminal A vs B/HER-2 neg. tumors was low (kappa=0.31), similar to the overall MINDACT results (kappa=0.35). Using clinical risk assessment 820/826 (99.3%) were low-risk (CL). Using genomic risk assessment 624/826 (75.5%) were low risk (GL) and 201/826 (24.3%) were high-risk (GH). Overall, 624/826 (75.5%) were both CL/GL and 196/826 (23.7%) were CL/GH. The percentage of discordant CL/GH cases among T1abN0 tumors (196/826, 23.7%) is higher than in all MINDACT cohort (592/6694, 8.9%) and among T1c-2-3N0 tumors (381/4462, 8.5%). T1ab tumors were mainly 720/826 (87.2%) treated with BCS and radiotherapy; adjuvant treatment and compliance rate will be available at the meeting.

Conclusions: 1) Results show biological characteristics are relevant for T1abN0 BC & that size/nodal status could be insufficient determinants of adjuvant systemic therapy. 2) A significant portion (23.7%) of such tumors, clinically classified as low risk, was genomically high risk by MammaPrint. 3) IHC surrogates don't always accurately reflect genomic molecular subtyping.
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Average Grade: 5.20

Title: Incidence of actionable genomic alterations in metastatic breast cancer: A prospective study

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Body: Introduction: Foundation Medicine Incorporated (FMI) uses next generation sequencing (NGS) to simultaneously identify oncogenic molecular events and match targeted therapies to these alterations. We conducted a prospective study of the FMI test to describe the incidence of actionable genomic alterations found in advanced breast cancer patients.

Methods: This is a prospective, single center, single-arm trial in advanced breast cancer patients designed to assess FMI testing’s feasibility and impact. Adult metastatic breast cancer patients with an estimated survival of ≥ 3 months were included, had tumor sample available for testing, and were within 10 weeks of starting their current therapy line. A massively-parallel sequencing platform (FoundationOne™) used the patient’s tumor (FFPE tissue) to sequence for 236 cancer-related genes plus 47 introns of 19 genes often rearranged in cancer to high depth (>500x). Genomic alterations were categorized as actionable if linked to an approved therapy in the solid tumor of study or another malignancy, a known or suspected contraindication to a given therapy, or a clinical trial linked to the alteration. The number of genomic alterations was quantified; genes with > 1 alteration were counted once.

Results: Forty-nine patients were consented to FMI testing. Forty patients (82%) successfully completed testing; analysis was insufficient in 6 tissue samples, 2 failed DNA extraction, and 1 patient’s tissue was not received. Of the 8 unsuccessful tests, sampling from the bone (50%) and liver (38%) were the most common sites of failure. Thirty-seven reports have been received; 27 (73%) samples were taken from metastatic tissues, 10 (27%) from the primary breast tumor. A total of 192 actionable alterations were detected in tumors tested, with a median of 5 (range 1-11) alterations found per patient. Amplifications comprised 87 alterations, 105 were a base substitution, insertion/deletion or other copy number alteration. Of the 192 alterations, the TP53 gene was the most often altered [n=22 (11%)] and most frequent in ER/PR/HER2-neu negative breast tumors. Alterations in PIK3CA [n=16 (8%)] and ZNF217 [n=8 (4%)] were the 2nd and 3rd most commonly altered genes. The NGS report provided 24 of the 37 patients (65%) with a recommended FDA approved breast therapy; an additional 24 patients (65%) were suggested a non-breast FDA approved therapy. At least 1 potential treatment or clinical trial was proposed to 36 patients (97%) with everolimus (n=30) and temsirolimus (n=30) the most commonly recommended FDA approved interventions, and trametinib (n=6), regorafenib (n=6), and pazopanib (n=6) the next most commonly suggested interventions. Patients had a median of 3 (range 0-7) genomic alterations paired with a recommended clinical trial.

Conclusions: This prospective study shows NGS testing in advanced breast cancer patients is successful in over 80% of patients screened. Two-thirds of patients were recommended a FDA approved therapy and nearly all patients were suggested at least one potential clinical trial. Further studies to assess barriers to patients’ access to these recommended targeted therapies are currently underway.
Title: Comparative analysis of somatic mutations in matched primary vs metastatic triple-negative breast cancers

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Body: Background. Treatment of triple-negative breast cancer (TNBC) is clinically challenging due to disease heterogeneity and aggressiveness which often result in high recurrence and mortality rates. We have previously reported on genomic alterations found in 14 metastatic TNBC tumors (Craig et al 2012) uncovered through whole-genome and transcriptome sequencing and found that majority of tumor changes in our cohort converged on RAS/RAF/MEK/ERK and PI3K/AKT/mTOR pathways revealing potential therapeutic targets. In this study, we interrogated patients’ matched primary and metastatic cancers using exome sequencing. Differentiating between primary and metastasis-specific genomic alterations will further our understanding of TNBC recurrence.

Methods. Tumor tissue was microdissected from 10μM FFPE sections from primary tumors of nine TNBC patients. DNA was extracted using Qiagen AllPrep kit. Whole-genome libraries were prepared from >250ng of DNA using Kapa-on–Bead method. Exomes were captured using All Exon V5 capture kit (Agilent Technologies) and sequenced on Illumina HiSeq. List of somatic variants (SNVs) in primary tissue was generated using Seurat variant caller and manually compared with the SNVs uncovered previously in metastatic tumors. Presence (or absence) of all reported somatic SNVs was visually confirmed in IGV genome viewer.

Results. Our analysis revealed the majority of somatic mutations found in metastatic lesions were also present in the primary tissue (64.3% on average). Patient TNBC03 had an unusually high number of new mutations in the lung metastatic lesion (72.7%). All TP53 mutations in our cohort were an early event present in both primary and metastatic tissues. In patient TNBC01, acquired mutation in NF1 - an upstream negative regulator of RAS pathway - in addition to pre-existing PTEN deletion, CTNNA1 underexpression and RB1 mutation - suggests RAS pathway addiction which could have been responsible for disease recurrence in this patient. Interestingly, NEDD4 mutation was found in a lung metastasis of another patient whose disease was refractory to BEZ235 (PI3K/mTOR inhibitor) treatment. NEDD4 has recently been shown to be an upstream regulator of the PI3K pathway and a possible therapeutic target in cancers with downregulated PTEN. Additional select acquired mutations in metastatic lesions of our cohort included FGF14, ABCB10, MDM2, KIF1C, ATAD2B, PTPLAD2, MDM20, TDRD1, and USP6. Mutated CDH5 was found in metastases in 2 of 9 patients.

Conclusion. Our findings demonstrate the complexity and variability in somatic events underlying TNBC recurrence, reinforcing the need to re-biopsy metastatic TNBC lesions and to employ combination targeted therapies wherever possible.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P2-03-05  
**Average Grade:** 5.50

**Title:** The heterogeneous clinical behavior of luminal breast cancers is associated with different mutational landscapes

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**Body:** Background: Genomic studies in estrogen-receptor (ER) positive breast cancer showed few commonly mutated genes and suggested that tumors with higher background of mutation rate were enriched in intrinsic aromatase-inhibitor-resistant tumors (Ellis MJ Nature 2012). Different combinations of proliferation (PROL) and estrogen-receptor related genes (ERS) allow the prediction of the risk of recurrence and endocrine treatment sensitivity (Bianchini G Breast Cancer Res 2013). The highPROL/lowERS is enriched in intrinsic endocrine resistant tumors. We explored whether different genomic alterations could contribute to the different clinical behavior of groups defined by PROL and ERS.

Method: We defined luminal breast cancers by PAM50 and retrieved gene expression and whole-exome sequencing data of 324 luminal breast cancer cases from the Cancer Genome Atlas (TCGA). PROL and ERS were calculated as previously reported. Low and high expression groups were defined by median cut-points (Bianchini G Breast Cancer Res 2013). As a metric of the background mutation rate of each sample, we used the overall mutation frequency in whole exome (Kandoth C Nature 2013). The most frequently mutated genes (TP53, PIK3CA, MAP2K4, MAP3K1, GATA3, CDH1) were also assessed for their association with PROL and ERS defined groups.

Results: The average number of mutations (Nmut) was 44.4 (5-366). High and low proliferation groups had different mutation frequency (average Nmut 53.6 and 35.3, respectively; p=6.1E-06). LowERS group also had a higher Nmut (average Nmut 54.4 vs. 34.5 in high ERS; p=1.2E-06). Notably, neither high or low expression of the ER gene (p=0.89), nor positive and negative progesterone receptor by immunohistochemistry (p=0.69) were associated with Nmut. LowERS group was associated with higher Nmut in luminal A (p=0.005) and B (p=0.006) subtypes. The group with highPROL and lowERS had the highest Nmut (average 62.1), which was not significantly different from basal-like tumors. HighPROL/lowERS was significantly associated with higher frequency of mutTP53 (32.3%) and lower rates of mutPIK3CA (28.1%), mutMAP2K4 and/or mutMAP3K1 (7.2%) and mutCHD1 (3.1%). The lowPROL/highERS had the lowest Nmut (average 29.8), with the higher rate of mutPIK3CA (61.4%) and mutMAP2K4 and/or mutMAP3K1 (29.2%), and lower rate of mutTP53 (6.7%).

Discussion: In ER-positive breast cancers, the highPROL/lowERS group, which is characterized by the highest risk of early recurrences despite endocrine treatment and chemotherapy, poor down-regulation of Ki67 after neoadjuvant aromatase-inhibitor (AI), but the highest rate of pCR after neoadjuvant chemotherapy, was also associated with the highest frequency of mutations, of the same order of magnitude of basal-like tumors. This data is in line with the observation that the number of mutations is linked to intrinsic resistance to neoadjuvant AlS (Ellis MJ Nature 2012). These data could be also informative for interpreting results of the combination of everolimus and endocrine therapy ( exemestane or tamoxifen) that suggest higher efficacy in tumors with acquired endocrine resistance (lower mutation rate) compared to intrinsic or primary endocrine resistance (higher mutation rate).
Title: FoundationOne profiling of TSC1 and TSC2-mutated advanced breast cancers

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Body: Background: The TSC1/TSC2 complex inhibits the mTOR pathway. Loss of function of this complex leads to hyperperactivation of the mTOR pathway which promotes growth and survival. Few studies have examined the role of TSC1 and TSC2 mutations in breast cancer and as a clinically relevant target for molecularly targeted therapy, such as everolimus, a rapamycin analog.

Methods: Hybridization capture of 3769 exons of 236 cancer-related genes and 47 introns from 19 genes that are frequently rearranged in cancer were fully sequenced to high, uniform coverage from a commercial CLIA-certified laboratory (Foundation Medicine).

Results: 30 of 2,208 breast cancer pt samples (1.3%) harbored either TSC1 (13/30, 43%) or TSC2 (17/30, 56%) genomic alterations (GAs). The samples with TSC1/2 consisted of breast carcinoma n.o.s. (17/30) and invasive ductal carcinoma (12/30); one invasive lobular carcinoma (ILC) harbored a TSC2 alteration. Sequencing was performed on metastatic (18/30, 60%) and primary (12/30, 40%) tumors. All TSC1 alterations resulted in truncation of the protein product; TSC2 GA consisted of truncations (14/17) and point mutations (3/17). TSC1/2 GAs frequently co-occurred with GAs in TP53 (19/30; 63%) and amplifications of MYC (9/30; 30%) and FGFR1 (7/30; 23%). Of the TSC1/2-mutant cases where hormone receptor and HER2/neu status was known (23/30), 65% were ER-/PR-/HER2- (TNBC), 26% were ER+/HER2- and 9% were ER-/HER2+.

Patient case: We describe a postmenopausal node positive ILC (ER+/PR+/HER2-) pt who received adjuvant chemotherapy followed by 9 yrs adjuvant anastrazole then with disease recurrence. On FoundationOne testing a TSC2 A1141T missense mutation, along with GAs in CDH1, ETV6, and CSF1R, were found in her metastatic disease in the soft tissues of her left orbit associated with substantial swelling resulting in loss of vision. While TSC2 A1141T has not been functionally characterized, in vitro and in vivo data suggest sensitivity to mTOR inhibitors. Her disease stabilized but did not decrease in size with vinorelbine + capecitabine. She was treated with letrozole + everolimus and had a near clinical complete response with restoration of vision for 5 + months. Collection of clinical data on additional pts is currently ongoing.

Conclusion: 1.3% of breast cancer cases harbored GAs in either TSC1 or TSC2. The majority of TSC1/2 GAs cause inactivation of these negative regulatory proteins leading to activation of the mTOR signaling pathway. TSC1/2 GAs were enriched for TNBC (65%) versus non-TNBC (35%) (p= 0.077; Fisher's exact test). Truncation of TSC1/2 occurred at multiple codons, underscoring the importance of examining the entire coding region of tumor suppressor genes for these inactivating events. Comprehensive genomic profiling has the potential to identify the broad spectrum of these inactivating events, a subset of which may be clinically relevant.
Deep clonal profiling identifies distinct mechanisms of heterogeneity and evolution in breast cancer

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Background: Breast tumors exhibit intratumor heterogeneity resulting in targeted therapy resistance and other challenges in disease management. To address the sources of heterogeneity, we performed a unique, in-depth analysis of clonal architecture in primary chemoradiation-naïve breast cancers. We combined DNA content-based flow cytometry and ploidy analysis with aCGH and next-generation sequencing (NGS) in multiple biopsies from the tumors and involved lymph nodes (LNs).

Material and methods: We used DNA content-based flow sorting to isolate nuclei from distinct populations of diploid and aneuploid tumor cells in surgical tumor samples from two chemoradiation-naïve patients. Each sorted tumor cell population was interrogated with aCGH and exome NGS. In Patient #1, we used 12 fresh frozen sections morphologically mapped from within a HER2+, ER+, PR- primary invasive ductal carcinoma (IDC) of histological grade 3 with LN involvement and 2-3 sections from 2 out of 5 LNs. In Patient #2, 11 morphologically mapped fresh frozen sections were analyzed from a grade 2, ER+, PR+, HER2+, BRCA2 mutant LN- IDC. In parallel, matching samples were processed for IHC assays.

Results: We identified multiple co-existing aneuploid populations within the biopsies. The 17 primary and LN biopsies from Patient #1 fell into 6 distinct ploidy groups albeit with aberrant but homogenous aCGH profiles, characterized by SARC amplification and homozygous deletions in ROBO1 and ROBO2. In contrast a dominant ploidy was identified throughout Patient #2 but with heterogeneous aCGH profiles. Mutation profiles obtained through exome sequencing further confirmed that ploidy was the main driver in Patient #1 whereas copy number aberrations played the key role in Patient #2 with the BRCA2 mutation (R3129X). A dendrogram based on exome variant calls of the aneuploid populations in Patient #1 strongly correlated with ploidy group and further revealed the specific clonal population characterized by a 5N ploidy and homozygous mutations in TP53 and PIK3CA as the progenitor to the ploidies present in the distant LNs. Strikingly, both patients had no HER2 amplification or mutation across their clonal populations, contradicting the initial IHC staining in a single core biopsy.

Conclusions: Rather than inferring the presence of distinct tumor cell populations, our novel flow-sorting based approach of first identifying the clonal populations and then interrogating their genomes, provides an objective method of exploring the sources and clinical significance of tumor heterogeneity. Our approach of clonal analysis has broad implications in the study of tumor heterogeneity and the identification of drivers in breast and other solid tumors that can advance more effective treatment and clinical management of patients with this disease.
Title: Mutational landscape of metaplastic breast carcinomas

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Body: Introduction: Metaplastic breast carcinoma (MBC) is an aggressive histologic type of breast cancer, which preferentially displays a triple-negative phenotype. These tumors are characterized by the presence of malignant cells exhibiting differentiation towards squamous epithelium or mesenchymal elements, including spindle, chondroid, osseous and rhabdoid differentiation. Unlike other rare histologic types of breast cancer such as adenoid cystic and secretory carcinomas, which are underpinned by the MYB-NFIB and ETV6-NTRK3 fusion genes respectively, pathognomonic genetic alterations have not been identified in MBC. It has been suggested, however, that the frequency of PIK3CA somatic mutations would be significantly higher in MBCs than in other forms of triple-negative disease. Here we sought to characterize the mutational landscape of MBCs by means of high-depth whole exome sequencing analysis.

Material and Methods: Twenty-one triple-negative MBCs were retrieved from the authors' institutions. Representative sections from frozen blocks were microdissected to ensure tumor cell content greater than 50%. DNA samples extracted from microdissected tumor and matched peripheral blood leukocytes were subjected to high-depth (250x) whole exome sequencing on an Illumina GAIIx or HiSeq2000. Somatic point mutations were called using MuTect and somatic insertions and deletions (indels) were called using Strelka, Varscan2 and Haplotype Caller. Potentially pathogenic mutations were predicted using computational algorithms including PolyPhen-2, Mutation Taster, Mutation Assessor, CHASM and FATHMM. Significantly mutated genes were identified using MutSigCV. Pathway and network enrichment analysis of mutations was performed with Ingenuity Pathway Analysis and HOTNET. The genomic landscape of MBCs was compared with that of triple-negative breast cancers (TNBCs) analyzed as part of The Cancer Genome Atlas project.

Results: A mean of 135 somatic non-synonymous point mutations and indels were identified per MBC. The most frequently mutated gene was TP53, found in 12/21 cases (57%), and the only significantly mutated gene as defined by MutSigCV (q<0.01). The repertoire of somatic mutations found in MBCs was qualitatively similar to that of TNBCs of no special type, and recurrently mutated genes were altered at similar frequencies in MBCs and TNBCs of no special type. When somatic mutations were annotated in pathways and networks, MBCs were found to have potentially pathogenic mutations affecting genes directly related to the PI3K pathway, including pathogenic non-synonymous mutations affecting PIK3CA, PIK3R1, PIK3R2, PIK3C2B, PIK3C2G and PTEN, significantly more frequently than TNBCs of no special type (10 out of 21 MBCs vs. 11 out of 62 TNBCs; Fisher's exact test p-value=0.0099).

Conclusion: The majority (57%) of MBCs harbored non-synonymous mutations affecting TP53. While the frequencies of mutations affecting recurrently mutated genes in MBCs are similar to those found in other forms of TNBCs, MBCs significantly more frequently harbor mutations affecting PI3K pathway-related genes than TNBCs of no special type.
Benchmarking mutation function prediction algorithms using validated cancer driver and passenger mutations

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Background: Massively parallel sequencing studies have identified large numbers of mutations of unknown biologic significance. There is a pressing need for computational methods to predict and distinguish neutral from potentially pathogenic mutations accurately, to help identify those mutations worth exploring experimentally and clinically. Although various bioinformatic algorithms are available, they are based on different methodologies and assumptions, and their predictions for the same mutations are not always concordant. In this study, we sought to benchmark the performance of 17 prediction algorithms using functionally validated and pathognomonic mutations.

Methods: We curated the literature for functionally validated and pathognomonic mutations as our positive dataset (i.e. pathogenic mutations). For the negative dataset (i.e. neutral mutations), we retrieved variants from the dbSNP database, including only those with minor allele frequency >25%. We compiled a total of 7975 mutations (875 pathogenic and 7100 neutral). The performance of each prediction algorithm, namely accuracy, specificity, sensitivity, positive predictive value (PPV) and negative predictive value (NPV), were defined using the positive and negative datasets described above. Confidence intervals were calculated by sub-sampling 2/3 of the functionally pathogenic mutations and equal number of neutral mutations 500 times. To reduce the bias introduced by mutations included in the COSMIC database, we excluded those found in COSMIC v67, resulting in 6048 mutations (212 pathogenic and 5835 neutral), and re-evaluated the performance of each prediction algorithm.

Results: Our analysis revealed that the overall accuracy varied considerably, with a median of 87% (range 78%-97%). In terms of accuracy, FATHMM (cancer) statistically outperformed all other prediction algorithms (97%, 95% confidence interval (CI) 96%-98%), followed by MutationTaster 2 (94%, 95% CI 93%-95%). Sensitivity and specificity also varied (median 85%, range 77%-96% and median 89%, range 71%-100%, respectively). The most sensitive prediction algorithm, FATHMM (cancer) (96%, 95% CI 95%-97%) statistically outperformed all others. The most specific prediction algorithm was CHASM (breast) (100%, 95% CI 94%-100%). While CHASM (breast) had the highest PPV (100%, 95% CI 99%-100%), FATHMM (cancer) had statistically better NPV than all other prediction algorithms (96%, 95% CI 95%-97%). When COSMIC mutations were removed, FATHMM (cancer) remained the most accurate (93%, 95% CI 91%-95%) though the difference was not statistically significant. In this context, CanDrA (breast) was the most sensitive prediction algorithm (95%, 95% CI 93%-97%) and had the highest NPV (93%, 95% CI 90%-96%), while CHASM (breast) was the most specific prediction algorithm (100%, 95% CI 99%-100%) and had the best PPV (99%, 95% CI 97%-100%).

Conclusions: Our results demonstrate that functional prediction algorithms varied in performance. Using this dataset of mutations, FATHMM (cancer) outperformed all other prediction algorithms in terms of accuracy, sensitivity and NPV, and remained the most accurate even when mutations catalogued in the COSMIC database were excluded.
Title: Comprehensive somatic SNV and CNV profiling for triple-negative breast cancer patients by targeted next-generation sequencing

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Body: Introduction: Triple-negative breast cancer is a subtype of breast cancer lacking expression of estrogen receptor, progesterone receptor and HER2/neu protein markers. Despite having the worse prognosis, currently there is no molecular target for this subtype. To discover the clues for therapeutic targets of TNBC, we examined somatic Single Nucleotide Variation (SNV) and Copy Number Variation (CNV) profiling of TNBC patients using targeted next-generation sequencing (NGS).

Methods: A total of 414 breast cancer and normal breast samples were collected at Seoul National University Hospital (SNUH) from 1995 to 2013. By sample condition, 94 samples were frozen tissues (47 tumor-normal matched pair), and 320 samples were formalin-fixed and paraffin-embedded (FFPE) tissues (155 tumor-normal matched pair; Tumor 164, Normal 156). Genomic DNA was extracted from samples and target enrichment was done by using Agilent SureSelect Human Kinome Panel (612 genes including over 500 kinases). The paired-end libraries were constructed and sequenced on Illumina HiSeq 2000 instrument with average 250x depth coverage. Generated sequence reads were aligned to human genome hg19 with bwa algorithm, and somatic SNV and CNV were identified using VarScan2 algorithm. To confirm the existence of identified somatic SNV and CNV, we carried out SNP microarray experiment (Illumina Human Omni5 Exome microarray) for 46 pairs of frozen tissue samples. There were 18,720 SNP probes in SNP microarray covering target region of kinome panel. If the same SNV and CNV were detected in both NGS and microarray data, we considered them validated.

Results: In 46 paired frozen samples, 610 somatic SNVs were detected. The most frequent and non-synonymous mutations were in PIK3CA (8.6%), BMPR1A (4.3%), FES (4.3%), TP53 (4.3%), ROCK1 (4.3%), PAK2 (4.3%), CDK18 (4.3%), OBSCN (4.3%), LRRK1 (4.3%), CDC6 (4.3%), PIKFYVE (4.3%), and FGFR3 (4.3%). Chromosomal aberration was detected in chromosome 1 (10 samples) and chromosome 8 (11 samples). 242 somatic CNV were found in 157 genes in 46 patients, but 99 gene amplifications showed only single occurrence. In other words, TNBC sample displayed highly heterogeneous CNV profile. However, we detected two frequently amplified genes; PKHD1L1 (13%) and NRPB2 (10.9%). Further analysis on NGS data of 155 pairs of FFPE samples is in process to validate the results.

Conclusion: We investigated comprehensive somatic mutation profile in matched pair TNBC samples. Although these samples showed highly heterogeneous mutation profile, it’s possible to detect some interesting SNV and CNV with over 4% frequency. Further analysis may illuminate clinical meaning of those alterations.
**Title:** Interaction between smoking history and gene expression levels impacts survival of breast carcinoma patients

James L Wittliff¹, Sarah A Andres¹, Mohammad A Alatoum¹, Katie E Bickett¹, Theodore S Kalbfleisch¹ and Guy N Brock¹.

¹University of Louisville, Louisville, KY.

**Body:** Our investigations explore the association of cigarette smoking on breast cancer risk of recurrence and progression, in contrast to studies that focused on tobacco use and risk of breast cancer occurrence. The goal was to decipher the interaction between smoking history and expression levels of 22 gene candidates selected from microarray data obtained from laser capture microdissected carcinoma cells from 247 de-identified patient tissue biopsies on disease recurrence and overall patient survival of breast cancer patients. qRT-PCR was used to determine expression levels for NAT1, NAT2, COMT, SOD1, SOD2, BRCA1, BRCA2, APOC1, ARID1B, CTNNBL1, MSX1, UBE2F, IRF2, NCOA1, LECT2, THAP4, RIPK1, AGPAT1, C7orf23, CENPN, CETN1 and YTHDC2 selected from a previous study for 50 breast cancer patients with a history of cigarette smoking and 51 patients who had never smoked. For smokers and non-smokers separately, L1-penalized multivariable Cox regression models were fit to predict patient disease-free and overall survival, with 1000 splits of the data into training (70%) and test (30%) sets to determine predictive accuracy based on the C-index. The LASSO penalty was used to perform variable selection in each of the training sets, and a permutation procedure was used to determine a significance threshold for the number of times a variable was kept in the model. Multivariable analyses using the LASSO revealed CENPN, CETN1, CYP1A1, IRF2, LECT2, and NCOA1 to be significant predictors for both disease recurrence and mortality among smokers. Additionally, COMT was highly associated with recurrence, and NAT1 and RIPK1 were associated with mortality. In contrast, only IRF2, CETN1, and CYP1A1 were significant for disease recurrence and mortality among non-smokers, with NAT2 additionally significant for survival. Median, 25th percentile, and 75th percentile for the C-indexes based on the gene expression models are given in Table 1.

**Table 1. C-indexes for the Four Gene Expression Models Based on 1000 Test Data Sets**

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Outcome</th>
<th>25th Percentile</th>
<th>Median</th>
<th>75th Percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current Smokers</td>
<td>Overall Survival</td>
<td>0.73</td>
<td>0.80</td>
<td>0.86</td>
</tr>
<tr>
<td>Current Smokers</td>
<td>Recurrence-free Survival</td>
<td>0.67</td>
<td>0.73</td>
<td>0.78</td>
</tr>
<tr>
<td>Never Smoked</td>
<td>Overall Survival</td>
<td>0.53</td>
<td>0.59</td>
<td>0.66</td>
</tr>
<tr>
<td>Never Smoked</td>
<td>Recurrence-free Survival</td>
<td>0.51</td>
<td>0.59</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Analysis of interaction between smoking status and gene expression values using the combined samples revealed significant interactions between smoking status and CYP1A1, LECT2, CETN1. Molecular signatures consisting of 7-8 genes were highly predictive for breast cancer recurrence and overall survival among smokers, with median C-index values of 0.8 and 0.73 for overall survival and recurrence, respectively. In contrast, the median C-index values for non-smokers was only 0.59. Hence, significant interactions between expression of crucial genes and cigarette smoking status appear to play a key role in predicting clinical outcomes of breast carcinoma patients. Supported in part by a grant from the Phi Beta Psi Charity Trust (TSK & JLW) and a Research of Women (ROW) grant to JLW from the EVP for Research and Innovation, University of Louisville.
Title: An academic cancer center’s experience in establishing a breast cancer-focused multidisciplinary genomic tumor board

Michelle McGowan¹, Shaveta Vinayak², Steven Maximuk², Roselle Ponsaran¹, Patricia Marshall¹, Lyndsay Harris² and Paula Silverman². ¹Case Western Reserve University, Cleveland, OH and ²University Hospitals Case Medical Center, Cleveland, OH.

Body: Background. Clinically, it is becoming increasingly important to assess genomic alterations in breast cancers using Next Generation Sequencing to find potential targets for personalized treatment. However, care paths for using such results to guide treatment have not yet been established. Previously reported studies suggest that developing an interdisciplinary review board to evaluate genomic findings can help to guide patient care. We report on a prospective qualitative study of a multidisciplinary genomic tumor board (MGTB) developed to discuss breast cancer patients’ genomic test results in the context of the clinical history and current practice guidelines. Our goal was to observe and describe the establishment of the MGTB and its members’ perceptions of a tumor board approach to interpreting genomic test results.

Materials and Methods. After IRB approval, we used qualitative case study methodology to study the MGTB and its participants, which include medical, surgical, and radiation oncologists, pathologists, geneticists, epidemiologists, bioinformaticians, pharmacologists, nurses, and bioethicists. MGTB meets on a monthly basis. Cases are anonymously presented by the treating physician. Genomic tumor findings from commercial vendors and in-house studies are discussed. Treatment recommendations are made by consensus, with level of evidence assigned based on Simon-Paik-Hayes biomarker guidelines. After obtaining consent from MGTB members, MGTB proceedings and interviews were recorded and transcribed for thematic analysis. Social dynamics within the MGTB, which includes interaction among MGTB members regarding interpretation and communication of genomic findings, were analyzed.

Results. MGTB began meeting monthly in 10/2013. As of 6/2014, 24 cases have been presented. All were female with metastatic breast cancer; 16/24 (67%) hormone receptor positive (ER or PR >1%), 7/24 (29%) triple negative, and 2/24 (9%) her2 positive. Analysis of meeting and interview transcripts revealed that though inherently multidisciplinary, the MGTB’s recommendations seem to be driven by medical oncologists with input from geneticists and pharmacologists when present. MGTB recommendations have become increasingly consistent over time. Based on genomic findings, the board often recommends referring patients to clinical trials (18/24, 75%) and to Medical Genetics (9/24, 38%) for evaluation of suspected germline abnormalities over other options such as drug therapies approved by the FDA for use in other tumor types.

Discussion and Conclusion. MGTB is a unique forum to discuss genomic test results and departs from typical tumor boards by involving non-clinicians. This case study suggests the feasibility of standardizing interdisciplinary evaluation and level of evidence recommendations for genomic results. The findings of this qualitative case study reveal MGTB members’ discomfort with recommending off-label use of FDA-approved therapies. Hence, on an institutional level, team dynamics will determine how testing and recommendations are established. Further study is required to assess institutional and professional variability in offering genomic testing and making treatment recommendations on the basis of genomic findings.
Title: Multiple breast cancer risk variants are associated with differential transcript isoform expression in tumors

Jennifer L Caswell¹, Scott Huntsman¹, Donglei Hu¹ and Elad Ziv¹. ¹University of California, San Francisco, San Francisco, CA.

Body: Introduction: Genome-wide association studies have identified over seventy single-nucleotide polymorphisms (SNPs) associated with breast cancer. A subset of these SNPs regulate expression of nearby genes, but the functional effects of the majority remain unknown. We hypothesized that some breast cancer risk SNPs may regulate alternative splicing of nearby genes. Methods: We used RNA sequencing data and matched germline genotypes from The Cancer Genome Atlas for 358 estrogen receptor (ER)-positive and 109 ER-negative breast tumors. For each risk SNP, we tested the association between genotype and exon-, junction-, and transcript-specific expression of genes within 500 kilobases, adjusting for overall gene expression. We excluded associations potentially related to error in mapping to pseudogenes or bias in mapping to the reference genome. Results: Six breast cancer risk SNPs were associated with differential isoform expression of seven nearby genes at FDR < 0.05 in ER-positive tumors; five of these associations replicated (P-value < 0.05) in ER-negative tumors (Table 1). At five loci, the pattern of association with alternative splicing mirrored the pattern of association with breast cancer. Of these five, two SNPs were associated with alternative splice site usage in nearby genes (STXBP4, BABAM1), one with exon skipping (DCLRE1B), and two with more complex exon usage patterns (PEX14, RAD51L1).

Associations between breast cancer risk SNPs and transcripts identified in ER-positive tumors at FDR < 0.05

<table>
<thead>
<tr>
<th>SNP rsID</th>
<th>SNP Location</th>
<th>Gene</th>
<th>SNP Distance from Gene</th>
<th>β (ER+)</th>
<th>P-value (ER+)</th>
<th>FDR (ER+)</th>
<th>P-value (ER-)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6504950</td>
<td>chr17:53,056,571</td>
<td>STXBP4</td>
<td>In gene: intron 1</td>
<td>-0.59</td>
<td>1.5E-23</td>
<td>2.6E-19</td>
<td>3.1E-07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exon 5:6 junction 1</td>
<td>0.59</td>
<td>1.5E-23</td>
<td>2.6E-19</td>
<td>3.1E-07</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exon 5:6 junction 2</td>
<td>-0.72</td>
<td>2.5E-23</td>
<td>2.6E-19</td>
<td>2.3E-10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transcript uc010docc</td>
<td>-0.42</td>
<td>1.5E-11</td>
<td>2.6E-08</td>
<td></td>
</tr>
<tr>
<td>rs11552449*</td>
<td>chr1:114,448,389</td>
<td>DCLRE1B</td>
<td>In gene: exon 1</td>
<td>-0.63</td>
<td>5.7E-13</td>
<td>1.4E-09</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transcript uc001eei</td>
<td>0.26</td>
<td>8.0E-10</td>
<td>1.2E-06</td>
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</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Transcript uc001eeg</td>
<td>-0.48</td>
<td>1.3E-07</td>
<td>1.3E-04</td>
<td>4.7E-03</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Exon 1:3 junction</td>
<td>0.24</td>
<td>1.2E-06</td>
<td>9.1E-04</td>
<td>1.0E-04</td>
</tr>
<tr>
<td>rs8170</td>
<td>chr19:17,389,704</td>
<td>BABAM1</td>
<td>In gene: exon 8</td>
<td>0.47</td>
<td>6.1E-08</td>
<td>6.5E-05</td>
<td>5.3E-05</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Transcript uc002nfv</td>
<td>-0.27</td>
<td>5.3E-06</td>
<td>3.3E-03</td>
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<td></td>
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<td>Transcript uc002nfu</td>
<td>-0.45</td>
<td>1.4E-07</td>
<td>7.3E-06</td>
<td>1.5E-02</td>
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<tr>
<td>rs11249433†</td>
<td>chr1:121,280,613</td>
<td>SRGAP2D</td>
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<td>-0.45</td>
<td>1.4E-07</td>
<td>7.3E-06</td>
<td>1.5E-02</td>
</tr>
<tr>
<td>rs11552449†</td>
<td>chr1:114,448,389</td>
<td>PHTF1</td>
<td>146 kB</td>
<td>-0.39</td>
<td>1.1E-06</td>
<td>8.8E-04</td>
<td>2.9E-02</td>
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<tr>
<td>rs616488</td>
<td>chr1:10,566,215</td>
<td>PEX14</td>
<td>In gene: intron 2</td>
<td>-0.39</td>
<td>1.1E-06</td>
<td>8.1E-04</td>
<td></td>
</tr>
</tbody>
</table>


Table showing association with transcript expression:

<table>
<thead>
<tr>
<th>SNP</th>
<th>Location</th>
<th>Effects</th>
<th>P-values</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs999737</td>
<td>chr14:69,034,682</td>
<td>-0.33</td>
<td>6.2E-05</td>
<td>3.4E-02</td>
</tr>
</tbody>
</table>

Location is for hg19 build. β is for effect of the breast cancer risk allele. FDR is when all exon, junction, and transcript tests are considered together. P-values for ER- shown when < 0.05. * SNP is associated with transcript expression of two different genes. † Pattern of association with transcript does not correspond to pattern of association with breast cancer.

Conclusion: Regulation of differential transcript expression appears to be an important functional mechanism of breast cancer risk SNPs. These associations allow us to identify likely candidate causal genes, and also specific exons and transcripts that may mediate breast cancer risk, opening a new window into the link between risk variants, gene function, and outcome.
Title: Long range expression patterns detected by RNASeq in breast cancer reveal cluster enriched with triple negative breast cancer

Alex Mankovich1, Vartika Agrawal1, Nilanjana Banerjee1 and Nevenka Dimitrova1. 1Philips Research, Briarcliff Manor, NY.

Body: Background: Breast cancer subtyping using gene expression is well established in breast cancer research. While it is known that there are large chromosomal regions affected by copy number polymorphisms in breast cancer, it is not clear whether expression patterns reflect large genomic events affecting larger portion of the chromosome. We present a method to quantify long range gene expression patterns of larger genomic regions at 23kb, 100kb and 1mb.

Methods: We used TCGA-level 2 breast cancer gene expression data (RNA-Seq) generated at the Carolina Center for Genome Sciences, UNC at Chapel Hill. We evaluated the long range expression patterns of 649 patients for which we had ER, PR and Her2 status data by IHC. For 221 samples we had ER, PR, Her2, age, menopausal status, p53 and PIK3CA mutation status. Our method defines long range expression within a window of a particular length (e.g. 23kb, 100kb). We take the mean expression scores for all genes that fall within each window and concatenate these windows to obtain larger chromosome-wide patterns. The final chromosome-wide vectors are concatenated to represent long range expression patterns across the entire genome. We then evaluate the variation of these window scores across all samples and keep top 2% varying windows. Then, we apply hierarchical clustering, and evaluate enrichment of clinically meaningful subtypes using hypergeometric test.

Results: Simple hierarchical clustering revealed clear separation of triple negative breast cancer samples at any level of resolution: 23kb, 100kb in the available data set. While the 100kb resolution showed two distinct clusters, the 23kb resolution showed three distinct clusters. Interestingly, the hierarchical clustering of samples (n=649) using the top 214 (2%) highly varying long 100kb regions revealed a cluster which contained 99 samples and was enriched with 75 TNBC samples (out of 102 TNBC in the entire set) (p=1.7E-53).

The long range expression of 23Kb regions on 221 samples revealed 319 (2%) such regions which further segregate the samples into three different clusters: Cluster1: Luminal enriched cluster with 147 samples out of which 104 are ER+PR+Her2- samples (p-value <<0.01), Cluster2: Her2+ enriched cluster with 31 samples out of which 23 are Her2+ (p-value<<0.01), and Cluster3: TNBC enriched cluster containing 33 samples out of which 26 are TNBC (p=1.5E-18). There are a total number of 38 TNBC samples. Furthermore, in the TNBC cluster with 33 samples, 28 had p53 mutations and 24 of those were both TNBC and had p53 mutations (p-value << 0.05). In this TNBC cluster, there is enrichment of samples lacking PIK3CA mutations (n=31)(p-value <0.01). We found no clear association with age and menopausal status.

Conclusions: Hierarchical clustering relying on long range expression regions produces clusters that are enriched with well known clinically relevant subtypes. The associations with known tumor biology are strong for different region sizes (23kb and 100kb). This is the first study to report long range gene expression patterns that reveal data-driven close association with tumor biology.
Title: Re-assessment of 21-gene breast cancer assay utilization as a quality indicator by the Florida Initiative for Quality Cancer Care (FIQCC) Consortium

Christine Laronga¹, Weihong Sun¹, Erin Siegel¹, Ji-Hyun Lee¹, William J Fulp¹, Paul B Jacobsen¹ and Jhanelle E Gray¹. ¹H. Lee Moffitt Cancer & Research Institute, Tampa, FL.

Body: Background: In 2006, the FIQCC conducted a comprehensive review of quality of care indicators specific to breast cancer (BC) based on QOPI/NCCN/ACOS and panel consensus, with feedback provided on indicator performance to encourage quality improvement efforts. In 2009, at re-assessment of adherence to the previous indicators, we introduced a pilot quality indicator from the NCCN treatment guidelines: discussion/recommendation of the 21-gene BC assay for treatment decisions. The current study assesses quality improvement efforts targeting specifically the 21-gene BC assay.

Methods: After disclosure of results in 2012, 5 FIQCC sites agreed to participate in a re-assessment sub-study focused on the 21-gene BC assay. Following IRB approval, each site developed and implemented a site-specific quality improvement plan focused on the discussion/utilization of this assay. Following this intervention, chart reviews were conducted for ER+ BC patients first seen by a medical oncologist in 2013 and eligible for the 21-gene BC assay based on NCCN criteria (n=130). Data collected included: 1) documentation of a discussion with the patient of the 21-gene BC assay; 2) documentation of ordering/performance/results of the 21-gene BC assay; and 3) treatment decisions based on results and/or documentation of reasons for non-compliance. Statistical comparisons of the previously collected 2009 data (n=262) for these 5 FIQCC sites and new 2013 data were performed using the Pearson Chi-Square exact test based on Monte Carlo estimation.

Results: Overall, the mean age of patients was 62 years (range 30-89) for the combined 2009 and 2013 years. More women had her2 negative BC in 2013 compared to 2009 (p=0.003). Since the 2009 dataset was not specific to the 21-gene BC assay eligibility, a smaller proportion of patients had tumor sizes staged as T1c/T2/T3 [80.3% vs. 92.2% for 2009 as compared to 2013, p=0.005] and less were node negative in 2009 [56.8% vs. 96.6%; p<0.001]. Based on NCCN criteria, only 60.9% of eligible patients had a documented discussion or recommendation for the 21-gene assay in 2009 with significant variability among the 5 sites (p=0.011). A significant improvement was seen in 2013 (p<0.001) with 91.4% of eligible patients having documentation of a discussion or recommendation for the 21-gene assay without significant variability amongst the practice sites. Significant improvement in the percentage of patients having the assay ordered from 2009 to 2013 was seen (p<0.001) but variability amongst the sites persisted (2009: p=0.002; 2013: p=0.018). Recurrence Score (RS) results were completely recorded in both 2009 and 2013. All patients with high-risk RS were referred for chemotherapy. A small fraction of patients with low-risk RS was referred for chemotherapy both years (2009: 5.4%, 2013: 3.8%; p=1.00).

Conclusions: Previously, the FIQCC identified quality improvement needs in multiple aspects of BC care, including recommendation and ordering of the 21-gene BC assay. A dedicated quality improvement effort can significantly increase both the discussion/recommendation and ordering of the 21-gene BC assay among eligible patients. These improvements have the potential to alter treatment recommendations.
Title: Examining 3-dimensional genome architecture and transcriptional profiles using an optimized HiC-seq protocol in breast cancer cells

Jennifer D Davis¹, Christian Ross², Hu Li² and Amy Brock¹. ¹University of Texas, Austin, TX and ²Mayo Clinic, Rochester, MN.

Body: The post-genomic era ushered in a cadre of techniques to study epigenetic mechanisms involved in disease progression and cancer. One understudied mechanism of gene expression/transcriptional control is 3-dimensional architecture of the genome. Several high-throughput technologies have been adapted to examine 3D genome architecture; in particular those derived from chromatin conformation capture (3C) sequencing technologies. Of 3C-derived techniques, HiC-seq, is the most extensive, and examines all chromatin-chromatin contact sites within a genome, elucidating the entire 3D genome architecture. Although, HiC-seq technique was published 2009, it has been under-utilized in research laboratories due to its technical complexity, expense, and requirement for large volumes of starting material (50 million cells/500 µg of DNA). Other barriers to use of HiC-seq include the requirement for specialized equipment and laborious extraction techniques. However, the benefits of HiC-seq are high for cancer researchers because a genome-wide "snap-shot" of the entire 3D genome of the cancer is displayed. This allows for analysis of higher-order epigenetic control of gene expression. Furthermore, understanding the chromatin conformation of the genome provides new insights into the cellular disease state and potentially novel therapeutic approaches to drug-resistant cancers. Our study presents significant technical innovations, permitting HiC-seq technique to be adopted in laboratories without extensive experience in 3C-seq technologies, and with the use of standard equipment and reagents, as well as smaller volumes of starting materials. Our modified technique utilizes up to 100 fold lower amounts of starting material (500,000 cells/5-10 µg of DNA) per reaction. This adaptation of HiC-seq preparation offers the potential for simultaneous extraction of total RNA and Hi-C processed chromatin for sequencing. To optimize our technique we utilized the estrogen-responsive breast cancer cell line, MCF7. This cell line is typically utilized to study potential drug therapies in the context of estrogen-sensitive cancers. We streamlined HiC-seq by optimizing nuclei extraction and allowing for collection of a cytoplasmic fraction for mRNA extraction and sequencing analysis. We also optimized enzymatic digestion, ligation and DNA extraction methods by utilizing newer enzymatic chemistries, commercially available silica column extraction methods and reagents in place of multiple phenol-based DNA extraction methods. Our quality control results suggest that sample processing for reduced amounts of material is similar if not superior to conventional HiC-seq. We leverage this powerful genomic high-throughput technique in order to simultaneously examine the 3D architecture and transcriptional profiles of MCF7 breast cancer cells. Our computational workflow includes further quality controls using HiC-User Pipeline software from the Bahraham Institute and the R Bioconductor package HiTC package. Overall our optimizations in MCF7 breast cancer cells, and post-acquisition work-flow, make HiC-seq an accessible technology. These innovations permit simultaneous analysis of architectural and transcriptional profiles in cancer cells.
Title: Assessing reproducibility of copy number arrays to assist breast cancer biomarker discovery

Cindy Q Yao1, Cheryl Crozier1, Mary Anne Quintayo1, Jane Bayani1, Melanie Spears1, Julie Livingstone1, Esther Jung1, Clement Fung1, Victoria Sabine1, Paul C Boutros1 and John MS Bartlett1. 1Ontario Institute for Cancer Research (OICR), Toronto, ON, Canada.

Body: Introduction:
Large-scale interrogation of the genome has emerged as an attractive method for identifying useful characteristics of cancer biology; in particular, the study of copy number aberrations (CNA) has recently received tremendous attention. A number of different technologies have been developed to assess the copy-number landscape, allowing us to better understand the role of CNA in cancer cells. The OncoScan CNA platform (Affymetrix Inc.) has been particularly appealing for oncology due of its ability to work well with formalin-fixed, paraffin-embedded (FFPE) materials, which is the primary form for storage of clinical samples. In addition, its high resolution, rapid analysis time and ability to interrogate different genomic characteristics (CNA, loss of heterozygosity or mutation) make the OncoScan platform highly popular: it has been widely cited in the literature for use in biomarker discovery, clonal evolution and sub-clonal detection, as well as population-based analyses. While CNAs identified by the OncoScan platform have shown good concordance with fluorescence in-situ hybridization (FISH) results, to date, no studies have been conducted to thoroughly assess the reproducibility of the assay. In this study, we have assessed the reproducibility of the OncoScan platform using identical samples performed in replicates across multiple chip batches. Moreover, we have assessed the effect on reproducibility of DNA treatment, including elution in water or TE buffer, as well as in the use of varying amounts of DNA.

Methods:
Affymetrix OncoScan FFPE Express 3.0 SNP Arrays were performed using the optimal input DNA as recommended by the manufacturer as well as fewer input amounts for comparison. CNAs were called using BioDiscovery Nexus Copy Number™ software (http://www.biodiscovery.com/software/nexus-copy-number/) using the SNP-FASST2 algorithm with modified parameters (significance threshold of 1 x 10^{-9} and minimum number of probes per segment of 10).

Results:
Initial reproducibility analysis involving 12 samples repeated either 2, 4 or 6 times both within a single batch and across different batches has revealed that CNA calls were concordant between replicates for the majority of the genome (ranges between 81% to 100%), suggesting high precision of the assay. In addition, we are in the process of assessing and comparing mutation calls across replicates to gain a more in-depth understanding of the platform.

Conclusion:
This is the first study examining the reproducibility of OncoScan FFPE assays; initial results have suggested that the assay is precise and has the potential for robust biomarker discovery. Additional characterizations would be interesting for evaluating its use as a clinical tool in the long term.
Discovery of novel amplified genes in primary breast cancer with copy number and gene expression analysis of whole exome and transcriptome sequencing data

Eunshin Lee¹, Woosung Lim¹, Kyung-Min Lee¹, Tae-kyung Yoo¹, Jongjin Kim¹, Han-Byoel Lee¹, Yun-Gyoung Kim¹, YoungJoon Kang¹, Min Kyoon Kim¹, Hyeong-Gon Moon¹, Dong-Young Noh¹ and Wonshik Han Han¹. ¹Seoul National University College of Medicine, Seoul, Korea.

Body: Introduction: Copy number alteration of genome is common in breast cancer and tend to have more driver role than single point mutations. Traditionally, genome-wide analysis of DNA copy number changes were done by array CGH or SNP array method. Here, we did DNA whole exome sequencing (WES) and RNA-seq using Next Generation Sequencing (NGS) technology to find common genes or chromosomal regions of which DNA copy was highly amplified and at the same time RNA expression was also upregulated.

Materials and Method: RNA and DNA were extracted from fresh frozen tissues of 93 breast cancer patients. WES and RNA-seq were done using NGS technology (Illumina HiSeq 2000). As a control, normal DNA from all matched patients were also sequenced. GATK was used to gain mean depth and coverage data for targeted regions. CNVs were calculated with ExomeCNV, a statistical method to detect somatic CNVs using depth-of-coverage information from mapped short sequence reads. To estimate expression levels, the relative transcript abundances were measured in FPKM using Cufflinks.

Results and Discussion: DNA of 1,737 genes were highly amplified (log R>1.0) in two or more samples. The two most commonly amplified chromosomes were chromosome 8 and 17. We applied a cut-off for higher gene expression as relative FPKM >1.5. ERBB2 amplifications and high expression were most common (21.5%) of all genes and it was in agreement with HER-2 IHC and FISH result. Among previously reported amplified genes, FGFR1 (5.4%) and PVT1 (8.6%) in chromosome 8, CCND1, PAK1 (3.2%) and EMSY (4.3%) in chromosome 11, CCNE1 (4.3%) in chromosome 19 were also identified in this study. IGF1R high amplification and expression was found in two samples, and ESR1, MDM2, KIT was found in only one sample each. We found uncommon but novel and recurrent highly amplified and expressed genes: CLK4 in 5q (3.2%), AHI/MYB in 6q (3.2%), MMP7(2.2%) and MALAT1 in 11q (1.1%), and NEK8 in 17q (4.3%) We designed FISH probe for this 5 new genes and confirmed the high amplifications in each sample with FISH. Functional study of these genes will be followed for the driver role of these genes in carcinogenesis and progression of breast cancer cells.
Title: Discovery of the genes that underpin the transition to malignant phenotype of breast tumors in highly consanguineous region

Ishita Gupta¹, Allal Ouhtit¹, Somya Shanmuganathan¹ and Hamad Al-Riyami¹. 'Sultan Qaboos University, Muscat, Seeb, Oman.

Body: Breast cancer (BC), a multifactorial and heterogeneous disease is a predominant women form of cancer worldwide affecting 22.9%. The rationale of this study is based on the following observations: 1) in Oman, a significantly increasing number of younger females (25-40 years) present to the clinic with advanced stage of BC; 2) in Oman, the rate of consanguinity is significantly high (52%); and 3) the transition from normal/beginin to malignant phenotype of breast tumor requires the involvement of a subset of specific genes. The long-term objective of this study is to identify and validate the subset of genes that are responsible for this malignant transformation using functional genomic studies, focus on this young age group of patients attending BC clinic (sporadic and familial). RNA samples were isolated from 40 Breast Tumors and 40 Normal tissues and analyzed by Microarray Gene Expression Profiling. Among a number of genes that were up and down regulated, BRIP1, HOXB3 and MAGED1 were identified as potential genes that might underpin the transition to the malignant phenotype; these genes were validated by RT-PCR using the same RNA samples that were examined by microarray. Pathway analysis was carried out to identify the major functional pathways connecting these genes. Ongoing sequencing of these genes using DNA extracted from the same samples will ultimately identify any genetic alteration that can affect the normal function of these genes. Functional validation assays aim to validate further the physiological relevance of these genes in tumor malignancy, and perhaps other novel genes specific to BC in the Omani population. Identification and validation of these genes will potentially pave the way towards the design of anti-cancer therapeutic strategies.
The signal transduction molecule MAP3K12 is critical for triple-negative breast cancer cells

Graham M Poage, Abhijit Mazumdar, Ivan P Uray, Jamal Hill, Yun Zhang, Gordon B Mills and Powel H Brown. 1University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Mitogen-activated protein kinase kinase kinases (MAP3Ks) are critical network hubs in signal transduction pathways that control many cellular responses including growth, inflammation, differentiation, apoptosis, and development. Recently, MAP3Ks have been implicated in the early and metastatic progression of solid tumors, including breast cancer. While some estrogen receptor (ER)-positive breast cancers have deletions or loss-of-function mutations in MAP3K1, consensus genetic alterations in triple receptor-negative breast cancers (TNBCs) have not been described for these important signal transduction molecules. Among the clinical subtypes, TNBCs carry the worst prognosis and no targeted interventions have yet been approved for routine use. Therefore, we sought to systematically determine which MAP3Ks are required for the growth of TNBC cells to discover novel targets for the treatment of this aggressive disease.

Materials and Methods: To determine which MAP3Ks are specifically required for proliferation of TNBC cells, we performed an RNAi screen by reverse transfecting siRNA pools targeting each of the 22 MAP3Ks (and the control Luciferase) across 5 triple-negative and 5 ER-positive breast cancer cell lines and measuring growth after 6 days. Candidate siRNAs that preferentially inhibited TNBCs were advanced to a secondary anchorage-independent growth screen to determine their ability to inhibit colony formation in soft agar. We developed doxycycline-inducible shRNA-expressing stable cell lines to determine whether the MAP3K is required for the growth of xenograft tumors in nude mice. Explanted xenograft tumors were analyzed by immunofluorescence for cleaved caspase-3 and immunohistochemistry for Ki-67. To determine how growth is inhibited by the candidate siRNAs, we performed FACS and immunoblot analysis to assess apoptotic initiation as well as cell cycle and thymidine-analogue incorporation experiments to determine proliferative dynamics.

Results: We identified multiple MAP3Ks, including MAP3K3, MAP3K4, MAP3K12, and MAP3K17, which were necessary for the growth of two or more TNBC cell lines but not more than one ER-positive cell line. Knockdown of these genes were also required for soft agar colony formation of TNBC cell lines. Further, knockdown of MAP3K12 in nude mouse xenografts significantly inhibited their growth rate and correlated with a decrease in Ki-67-positivity. In vitro experiments demonstrated that inhibition of cell proliferation is a result of an increased persistence of cells in the G0/G1-phase, evident by decreased thymidine-analogue incorporation and cell cycle analysis, and is not due to alterations in apoptotic activity.

Discussion: This study identified MAP3Ks that are required for the growth of specific breast cancer cell lines, specifically TNBCs. No one MAP3K is critical for the growth of all TNBC cells. Instead, different TNBC tumor cells utilize distinct MAP3Ks for their growth. These data support the development of inhibitors targeting specific MAP3K proteins, particularly MAP3K4, 12, and 17, for the treatment of women with TNBC.

This study was supported by a grant from the Breast Cancer Research Foundation (P.B.) and a Susan G. Komen for the Cure Promise Grant (P.B., G.M., KG081694).
Title: Identifying molecular targets and mechanisms of treatment resistance in inflammatory breast cancer (IBC) using reverse-phase protein microarrays (RPMA)

Laura Austin¹, Kimberly Limentani¹, Juan Palazzo¹, Tiffany Avery¹, Rebecca Jaslow¹, Ron Hencin³, Emanuel F Petricoin²,³ and Massimo Cristofanilli¹,⁴. ¹Thomas Jefferson University Hospital, Philadelphia, PA; ²George Mason University, Fairfax, VA; ³Theranostic Health, Inc, Rockville, MD and ⁴Inflammatory Breast Cancer International Consortium.

Body: Background
Inflammatory Breast Cancer (IBC) is a clinicopathologic diagnosis characterized by rapid progression and poor prognosis. Even with the advent of targeted therapies and a multimodal approach, IBC is often treatment refractory and a therapeutic challenge for all subtypes, including ER+ and HER-2 amplified (HER2+) disease (Masuda et al). Therefore identifying mechanisms of resistance to molecularly targeted therapy could provide clues to improve management and outcome. Recent studies comparing the gene expression profiles of IBC tumors with non-IBC demonstrated that HER2+ IBC have increased mTOR signaling compared to their non-IBC counterparts (Iwamoto et al). mTOR activation is a mechanism for Trastuzumab resistance and may contribute to treatment resistance in HER2+ IBC. The availability of molecular diagnostics evaluating phosphoproteins is an appealing approach to predict treatment-sensitivity and select more effective combinations.

Methods
This is an observational analysis of 12 IBC patients who had tissue biopsy after progression on standard therapies including HER-2 targeted therapies. Tissue analysis for expression of cancer-related phosphoproteins was performed using TheraLink™. The TheraLink™ assay uses reverse-phase protein microarrays (RPMA) to quantify HER1, HER2, and HER3 receptor overexpression; it also evaluates for phosphorylation of the receptor which indicates activation. Phosphorylation of HER downstream signaling pathways such as JAK2, AKT/mTOR and MEK1/2 are also detected. Additionally, next generation sequencing (NGS) using FoundationOne™ was performed if sufficient tissue was available.

Results
All patients had IBC and most had metastatic disease (83%). According to subtype 25% of patients were ER+/HER2-, 42% ER+/HER2+, 25% ER-/HER2+, 8% ER-/HER2-. 58% of tumors demonstrated HER1 activation, 75% had HER2 activation and 58% had HER3 activation. Interestingly, 83% had mTOR activation, and most of these patients also had accumulation of its downstream proteins, S6 ribosomal protein and 4E-BP-1. 78% of patients with HER2 activation also had mTOR activation. Two of the 4 patients who were HER2- by IHC/FISH had HER2 activation by RPMA. Six patients also had NGS on tissue; 75% had concordance between HER2 activation on TheraLink™ and ERBB2 amplification on NGS, 67% had concordance with mTOR activation on TheraLink™ and mutation in the mTOR pathway (PIK3CA mutation or PTEN loss) on NGS. One patient with triple negative, chemo-refractory IBC who underwent 3 lines of neoadjuvant therapy prior to bilateral mastectomy was found to have HER1, HER2, HER3 and mTOR activation; she was started on lapatinib and capecitabine and remains with no recurrent disease and on treatment.

Conclusions
Patients with IBC often have activation of members of the HER family and mTOR pathway indicating molecular targets and potential mechanisms of resistance in IBC. The concomitant use of NGS and RPMA is an intriguing approach to molecular diagnostics in this aggressive and treatment refractory disease providing additional information on pathway activation leading to expanded therapeutic options. Future prospective studies should clarify the potential impact in treatment selection and outcome.
Title: Circulating tumor DNA (ctDNA) provides molecular monitoring for inflammatory breast cancer (IBC)

Laura Austin¹, Paolo Fortina¹, Dragan Sebisanovic², LaiMun Siew², Aubrey Zapanta², Benjamin J Schiller², Gangwu Mei², Helmy Eltoukhy², AmirAli Talasaz³ and Massimo Cristofanilli¹,³. ¹Thomas Jefferson University Hospital, Philadelphia, PA; ²Guardant Health, Inc, Redwood City, CA and ³Inflammatory Breast Cancer International Consortium.

Body: Background
Inflammatory Breast Cancer (IBC) is a clinicopathologic diagnosis characterized by rapid progression, resistance to treatment and poor prognosis. It is often an incurable disease with complex molecular features including somatic mutations that evolve in relation to genomic instability and selective treatment pressure. Monitoring disease by performing multiple biopsies may not be feasible and puts the patient at risk with each invasive procedure. Circulating DNA fragments carrying tumor-specific sequence alterations (ctDNA) are found in blood and offer the possibility of longitudinal non-invasive molecular monitoring of the disease by detecting actionable mutations.

Methods
This is an observational analysis of 35 IBC patients who failed standard therapies and had plasma analyzed for ctDNA detection. Selection criteria included progression of disease after standard therapies, need to detect novel molecular abnormalities for possible therapeutic targeting, or confirmation of genomic abnormalities already demonstrated in tissue analysis. Guardant Health performed the plasma analysis (Guardant360®); first ctDNA was isolated from plasma, then a panel of 54 gene mutations associated with solid tumors as reported in the COSMIC database were sequenced to concurrently analyze somatic mutations and gene amplification using single-molecule digital sequencing technology.

Results
All patients had IBC and 80% had metastatic disease; 37% of patients were ER+/HER2-, 14% ER+/HER2+, 23% ER-/HER2+, and 26% ER-/HER2-. 94% of patients with stage III or IV tumors had ctDNA alterations detected. The most common mutations were TP53 (49%), PIK3CA (20%), ERBB2 (17%), NOTCH1 (17%), and ALK (11%). Twelve patients also had next generation sequencing (NGS) analysis of tissue biopsy and 75% of these patients demonstrated at least one concordant mutation. The genomic information obtained from ctDNA, NGS or both was used to select treatments in 11 cases (31%). HER2 targeted therapy was continued in four patients with HER2+ disease after ctDNA confirmed ERBB2 alteration or amplification. A patient with ER+/HER2+ disease who progressed on HER2 targeted therapies, demonstrated ERBB2 and PIK3CA mutations on ctDNA; she was changed to exemestane/everolimus with objective response for several months. Repeat ctDNA at time of progression showed ERBB2 mutation only; she was changed to Trastuzumab/everolimus/vinorelbine. Moreover, a combination of lapatinib and capecitabine was initiated on a patient with a triple negative, chemo-refractory tumor that on ctDNA revealed EGFR and ERBB2 mutations; a repeated ctDNA after 5 months of therapy EGFR and ERBB2 mutations were not detected and she remains without evidence of progression.

Conclusions
Evaluation and longitudinal monitoring of IBC patients using ctDNA allows for identification of genomic abnormalities in all patients with advanced disease and to perform real-time molecular monitoring. The discovery of actionable genomic abnormalities is driving the management of this aggressive and treatment refractory form of breast cancer with potential future impact on outcome.
Body: Background: While mammographic density is linked to increased breast cancer risk, limited yet conflicting data exists on an association between density and developing specific molecular subtypes of breast cancer.

Methods: Eligible patients were enrolled in a larger study on breast density, diagnosed with cancer between 2003-2013, and had pathology and films available for review. Density was classified qualitatively from existing radiology reports according to Breast Imaging Reporting and Data System (BIRADS) classification and quantitatively by volumetric breast density measurements using Volpara Solutions™ software. Subtype was assigned by hormone receptor status, tumor grade and mitotic score (MS). Subtype categories included: Luminal A (ER/PR + & grade 1; ER/PR + & grade 2 & MS=1; ER+/PR- & grade 1); Luminal B (ER+ & grade 3 or MS=3; ER+/PR- & grade 2; ER/PR + & grade 2 & MS=2); Her-2 + (ER+ or ER - & Her-2 +); Triple Negative (ER/PR-, Her-2 -). Relevant pre-cancer factors including patient age, race, BMI, family history of breast cancer, and biopsy showing LCIS were included in analysis.

Results: Of 604 patients with invasive cancer, 457 had sufficient information for analysis. Among these, 233 (51%) had Luminal A, 79 (17%) Luminal B, 59 (13%) Her-2 +, and 86 (19%) Triple Negative tumors. Younger women and those with denser breasts based on quantitative measurements were more likely to have Her-2+ tumors (Table 1); this association was not seen using the standard BIRADS classification. Triple Negative tumors were less common in patients with LCIS and more common in African Americans. A multinomial logistic regression model controlling for pre-cancer patient factors demonstrated that while quantitative breast density does not significantly differentiate between all molecular subtypes (p=0.140), the association between Her-2+ tumors and denser breasts using continuous quantitative measurements is significant (p=0.035).

Conclusion: Women with denser breasts by continuous-scaled quantitative measurements are at higher risk for Her-2+ tumors; an association not delineated using standard BIRADS density classification. Delineating risk factors specific to molecular breast cancer subtype may promote individualized risk prediction models and prevention strategies.

Table 1. Association between patient factors and molecular breast cancer subtype

<table>
<thead>
<tr>
<th>Variable</th>
<th>Molecular Subtype</th>
<th></th>
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</tr>
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<tr>
<td></td>
<td>Luminal A (n=233)</td>
<td>Luminal B (n=79)</td>
<td>Her-2+ (n=59)</td>
<td>Triple Negative (n=86)</td>
<td>P value</td>
<td></td>
</tr>
<tr>
<td>Age (median,IQR)</td>
<td>61 (54,70)</td>
<td>58 (50,67)</td>
<td>54 (46,70)</td>
<td>59 (48,67)</td>
<td>0.006</td>
<td></td>
</tr>
<tr>
<td>BMI (median,IQR)</td>
<td>27.1 (22.9,30.5)</td>
<td>25.7 (23.0,30.0)</td>
<td>26.0 (22.7,32.6)</td>
<td>28.1 (24.3,32.6)</td>
<td>0.173</td>
<td></td>
</tr>
<tr>
<td>Volpara density (median,IQR)</td>
<td>7.18 (4.74,11.25)</td>
<td>8.68 (5.68,14.34)</td>
<td>10.25 (5.96,16.51)</td>
<td>7.00 (4.97,11.89)</td>
<td>0.002</td>
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<tr>
<td>BIRADS density (n,%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.183</td>
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<tr>
<td>Fatty</td>
<td>42 (18.0%)</td>
<td>15 (19.0%)</td>
<td>5 (8.5%)</td>
<td>16 (18.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Scattered</td>
<td>103 (44.2%)</td>
<td>27 (34.2%)</td>
<td>20 (33.9%)</td>
<td>36 (41.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heterogeneous</td>
<td>74 (31.8%)</td>
<td>30 (38.0%)</td>
<td>25 (42.4%)</td>
<td>26 *30.2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extreme</td>
<td>14 (6.0%)</td>
<td>6 (7.6%)</td>
<td>9 (15.2%)</td>
<td>7 (8.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unspecified</td>
<td>0</td>
<td>1 (1.2%)</td>
<td>0</td>
<td>1 (1.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Race (n,%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
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</tr>
<tr>
<td>Caucasian</td>
<td>202 (86.7%)</td>
<td>65 (82.3%)</td>
<td>51 (86.4%)</td>
<td>61 (70.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>23 (9.9%)</td>
<td>13 (16.5%)</td>
<td>6 (10.2%)</td>
<td>23 (26.7%)</td>
<td></td>
<td></td>
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<tr>
<td>Category</td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 3</td>
<td>Group 4</td>
<td>p-Value</td>
<td></td>
</tr>
<tr>
<td>-----------------------------------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td></td>
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<tr>
<td>Other</td>
<td>2 (0.9%)</td>
<td>0</td>
<td>2 (3.4%)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unspecified</td>
<td>6 (2.6%)</td>
<td>1 (1.2%)</td>
<td>0</td>
<td>2 (2.3%)</td>
<td></td>
<td></td>
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<td>LCIS (n,%)</td>
<td>48 (20.6%)</td>
<td>8 (10.1%)</td>
<td>8 (13.6%)</td>
<td>5 (5.8%)</td>
<td>0.004</td>
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<td>Family history of breast cancer (n,%)</td>
<td>107 (45.9%)</td>
<td>34 (43.0%)</td>
<td>21 (35.6%)</td>
<td>39 (45.35%)</td>
<td>0.625</td>
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</table>
Title: Modeling luminal breast cancer heterogeneity: Combination therapies to suppress hormone receptor-negative subpopulations among receptor-positive ones

Allison L Scaling, Aaron J Knox, Mauricio P Pinto, Brian S Bliesner, James M Haughian, Hany A Abdel-Hafiz and Kathryn B Horwitz. 1University of Colorado Anschutz Medical Campus, Aurora, CO.

Body: All Luminal breast cancers are heterogeneous, containing substantial numbers of estrogen (ER) and progesterone (PR) receptor-negative cells among the ER+PR+ ones. We have identified two such Luminal-derived ER–PR– cells: The first, we call "Luminobasal" (LB), are ER–PR– and cytokeratin 5 (CK5)-positive. The second, we call "Double Negative" (DN), are ER–PR– and CK5–. Currently, neither is targeted for treatment. Luminobasal cells: To address the relationships between true Luminal ER+PR+CK5– and Luminobasal ER–PR–CK5+ cells in Luminal cancers and tightly control their ratios we generated isogenic pure Luminal (pLUM) and pure Luminobasal (pLB) cells from solid tumor xenografts starting from the same parental T47Dco Luminal human breast cancer cell line. Cells were gene profiled and unique immunohistochemical (IHC) biomarkers for each were confirmed. Interaction dynamics studies show that in mixed-cell 3D colonies, pLUM or Luminal MCF-7 cells suppress growth of pLB cells. Similarly, in mixed-cell solid tumor xenografts, pLUM cells suppress pLB proliferation. Alarming, in mixed-cell models, monotherapy of a pLUM or MCF-7 subpopulation with the antiestrogen Fulvestrant inadvertently expands the number of pLB cells. This suggested that pLB cells also need to be treated. An 89 drug FDA-approved oncology library and high throughput screening methods were therefore used to show that pLB cells are specifically targeted by the EGFR inhibitors Gefitinib and Erlotinib. We then showed, in both mixed-cell 3D colonies and mixed-cell solid mouse tumors, that combination therapy using Fulvestrant plus Gefitinib constitutes a robust treatment strategy that targets both cell populations simultaneously. Double Negative cells: DN cells are EGFR– and therefore unaffected by EGFRi. However they express the Luminal marker Claudin-3 (CLD3). Among other conditions, DN cells arise as a discrete CLD3+ subpopulation within CLD3– pLB xenografts. This allowed us to use CLD3 for FACS isolation and purification of DN cells from xenografts. Purified cells were gene expression profiled, which showed that DN cells have a unique genomic signature that distinguishes them from LUM and LB cells. They express diagnostic markers suitable for IHC and FACS-sorting, some of which also present potential therapeutic targets. We propose that heterogeneous Luminal breast cancers may be best treated with combination therapies that include endocrine, EGFRi and/or DN inhibitors. Importantly, optimizing combination regimens that eradicate or suppress not only ER+PR+ cells but also diverse ER–PR– cells in Luminal disease requires that primary tumors be pre-screened for appropriate biomarkers, including but not limited to ER, PR, CK5 and EGFR.
Demonstration of immune cell and pathway heterogeneity in Singapore DCIS samples using novel hyperplexing method (MultiOmyx®)

Nicole E LaPlante¹, Yunxia Sui¹, Michael J Gerdes¹, Sean Dinn¹, Rong Zhang¹, Sireesha Kaanumalle¹, Elizabeth McDonough¹, Christina Lowes¹, Craig Allred², Fiona Ginty¹, Thomas Foo¹ and Puay-Hoon Tan³. ¹GE Global Research, Niskayuna, NY; ²GE Healthcare, Aliso Viejo, CA and ³Singapore General Hospital, Singapore, Singapore.

Breast cancer is the most common malignancy in Singapore women with rising incidence across all ethnic groups (Chinese, Malays and Indians). Ductal carcinoma in situ (DCIS) is the earliest non-invasive stage of disease and has been shown to account for approximately 26% of diagnoses in all women participating in the Breast-Screen Singapore program. Despite availability of breast screening, there are still Singapore women presenting with locally advanced breast cancer. The goal of the current study was to investigate pathway and immune cell heterogeneity in low, intermediate and high nuclear grade DCIS using a newly developed method for in situ hyperplexed analysis of multiple proteins in a single FFPE tissue (MultiOmyx). FFPE samples from patients (n= 15) diagnosed with DCIS were provided by Singapore General Hospital. Patients were of Chinese origin, ranged from 50-59 years, were all post-menopausal, and included low (n=5), intermediate (n=5) and high grade (n=5) samples. All histological diagnoses were reviewed by a single pathologist. Following a single antigen retrieval step, DAPI and cytokeratin staining was conducted and imaged at 10X. Based on DAPI, cytokeratin and autofluorescence, an H&E-like image was generated for each sample. Using this image, approximately 30 regions of interest (ROI) were selected per slide/patient, including normal and DCIS regions with and without immune cells. In total, 15 biomarkers were imaged following an iterative process of IF staining and dye inactivation. These included CD4 (T-cells), CD8 (T-cells), CD20 (B-cells), CD68 (macrophages), CD10 (myoepithelial cells), CD44v6 (cancer stem cell), Her2, Her4, EGFR (ErbB family proteins), pmTOR (cell growth), SLC7A5 (Mammostrat) and epithelium and cell segmentation markers (pan-cadherin, pan-cytokeratin, S6 and Na+K+ATPase). Images were evaluated for quality and processed to generate single cell quantification data for each marker in the epithelium and stroma. Single cell data was compared with clinical and histological features (grade, DCIS/ low immune cells, DCIS/ high immune cells). Cell-based k-means clustering was then performed for biomarkers in both the epithelial cells (Her4, Her2, panCK, SLC7A5, EGFR, NaKATPase, pmTOR, and CD10) and stromal cells (CD4, CD8, CD20, CD68). Using consensus clustering, the optimum number of clusters was found to be six in both the epithelium and stroma. No distinct clusters were associated with histological grade. Normal ROIs tended to be associated with higher CD10 and EGFR, moderate CD20 and low or negative for other markers. DCIS with low immune cells tended to have higher Her4, pmTOR and moderate CD68 while DCIS with high immune cells tended to have high Her2 and moderate to high CD4, CD20 and CD68. A larger study is needed to associate the findings with outcome. This technology provides a way to elucidate mechanisms of early disease and illustrates the complex inter-play between grade, pathway activation and immune response.
Body: BACKGROUND
Recently, it has been shown that annotation of triple-negative breast tumours (TNBC) by means of immunohistochemistry (IHC) or gene-expression signatures (GES) gave different results: 20 to 30% of these tumours were not basal-like, but luminal A, B or HER2-E. In this study, we aimed at identifying gene-expression molecular subclassification of IHC-TNBC.

MATERIALS AND METHODS
Patients
Studied bi-centric cohort retrospectively included 107 randomly selected women whose primary breast tumours lacked IHC expression of estrogen receptor, progesterone receptor and HER2. Patients were diagnosed and treated primarily between 1998 and 2007 at the ICO-Gauducheau (n=65) and the ICO-Papin (n=42).

Gene expression profiling
Gene expression analysis was performed using Affymetrix Human Genome U133 Plus 2.0 Arrays that correspond to approximately 20,000 genes.

Statistical analysis
Unsupervised analysis was performed by means of fuzzy clustering. Independent IHC-TNBC cohort (GSE21653; n=87) was used for external validation.

"Fuzzy cluster“ functional annotation
To annotate “fuzzy-clusters”, we used clinicopathologic characteristics, gene-expression signatures (GES) (PAM50, Proliferation score, TNBCtype, Immune response, Claudin-low), Gene Ontology enrichment and IHC (CK5, CK5/6, HER1, AR, Ki-67, FOXA1).

RESULTS
Fuzzy clustering partition individualized 3 clusters in our cohort: C1, C2 and C3. “Fuzzy cluster“ functional annotation results are displayed in Table 1. C1 was composed of a mixture of non-basal-like subtypes. C2 and C3 were basal-like and IHC-TNBC. Except an adenoid cystic case, C2 was exclusively composed of basal-like subtypes. C3 was essentially characterized by immune response and included 26% of claudin-low subtypes. For this reason, C3 should rather be named “immune response cluster“. External validation confirmed our results.

Table1. “Fuzzy cluster“ functional annotation results.
### Gene Ontology enrichment

<table>
<thead>
<tr>
<th>Biological processes</th>
<th>Epithelial cell differentiation, hormone metabolic process</th>
<th>Cell adhesion, locomotion, chemotaxis</th>
<th>Immune response</th>
</tr>
</thead>
</table>

### Immunohistochemistry

<table>
<thead>
<tr>
<th></th>
<th>CK5 and/or HER1 positive</th>
<th>AR positive</th>
<th>Ki-67 positive</th>
<th>FOXA1 positive</th>
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<tr>
<td></td>
<td>50%</td>
<td>91%</td>
<td>5%</td>
<td>83%</td>
</tr>
<tr>
<td></td>
<td>73%</td>
<td>5%</td>
<td>87%</td>
<td>9.6%</td>
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<tr>
<td></td>
<td>&lt;0.05</td>
<td>&lt;0.05</td>
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</tr>
</tbody>
</table>

### CONCLUSION

Heterogeneity within basal-like and IHC-TNBC is still controversial and require further research to understand the complexity of the disease, and to identify molecular drivers that can be therapeutically targeted. Annotation discordances between IHC and GES clearly indicate that a robust molecular subtyping method must be found. Correct molecular assignment would permit to orientate 20 to 30% of IHC-TNBC patients towards targeted therapy (hormonotherapy or anti-HER2).
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-04-08
Average Grade: 5.67

Title: Accuracy of estrogen receptor, progesterone receptor, HER2 Status and Ki67 labeling index between core needle and surgical excisional tumour in 910 patients with breast cancer

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Body: Purpose
Accurate determination of ER, PgR, HER2 status and Ki67 labeling index was very important in making decision for adjuvant and neoadjuvant treatment for patients with breast cancer. However discordance of ER, PgR, HER2 status and Ki67 labeling index (Ki67-LI) between core needle biopsy (CNB) and surgical specimens (SSp) varied among reported studies. The aim of the present study was to compare the accuracy of CNB with that of SSp for ER, PgR, HER2 status, Ki67-LI detection in breast cancer.

Patients and methods
All patients diagnosed with an early breast cancer in our Cancer Center Hospital between January 2005 and May 2014 was included but exclusion criteria of patients with large tumour requiring neoadjuvant chemotherapy. All CNB were performed under ultrasound guidance, with at least four 14-gauge core biopsies being obtained for pathological examination. ER, PgR, HER2 status and Ki67-LI were assessed in CNB and in SSp. ER and PgR were determined by Immunohistochemistry (IHC). The cut points for ER and PgR positive was10%. The cut point for Ki67-LI high expression was 20%. HER2 was determined by IHC and scored from 0 to 3+. FISH analysis was carried out in HER2 2+ cases and in discordant cases.

Results
A total of 910 patients were assessed. CNB can be used with confidence for ER and HER2 determination. Rates for sensitivity, specificity, negative predictive value and positive predictive value for CNB compared with SSp are 99.3%, 95.6%, 97.2%, 98.5% for ER, 94.5%, 81.8%, 88.2%, 90.7% for PgR, 93.0%, 99.1%, 99.6%, 93.9% for HER2, and 87.7%, 69.8%, 81.1%, 79.4% for Ki67-LI, respectively. Specially, the most impact factor of discordance for Ki67-LI and HER2 is Tumour size and that for ER and PgR is lobular carcinoma.

Conclusion
CNB can be used with confidence for ER and HER2 determination. For PgR and Ki67-LI due to substantial discordance results from CNB should be used with caution.
Title: Utility of simultaneous HER2 protein and gene assessment for the evaluation of discrepancy and intratumoral heterogeneity of HER2 status and the prediction of prognosis in invasive breast cancer using the gene-protein assay (GPA)

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Body: Background:
The eligibility of patients for HER2-targeted therapies is determined by the evaluation of HER2 gene amplification and HER2 protein overexpression. The gene-protein assay (GPA, Ventana Medical Systems, Inc., USA) is a new method for simultaneous evaluation of HER2 immunohistochemistry (IHC) and dual in situ hybridization (DISH) using a single tissue section. In this study, we investigated the relationship between HER2 IHC and DISH results evaluated by GPA. In addition, we analyzed the correlation between HER2 status and prognosis of invasive breast cancer patients.

Patients and Methods:
In this study, invasive carcinoma tissues of 280 consecutive patients treated in Saitama Cancer Center in 2000-2001 (median follow-up: 130 months) were examined. Among HER2 positive initial samplings, no patients originally received adjuvant trastuzumab therapy. However, 76% of HER2 positive cases of recurrence received trastuzumab therapy. GPA was performed on a section of routinely processed primary tumor and the status of HER gene and protein were separately evaluated in whole areas of tumor sections using the following FDA criteria: DISH (negative: HER2/CEN17 < 2, positive: HER2/CEN17 ≥ 2.0) and IHC (score 0 to 3+). In IHC score 2+ patients group, final HER2 positivity was decided according to DISH results using criteria of FDA criteria. Recurrence-free survival (RFS) and cancer-specific survival (CSS) stratified by IHC and DISH results were analyzed. In addition, patterns of heterogeneity were grouped according to the following 4 phenotypic and genotypic types: A) IHC 2+/DISH+; B) IHC 2+/DISH-; C) IHC 1+ & 0/DISH+; and D) IHC 1+ & 0/DISH-. The presence of heterogeneity in relation to prognosis was analyzed in the IHC 0 & 1+/DISH- group.

Results:
The HER2 IHC 3+ group (27.5%), both with or without trastuzumab therapy, had significantly worse survival than HER2 IHC 1+ & 0 group (RFS: P=0.0039; CSS: P=0.0362) and HER2 DISH+ group (27.5%) had significantly worse survival than HER2 DISH- group (RFS: P=0.0056; CSS: P=0.0497). HER2 positive group defined by FDA criteria had significantly worse RFS than HER2 negative group (P=0.0211). HER2 IHC 1+ & 0/DISH+ group had significantly worse RFS than IHC 1+ & 0/DISH- group (P=0.0208). In the HER2 IHC 1+ & 0/DISH- group, patients with heterogeneity (33 cases) had significantly worse survival than those without heterogeneity (RFS: P=0.0176; CSS: P=0.0199).

Conclusions:
HER2 GPA technology might be useful for evaluating the discrepancy and heterogeneity of HER2 IHC and DISH results at single cell levels simultaneously and the presence of HER2 tumor cell heterogeneity might be a potent prognostic factor in HER2 negative breast cancer patients. Further clinical research must be conducted for clarification of the relationship between the presence of HER2 intratumoral heterogeneity and the effectiveness of HER2-targeted therapies.
Title: HER2/Neu genetic heterogeneity analysis in breast carcinoma

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Body: Both immunohistochemistry (IHC) for detecting protein expression and fluorescence in situ hybridization (FISH) for gene amplification are accepted methods of determining clinical HER2 (or ERBB2) status. However, analysis of traditional clinical and pathological prognostic markers often misses the inherent heterogeneity of breast carcinoma. The reliability of IHC and FISH is questionable, and less than half of the patients with HER2-positive cancer will respond to trastuzumab therapy. The genetic heterogeneity (GH) of breast carcinoma is a major cause that makes its diagnosis and treatment far from being optimal. The impact of GH on patient outcome and the underlying mechanisms are lacking. To clarify the impact of FISH and IHC testing on HER2 GH, a total of eight studies with 11478 patients retrieved from PubMed and Embase were performed in this study. HER2 GH was noted in 27.8% of breast carcinomas, and the percentage ranged from 2.5% to 33.5%. HER2 GH existed in 84.0% FISH equivocal test and 30.8% IHC equivocal (IHC 2+) test. Further analysis showed the significant statistical differences between GH and different HER2 expression by FISH test ($\chi^2=864.23$, $P<0.001$), and between GH and different HER2 expression by IHC test ($\chi^2=206.52$, $P<0.001$), respectively. Forest plots displayed the discrepancy of GH frequency existed in the different ethnicities. Networks revealed that ERBB2 regulates different downstream proteins to coordinate numerous processes. Our results revealed that GH of HER2 differentially presented in a subset of HER2 amplified breast carcinomas, especially in cases with HER2 expression (FISH equivocal and IHC equivocal). There is a substantial difference in the frequency of GH among different ethnicities. HER2 GH is more likely to exist in FISH equivocal test than IHC equivocal test, and more convenient to document GH status by FISH test. Various HER2 genetic heterogeneity may activate diverse downstream signaling processes and play a different role in the pathogenesis of breast carcinoma.
Title: The non-coding transcriptome of hypoxic breast cancer: Novel insights of clinical relevant long non-coding RNA in hypoxia signalling

Hani Choudhry¹,³, Johannes Schodel², Ashwag Albukhari³, Syed Haider³, Francesca Buffa³, Peter J Ratcliffe⁴, David R Mole⁴, Ioannis Ragousis⁵ and Adrian L Harris³. ¹King Abdulaziz University, Jeddah, Saudi Arabia; ²Friedrich-Alexander-University, Erlangen-Nuremberg, Germany; ³University of Oxford, Oxford, United Kingdom; ⁴Henry Wellcome Building for Molecular Physiology, University of Oxford, Oxford, United Kingdom and ⁵McGill University and Genome Quebec Innovation Centre, Montreal, Canada.

Body: Hypoxia is associated with aggressive and poor prognosis of breast cancer. Generally, pan-genome analyses of hypoxia have focussed on protein-coding genes, however, the role of non-coding RNAs, in particular long non-coding RNAs (lncRNA) in hypoxia is not well characterised. We undertook an integrated genomic analysis of the hypoxic transcriptome in MCF7 breast cancer cells, employing total RNA-seq together with ChIP-seq for the hypoxia-inducible transcription factor (HIF) and for epigenetic marks of transcriptional activation (RNApol2 and histone H3K4me3).

Analyses revealed that all classes of RNA are significantly regulated by hypoxia including piwiRNA, miRNA, tRNA, and sn/snoRNA. Significant numbers of lncRNAs were upregulated in hypoxia and were associated with increased RNApol2 and H3K4me3 markers and with HIF binding, indicating direct transcriptional activation of lncRNAs by HIF. The most hypoxically upregulated IncRNA was NEAT1, which is a direct transcriptional target of HIF-2a but not HIF-1a. The role of NEAT1 in cancer has not been previously studied.

We demonstrated that hypoxic NEAT1 induction is common in breast cancer cell lines and xenografts models treated with bevacizumab. NEAT1 directly induces the formation of nuclear paraspeckle bodies in hypoxia. Moreover, it contributes to tumourigenicity by increasing cell proliferation, colony formation, and reducing apoptosis. In addition, we report that NEAT1 is required to retain hypoxia induced hyper edited Junctional Adhesion Molecule A (JAM-A) mRNA in the nucleus, thus preventing export into the cytoplasm for translation. Finally, in a large cohort of 2000 breast cancers, high levels of NEAT1 were associated with poor clinical outcomes and clinicopathological features.

Our results extend knowledge of the hypoxic transcriptional response into the spectrum of non-coding transcripts. These findings provide novel mechanisms of transcriptional regulation in hypoxia and open new avenues to find novel pathways and targets to develop therapies for breast cancer.
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-05-02
Average Grade: 5.50

Title: Preliminary translational results from PROMIX, a phase II trial of preoperative chemotherapy with the addition of bevacizumab in large operable and locally advanced HER2-negative breast cancer

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Body: Background: Preoperative chemotherapy in breast cancer (bc) provides unique possibilities to evaluate effects of therapy by studying response and changes in the tumor during the course of treatment. A pathologic complete response (pCR) correlates positively with long term prognosis in high-proliferating bc. In triple negative bc (TNBC) the prognosis was still relatively serious in cases with pCR in one large meta-analysis (Cortazar Lancet 2014).

Methods: 150 cases were included in this multicenter study, and treated with six cycles of epirubicin and docetaxel, adding bevacizumab from cycle 3, before surgery. Core needle biopsies were collected at free hand or with ultrasound (US) guidance and snap frozen at base-line and after cycle 2, tissue was also collected at surgery. Subtyping was performed using immunohistochemistry (IHC) of ER, Ki67 and HER2 according to modified St Gallen criteria; and using bead array gene expression profiling (GEX) according to PAM50.

Results: Biopsies were successfully retrieved from 145/150 pts at baseline, 138 after cycle 2 and 139 at surgery. The mRNA yield was adequate for GEX from 123/145 (85%) at baseline, 82/138 (59%) at cycle 2 and 71/139 (51%) at surgery, the decrease being a result of tumor shrinkage during treatment.

Initial PAM50 subtypes were as follows: luminal A (LA) 20%, luminal B (LB) 45%, HER2 5 %, basal like (BL) 22% and normal like (NL) 8%. PAM50 at baseline differed compared to IHC subtypes. Among IHC defined LA-like cases 15/33 (45%) were classified as LB by PAM50. Similarly, among IHC LB-like 22/57 (39%) were classified as non-LB (6 basal, 8 LA, 3 HER2 and 5 NL), while among IHC TNBC 7/28 (25%) were classified as non-BL subtypes (1 LA, 3 HER2 and 3 NL).

Of the pts with a baseline GEX analysis, 17 (14%) achieved a pCR. The observed pCR rates among PAM50 subtypes were: LA 8%, LB 5%, HER2 17%, BL 53% and NL 20%. For non-pCR cases, 39/52 (75%) of the tumors changed PAM50 subtype between baseline and surgery. The majority changed to the NL subtype. 33% of the LB tumors changed to the LA subtype. Currently, after 2.2 years of follow-up, 16 pts are deceased due to bc. Among BL cases, 6/9 pts with a pCR at surgery remain alive; while 3/9 have died from bc. Exploratory analyses using functional gene modules (Desmedt Clin Cancer Res 2008) suggest that patients with BL tumors who have died have higher scores for PLAU/invasion and lower scores for STAT1/immune response compared with those who are still alive. Tumor size at baseline did not obviously correlate with outcome.

Conclusion: We show that biological material can be retrieved from a substantial fraction of cases treated within a multicenter study of preoperative chemotherapy. The success rate may be ameliorated by routine use of US guidance. The distribution of subtypes differs between modified IHC St Gallen criteria and PAM50, especially within the luminal subtypes. The pCR rate is highest among cases with a BL tumor at baseline. Shift of the gene signature between different subtypes during the course of treatment is frequent. In this set of relatively large tumors, the prognosis among BL bc appears to be adverse in spite of a pCR.
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-05-03
Average Grade: 5.00

Title: Anastrozole and everolimus in hormone receptor-positive metastatic breast cancer: Safety profile, activity and associations of molecular alterations in the PI3K/AKT/mTOR pathway

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Body: Background
Combining aromatase inhibitors with PI3K/AKT/mTOR inhibitors in patients with hormone receptor (HR)-positive metastatic breast cancer has demonstrated clinical efficacy. There is limited data on associations between molecular signatures and activity.

Patients and Methods
We evaluated the combination of anastrozole and everolimus in 56 patients with HR-positive, metastatic breast cancer. The primary objective was to establish safety and maximum tolerated dose (MTD). Dose limiting toxicities (DLTs) were defined as serious grade 3 or 4 toxicities related to treatment that occurred during cycle 1. Dose level 1 was anastrozole 1 mg PO QD and everolimus 5 mg PO QD and dose level 2 was anastrozole 1 mg PO QD and everolimus 10 mg PO QD (a dose level -1 included everolimus 2.5 mg PO QD). Secondary endpoints included evaluation of antitumor activity and molecular associations with response. When tissue was available, Next Generation Sequencing (NGS) was performed using genomic libraries selected for all exons of 236 (or 182) cancer-related genes sequenced to average depth of >500× in a CLIA laboratory (Foundation Medicine, Cambridge, MA, USA). An analysis was then performed for all classes of genomic alterations.

Results
The median age was 59 (range, 37-82) and the median number of prior therapies in the metastatic setting was 3 (range, 0-13). The initial oral daily dose of anastrozole 1 mg oral and everolimus 10 mg PO daily was well tolerated. Five dose-limiting toxicities (DLTs) were seen at full doses, including grade 3 thrombocytopenia (1 patient), grade 3 neutropenia (1 patient), grade 3 increased liver enzymes (1 patient), grade 3 hyperglycemia (1 patient) and, grade 3 mucositis (1 patient). The most common grade 3 or 4 treatment-related toxicities were neutropenia (5%), increased liver enzymes (5%), and hyperbilirubinemia (3%). Of the 56 patients on study, 36 were tested for at least one molecular alteration in the PI3K/AKT/mTOR pathway. Twelve of these 36 patients had NGS analysis of their tumor tissue. Eighteen of 36 patients (50%) tested had at least one alteration in the pathway, including mutations in PIK3CA (n=16), PIK3R1 (n=1), and AKT1 (n=2); PTEN protein loss (n=1); and, AKT3 amplification (n=1). Sixteen of 56 evaluable patients (29%) achieved stable disease (SD) / partial response (PR) / complete response (CR) ≥ 6 months (n = 3 (5%) with PR/CR). Thirteen of the 16 patients who achieved SD/PR/CR ≥ 6 months were tested for a genetic alteration in PI3K/AKT/mTOR pathway and 7 of these patients (54%) had at least one alteration in the pathway, including mutations in PIK3CA (n=6), PIK3R1 (n=1), and AKT1 (n=1); PTEN loss (n=1); and AKT3 amplification (n=1).

Conclusions
Combination anastrozole 1 mg and everolimus 10 mg is well tolerated and is active in heavily-pretreated patients with HR-positive breast cancer. The presence of a molecular alteration in the PI3K/AKT/mTOR pathway did not predict for clinical activity of this combination.
**Title:** Gene expression associated with poor prognosis of young TNBC patients

Thomas Karn¹, Lajos Pusztai², Cornelia Liedtke³, Giampaolo Bianchini⁴, Balazs Győrfy⁵, Christos Hatzis², Achim Rody³, Volkmar Müller⁶, Marcus Schmidt⁷, Uwe Holtrich¹ and Sven Becker¹. ¹Goethe-University, Frankfurt, Germany; ²Yale Cancer Center, New Haven, CT; ³University Hospital Lübeck, Germany; ⁴Ospedale San Raffaele, Milan, Italy; ⁵Hungarian Academy of Sciences, Budapest, Hungary; ⁶University Hospital, Hamburt, Germany and ⁷Johannes Gutenberg University Mainz, Mainz, Germany.

**Body:**

**Background:**
Among TNBC patients those of very young age (<40 years) display a significantly worse prognosis (Liedtke et al. 2013 Breast Cancer Res Treat). We verified this result in 1161 TNBC samples with full Affymetrix gene expression data of which 845 patients had both detailed age and follow up information.

**Materials and Methods:**
We split the full sample set into a finding cohort of 394 TNBC and a validation cohort of 767 TNBC encompassing 309 and 536 samples, respectively, with both age and follow up data. We then used significance analysis of microarrays (SAM) in the finding cohort to look for genes whose expression is associated with young age (<40 years). Identified genes were analyzed for their correlations to known metagenes characteristic for different TNBC subtypes. Subsequently the whole analysis was repeated in the validation cohort.

**Results:**
We identified 98 and 222 probesets by SAM in the finding and validation cohort, respectively. Only a subset of identical probesets (19.4%) was re-identified in the validation. However, the gene lists were similarly enriched for correlations to specific metagenes and the respective TNBC subtypes. We found that young age among TNBC is positively correlated with increased proliferation and a basal-like subtype, but negatively correlated with the molecular apocrine phenotype and the claudin-low subtype. We also observed a negative correlation of young age with stromal enrichment and EMT but no difference in lymphocyte infiltration as judged by specific metagenes. However, despite that TNBC of patients <40 years have a poor prognosis (P=0.006) and are clearly enriched in basal-like subtype (70.7% vs 55.3%; P=0.001), still basal-like TNBC do not differ from non-basal-like TNBC in prognosis. Moreover, even within the group of basal-like TNBC the prognostic effect of age<40 was still observed (P=0.008). Interestingly, the changes in expression of single genes and metagenes were observed over a wide age range of 35-85 years. In contrast the difference in prognosis was markedly pronounced at the 40 year age limit. Thus, potential critical differences in gene expression associated with poor prognosis of TNBC <40 years seem to be concealed by many age-associated changes without prognostic value.
Title: A four gene signature predicts anthracycline benefit: Evidence from the BR9601 and MA5 breast cancer trials

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Body: Background: Chromosome instability (CIN) in solid tumours is associated with poor prognosis and results in numerical and structural chromosomal aberrations. Recent evidence from both the BR9601 and MA.5 trials has demonstrated CEP17 duplication as a predictive marker of anthracycline benefit. CIN25 and CIN70 gene expression profiles have previously been published and predict survival response. An analysis of the BR9601 and MA5 clinical trials was performed to test the role of CIN gene expression signatures as a marker of anthracycline sensitivity.

Methods: RNA was extracted from patients in both the BR9601 and MA5 studies and analysed through Nanostring technology. Log-rank analyses explored the prognostic values of the signatures on distant relapse-free survival (DRFS). Cox-regression models tested independent prognostic value on DRFS in the presence of treatment, age, tumour size, nodal status, ER status and grade, and treatment by marker interactions.

Results: Of the 761 samples available from the BR9601 and MA5 cohorts we successfully analysed 703 (92.4%). High CIN25 and CIN70 scores were associated with age (p<0.0001), grade (p<0.0001), PgR negativity (p<0.0001) and ER negativity (p<0.0001). In univariate analysis, high CIN25 score was associated with decreased DRFS (HR: 0.74, 95% CI 0.54-0.99, p=0.046). In a multivariate analysis with adjustment for size, nodal status, ER, pathological grade, HER2, CIN25, treatment and treatment by marker only pathological grade, nodal status and tumour size were significant predictors of outcome.

A more limited set of genes that reflected CIN was established by examining the expression profile of the genes and clustering them. The combined cohort was split into a 60% training and 40% validation set. The area under the curve (AUC) was calculated and the gene signature with the greatest AUC was selected and termed CIN4. Patients with low CIN4 score benefited from anthracycline treatment compared to those that had high CIN4 score (HR 2.72, 95% CI 1.48-5.02, p=0.001). No significant benefit with CMF treatment was observed in (HR: 1.02, 95% CI 0.58-1.82, p=0.92). After multivariate analysis the treatment by marker interaction for CIN4 had a hazard ratio of 2.10 (95% CI 2.18-30.38, p= 0.001).

Conclusion: High CIN70 and CIN25 scores were associated with an aggressive phenotype and showed a potential increased sensitivity to anthracycline therapy compared to those with low CIN scores. CIN4 was an independent predictor of anthracycline benefit for DRFS. However, further work in larger patient cohorts such as NEAT is warranted.
Identification of specific gene signatures and alternative splice variants using exon array in Inflammatory breast Cancer

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Body: Inflammatory Breast Cancer (IBC) is a rare form of breast cancer with a particular phenotype and aggressiveness. Although significant progress has been made in the management of IBC, the prognosis remains poor. Intensive research on IBC has allowed identification of several candidate genes and pathways, but molecular findings unique to IBC have yet to be identified to improve treatment and survival. Transcriptomic studies have revealed a marked heterogeneity of IBC with limited gene overlap between studies, preventing the identification of a sensitive and specific signature in IBC.

In order to detect both gene expression levels and alternate RNA splice isoforms, we chose to perform splice-sensitive array profiling using Affymetrix Exon Array in a well-defined series of 33 IBC (core biopsies from previously untreated T4d carcinomas) compared with 28 stage I to non-inflammatory stage III breast cancers.

Gene expression analysis allowed the identification of classical molecular subtypes in both IBC and non-IBC, with overrepresentation of basal-like (17/33 vs 9/28) but no luminal A in IBC (vs 11/28). Based on Fold-Change (FC) ≥ 1.5 and p-value < 0.05, 495 genes were significantly dysregulated between IBC and non-IBC, including in particular up-regulated hemoglobin genes and down-regulated ER-related genes in IBC compared to non-IBC. Most activated pathways were hematopoietic cell lineage, cytokine-cytokine receptor interaction, chemokine signalling pathway and complement and coagulation cascade. We defined a 21-gene signature discriminating IBC from non-IBC with 8% error rate. To get rid of genes associated with molecular subtypes like ER-related genes, an analysis restricted to basal-like BC (17 IBC compared to 9 non-IBC) was performed allowing the definition of a 29-gene signature discriminating the whole groups of IBC from non-IBC with 3.3% error rate. Validation of this signature in the gene expression datasets from the World IBC Consortium will be presented.

Specific exon expression analysis revealed that when based on FC splicing-index and p-value, 266 exons representing 177 distinct genes were differentially regulated between IBC and non-IBC. After manually curation of results, 13 splice events representing 12 distinct genes were retained as good candidates for alternative splicing in IBC (EVL, RPL10, MYH10, HSPA8, DOCK7, DIDO1, RPL4, TRAK1, RGS1, LMO4, SMARCA4, ZNF337).

To confirm gene-signatures specific to IBC, altered pathways and major splice variants, we are performing a validation study using quantitative RT-PCR in the screening set (n=33) and in an independent series of 140 IBC compared to 200 non-stage-matched non-IBC. Finally the most dysregulated genes will also be studied at the protein level using immunohistochemistry.
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Publication Number: P2-05-07
Average Grade: 5.40

Title: Feasibility and sensitivity of fine-needle aspiration biopsies for the detection of somatic mutations using next-generation sequencing in breast cancer

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Body: Background/Purpose: Next-generation sequencing (NGS) is being incorporated rapidly into clinical practice. Fine-needle aspiration biopsy (FNAB) specimens have been used feasibly in molecular analysis including direct sequencing and microarrays. They are readily available and enriched in malignant cells, thus providing opportunities for genomic analysis for more clinical samples. In this study, we assessed the feasibility and sensitivity of FNAB for the detection of somatic mutations by NGS compared to bulk tissue.

Methods: Bulk tissue and FNAB was sampled via skin superficial to the palpable tumor from surgically resected breast cancer specimen. DNA was extracted from the bulk tissues and FNAB samples obtained from twelve patients. Somatic mutations detected from whole exome sequencing (WES) by next-generation sequencing (NGS) (HiSeq 2500, Illumina) were analyzed for corresponding pairs of bulk tissue and FNAB. Verification of somatic mutations detected exclusively from FNAB and known to be clinically relevant to breast cancer was carried out by Sanger sequencing. Invasive tumor percentages of bulk tissues were evaluated using hematoxylin and eosin (H&E)-stained sections.

Results: Average depth of coverage were 158.8x and 158.3x for bulk tissue and FNAB, respectively. Number of detected somatic mutations ranged from 2 to 153 (median 18.5) and 19 to 210 (median 39.5) for bulk tissue and FNAB, respectively. Ten specimens had more mutations detected exclusively from FNAB than from bulk tissue. Allele fractions plotting of corresponding pairs of bulk tissue and FNAB showed good, intermediate, and poor correlation in five, two, and five specimens, respectively. H&E-stained sections of bulk tissue from the five specimens with good correlation contained an invasive tumor percentage of 45 to 98%, whereas those from five specimens with poor correlation contained 0 to 25%. Three of the poorly correlated bulk tissues were judged to have 0% of invasive tumor. Among mutations detected exclusively from FNAB, eighteen different genes of interest in 22 foci were evaluated for both FNAB and corresponding bulk tissue by Sanger sequencing. In the results, three mutations (PIK3CA, TP53 x2) were verified in FNAB samples but not in the bulk tissue.

Conclusion: WES was successfully carried out in all pairs of bulk tissue and FNAB from twelve breast cancer patients. In samples with high tumor content somatic mutation profiles showed high correlation between the two samples whereas samples with low tumor content failed to show correlation. The failure was mostly due to the scarcity of tumor portions in the bulk tissues, indicating that FNAB more reliably retained malignant tumor portion. This study suggests that FNAB is an easy and feasible method, and furthermore, provides a more reliable specimen for NGS analysis where somatic mutations could be identified for potential prognostic or therapeutic benefits.
Title: The molecular landscape of breast ductal carcinoma in situ (DCIS)

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Body: Ductal Carcinoma in situ (DCIS) is by definition a local cancerous condition with no regional or lymph node involvement and is a precursor lesion to invasive ductal carcinomas (IDC). However, we have no understanding on why only some DCIS lesions evolve to IDC while others appear not to do so during the life span of the patient. The massive use of screening mammography has led to a dramatic increase in DCIS detection in the last three decades. This significant increase in DCIS detection has led to an 'overdiagnosis and overtreatment problem'. It has been argued that in 2008 breast cancer was overdiagnosed in > 70,000 women in the US and that the increase in DCIS and localized breast cancer detection was not followed by a corresponding decrease in the incidence of late stage cancer. As a second issue, most DCIS patients are recommended to receive post-surgery adjuvant therapies that may be unnecessary for most patients and this in itself constitutes an additional overtreatment conundrum.

Importantly, no biomarkers exist for facilitating the distinction of DCIS lesions with higher chance of progression vs. lesions unlikely of progressing. A detailed analysis of the mutational landscape of ‘pure’ DCIS lesions, coupled with a deeper understanding of the transcriptome and epigenome of these lesions, will be instrumental to uncover the molecular mechanisms and pathways that govern the natural progression of pre-malignant breast lesions. To this end we are profiling the genome (Exome-seq) transcriptome (RNA-seq) and methylome (RRBS) of normal and DCIS matched samples as well as normal organoid specimens. Our DCIS Exome-seq data showed an average mutation rate of 9.1 mutations/Mb, with a range of 4.2 to 19.5, indicating that some DCIS have significantly higher mutation rates than others.

All ‘pure’ HGDCIS samples showed somatic mutations affecting multiple genes and 75% of cases displayed mutations affecting targets described as ‘cancer gene drivers’ and candidate drivers. Among these we detected mutations affecting TP53 (22% of cases), PIK3CA (22%), GATA3 (9%), MLL3 (9%) and mutations affecting CDH1, MAP2K4, USH2A, NF1, ATM and various epigenetic regulators were also observed. Interestingly, no mutations were detected so far in some genes reported as commonly mutated in invasive breast cancer. Exome-seq results were validated by RNA-seq data.

Integrated pathway-based modeling analysis (PARADIGM) of RNA-seq data allowed us to identified DCIS subgroups that differ in the activity of a plethora of signaling pathways such as FOXM1, AP1, miR-34a, E2/ER, and immune signatures. Comprehensive analysis of RNA-seq and RRBS data allowed us to identify specific genes and pathways commonly silenced by CpG islands promoter's methylation.

Our final goal is: to frame at what stage in the DCIS progression specific mutations take place, define which molecular abnormalities are cause (drivers) or consequence of progression, and ultimately better stratify DCIS subgroups based on the biomarker profiles obtained.

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Title: Functional mapping of the oncogenic kinome activity of triple-negative breast cancer cells

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Body: Background: Triple-negative breast cancer (TNBC) accounts for approximately 10 to 15% of all breast cancer cases. The management of TNBC cases will significantly improve once molecular mechanisms specific to TNBC cells will be identified and treated accordingly. Evidence suggests that TNBC cells display deregulated kinase-dependent signaling cascades that differ from non-triple-negative breast cancer cells. We hypothesized that uniquely divergent phospho-circuits could be distinguished between TNBC and non-TNBC cell lines. By revealing such unique, dysfunctional phospho-signaling network, our long-term objective is to identify kinases that underpin triple-negative breast cancer development, and can be inhibited using targeted therapy.

Methods: The kinome activity of TNBC and non-TNBC cell lines was identified (HCC70, MDA-MB-231, MDA-MB-436 compared to AU565, MCF-7, T47D). The functional phospho-signature of each breast cancer cell was analyzed using a high throughput experimental platform that monitors the level of activity of myriad kinases at once. This technique uses 242 phospho-sensing probes and 78 controls in an aqueous-based assay to simultaneously and directly measure the phospho-catalytic activity of phosphorylating enzymes in cell lysates. We mapped the most significantly deranged phospho-signaling cascades and the related kinases.

Results: Using 6 cell lines tested under various conditions, we generated 72 phospho-signatures, out of a total of 23,040 data points. After validating the repeatability and robustness of the assay, the kinase activity signature of each breast cancer cell line was analyzed and compared to each other using unsupervised hierarchical clustering. The phospho-sensing assay revealed the heterogeneity of kinase activity networks among breast cancer cells. These data also established that phospho-signaling cascades related to AKT, ERK, and SRC kinases were differentially altered in TNBC and non-TNBC cell lines.

Conclusions: We successfully identified unique phospho-circuits of TNBC and non-TNBC cell lines. Our goal is now to test whether specific kinase inhibitors can efficiently kill or prevent the growth of TNBC cell lines in culture and animal models. We will expand our approach into a high-content, functional kinome-screening platform to characterize the phospho-fingerprint of breast cancer cells and tissues, and explore the druggable, kinase-dependent mechanisms critical to triple-negative breast tumors.
Title: Explore the differences and relationships between normal and ADH, DCIS and IDC tissues in breast based on Raman spectroscopy

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Background: Atypical ductal hyperplasia (ADH) is a premalignant breast lesion associated with an increased risk of breast cancer which shares some but not all the features of ductal carcinoma in situ (DCIS). The mechanism that ADH changes to DCIS and even invasive ductal carcinoma (IDC) is still not clear now. There is a pressing need to develop screening techniques that are accurate, minimally invasive and, more sensitive to identify early neoplastic changes and hence improve early breast cancer diagnosis. Our aim was to explore the differences and relationships between normal, ADH, DCIS and IDC lesions of the breast based on biochemical characteristics determined by Raman spectroscopy.

Methods: Frozen sections were collected from 39 patients (all ladies) who underwent surgical resection or mammotome biopsy at the Department of Breast Surgery, the First Hospital of Jilin University. After operation the samples were immediately frozen at -20-25 ° and two contiguous sections (6 µm thickness) were cut from a sample by freezing microtome. One was stained with haematoxylin and eosin for routine histopathological analysis; the same position of the other section was detected by Microscopic confocal Raman spectrometer (HORIBA JY Lab800, 633nm) with its mirror image (the H&E section). After Raman measurement, the sections were routinely processed, stained with H&E and histologically examined. Support vector machine (SVM) was used to differentiate different breast lesions.

Results: A total of 475 Raman spectra were obtained from 9 normal breast tissues, 7 ADH, 8 DCIS, and 15 IDC breast tissues. Pronounced mean Raman spectra differences were observed between normal tissues, ADH, DCIS and IDC tissues. The significant features of normal tissues are 1301, 1438, 1652, and 1743 cm⁻¹, these peak positions are attributed to lipids, and the spectra profiles of normal tissues have no strong protein peaks. Most noticeable was the increased protein and reduced lipid levels of ADH tissues compared to normal tissues. The peak relative intensity of 1158 cm⁻¹ which is attributed to the vibrational modes of C-C stretch of proteins has significantly increased from normal tissues to DCIS and IDC in breast. The major spectra differences in ADH, DCIS and IDC spectra were evidenced by a red shift with a broad peak of CH2, the intensity of the stretching vibration peak of carotenoids, a relatively strong band of amide-I, and the nuclear acid peak. ADH tissues had the largest constituent variations between subjects. During the disease progression, IDC tissues have smaller inter-subject constituent variations than DCIS and ADH tissues. The accuracies of SVM are 93% and 95% in discriminating normal and IDC, while only 50% and 51% in discriminating ADH and DCIS.

Conclusion: Malignant transformations in tissue are associated with complex biochemical changes and may provide an effective way to evaluate the malignancy by Raman spectroscopy as it reveals differences between normal tissues, ADH, DCIS and IDC tissues in the breast. Further study to explain the biochemical relationships between these differences will shed more light into a better understanding of the mechanism by which ADH and DCIS convert to IDC.
Title: Genetic profiling of breast cancer confirms a pivotal role of EGFR pathway in the development of acquired resistance to tamoxifen

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Body: Background: Acquisition of resistance to tamoxifen remains a major drawback in the treatment of oestrogen receptor (ER)-positive breast cancers. Aberrant expressions of some genes in the EGFR pathway were associated to the acquisition of resistance in some studies.

Patients and methods: This prospective study was carried out on 157 female patients with hormone receptor positive, locally recurrent inoperable and/or metastatic breast cancer who presented to the National Cancer Institute, Cairo University during the period from October 2010 to October 2012. All patients received tamoxifen. Patients were divided into tamoxifen responsive and refractory groups according to their response to therapy.

In an attempt to understand the contribution of EGFR pathway to the development of resistance to Tamoxifen, we assessed the genetic profile of the EGFR pathway genes in the 2 groups. RNA was extracted from tumor and normal tissue samples obtained from all patients and the expression level of 92 genes was evaluated using the SABioscience array (Qiagen) with four house-keeping genes.

Results: Age ranged between 29 and 79 years but age ≤ 50 or > 50 did not correlate to resistance. Evaluation of Hormone receptor status showed that 58.59% were positive for both ER and PR, 32.48% were ER positive, PR negative and 8.91% ER negative PR positive. Neither hormone receptor status nor nuclear grade was correlated to drug resistance. There was a strong correlation between response to tamoxifen and disease site as patients with bone only disease demonstrated noticeable good and maintained response compared to those who had visceral involvement (p- 0.005). The expression levels of all genes were assessed in both studied groups: the responders (the control) and the refractory (tested) groups. Fifty six genes were differentially over-expressed in the refractory group compared to the responding group, among which only JAK1, COL1A1, GAB1, FN1 and MKNK1 showed a significant difference between responders and refractory groups. Thirty four genes were differentially expressed (reduced expression) in the refractory compared to the responders. Moreover, CYP2D6 *3, *4 were significantly prevalent in the refractory group (86.6%), whereas variants *10/*10 and *10/*3 were more common in the in the responding group (85.5%) (p = 0.027)

Conclusion: A panel of 5 genes (M JAK1, COL1A1, GAB1, FN1 and MKNK1) in EGFR pathway together with CYP2D6 polymorphisms could be used to predict patient’s response to tamoxifen though this has to be verified in an extended study including larger sample.
Title: Signatures of endocrine resistance in the tamoxifen and exemestane adjuvant multinational trial (TEAM)-UK cohort

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Body: Introduction: There are a number of commercially-available tests to stratify risk for women diagnosed with early breast cancer. While such “Generation I” tests are increasingly being used, a consensus is growing that these tests are moderately accurate in assessing risk. Moreover, Generation I tests fail to direct more personalized treatment. There exists, therefore, a clear need for more informative "Generation II" tests that have theranostic targets. To this end, we have performed an mRNA abundance-based analysis using the UK cohort of the TEAM trial to identify signatures of endocrine resistance, from which pathways for putative therapeutic intervention may be identified.

Methods: RNA extracted from 790 patients in the UK-TEAM cohort were profiled using a 165-gene NanoString codeset. The gene list was compiled from targets that comprise many of the existing risk assessment tests, in addition to genes known to be of importance for breast cancer pathogenesis. Signal intensities were normalized using the R statistical environment; 336 different combinations of preprocessing methods were assessed and the most optimal method selected using unbiased criteria.

Results: Univariate survival analysis revealed a number of significantly prognostic candidates. Using inter-gene correlation and consensus clustering, we identified five gene clusters. Not surprisingly, these clusters included a strong proliferation, hormone signalling and cell migration component. Derivation of risk scores using Cox proportional hazards model, with the inclusion of age and nodal status, generated a signature identifying patients with differences in distant relapse-free survival (DRFS). Moreover, the composition of the gene-list made it possible to characterize the patients into their intrinsic subtypes and to determine their relative risk for recurrence relative to assessment tools available today. The added value of subtyping and the gene clusters identified in this discovery cohort will be discussed.

Conclusions: The impact of test-guided therapy using multi-parametric tests is increasingly being felt in the clinic, and is reshaping modern health-care economics. A successful Generation II multi-parametric test will better discriminate those that are truly at high risk for recurrence following endocrine therapy and offer potential therapeutic options for intervention for those who would not benefit from current modalities.
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Title: Transcriptomic profiling of patient sequential tumours provides cutting edge view of global metastatic expression changes following endocrine therapy

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Body: Disease recurrence is a common problem in breast cancer and yet the mechanisms enabling tumour cells to evade therapy and colonise distant organs remain unclear. Here, for the first time, RNAsequencing has been performed on matched primary, nodal and liver metastatic tumours from three tamoxifen-treated patients following metastatic disease progression. Despite all primary tumours being of a luminal subtype and all cancers metastasising to the liver, the extent of patient heterogeneity at the gene level was striking. Less than 3% of the genes differentially expressed between sequential tumours were common to all patients. Larger divergence was observed between primary and liver tumours than between primary and nodal tumours, reflecting both the latency time to disease progression and the genetic impact of endocrine therapy. Furthermore, a xenograft model demonstrated the ability of tamoxifen to drive disease progression and establish distant metastatic disease in the endocrine resistant setting. Common functional pathways altered during metastatic, endocrine-resistant progression included ECM receptor interactions and focal adhesions. This novel global analysis highlights the influence of primary tumour biology in determining the transcriptomic profile of metastatic tumours, as well as the need for adaptations in cell-cell communications in order for tumour cells to successfully colonise distant host organs.
Title: New treatment options for metastatic breast cancer revealed by reverse-phase protein microarray and genomic profiling

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Body: Background: The optimal treatment strategy for patients with metastatic breast cancer (MBC) is currently unknown. Resistance to standard therapies like anthracyclines and taxanes limit the number of treatment options in many patients to a small number of non-cross-resistant regimens. We hypothesized that genomic and proteomic profiling of clinical MBC samples would identify genomic alterations that are linked to targeted therapies, and that this could facilitate a personalized approach to therapy for our patients.

Methods: We retrospectively analyzed 31 consecutive metastatic breast cancer patients that had genomic and/or proteomic studies sent over a 4 month period from February to May 2014. All patients were seen in our Genomic Oncology Clinic and subsequently underwent a rebiopsy of a metastatic site. Formalin-fixed, paraffin-embedded (FFPE) tissue was sent to either Foundation Medicine for genomic profiling, Theranostics for reverse-phase protein microarray, or both. Standard immunohistochemistry for ER, PR, and HER2 was also performed on all patients.

Results: Genomic or proteomic alterations were identified in all 31 patients. All patients harbored at least one actionable target and a treatment recommendation for a currently available FDA approved drug or drug combination was able to be suggested in all but one patient. The most commonly observed genomic alterations were within PIK3CA (26%), TP53 (23%), CCND1 (19%), and MYC (16%). Over 30 distinct genomic alterations were identified. Proteomic results were available from 16 patients. Activation of the AKT/mTOR pathway was evident in a majority of patients. A change in HER2 status was also found in 26% of patients. 16% of cases underwent a negative to positive conversion in HER2 status while 10% of cases underwent a positive to negative conversion. It is notable that all 5 patients that underwent a negative to positive conversion in HER2 status had biopsies taken from metastatic disease in the liver.

Conclusions: All patients in this retrospective study harbored genomic or proteomic alterations that are associated with targeted therapies. Treatment recommendations were suggested in all but one patient and a majority of patients are receiving the suggested therapy. Our data demonstrate that routine genomic and proteomic analysis in a clinical setting makes a significant positive impact for patients.
Title: Exploring type II microcalcifications in benign, premalignant and malignant breast lesions by shell-isolated nanoparticle-enhanced Raman spectroscopy (SHINERS)

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Body: Background: Type II microcalcifications, most often seen in proliferative lesions, including both benign and malignant pathologies. But whether the emergence of type II microcalcifications associated with cell canceration is still not clear now. Raman spectroscopy is a powerful, non-invasive analytical tool which can provide detailed and meaningful information about biochemical composition of tissues at molecular level. Our aim was to find the differences and relationships of type II microcalcifications between fibroadenoma, ADH tissues, and DCIS, IDC in breast based on their various biochemical characteristics by Raman spectroscopy.

Methods: The frozen sections were collected from 15 patients (all female; ages 25-57) who underwent surgical resection or mammotome biopsy at the Department of Breast Surgery, the First Hospital of Jilin University. After operation the samples were immediately frozen at -20°C to -25°C and two contiguous sections (6 µm thickness) were cut from a sample by freezing microtome. One was stained with haematoxylin and eosin for routine histopathological analysis and found the microcalcification locations by three expert breast pathologists; the same position of the other section was detected by Microscopic confocal Raman spectrometer (HORIBA JY Lab800, 633nm) with its mirror image (the H&E section). After the spectra we needed had been obtained, the Au@SiO2 shell-isolated nanoparticles (SHINs) were added to the surface of frozen sections immediately and then the spectra with SHINs were collected.

Results: A total of 122 Raman spectra and 119 SHINERS spectra were obtained from the microcalcifications in 3 fibroadenoma tissues, 3 ADH, and 5 DCIS, 4 IDC breast tissues. The Raman signals were significantly enhanced by SHINs. Except the major peak at 958-960 cm⁻¹ which attributed to the vibrational modes of calcium hydroxyapatite, the results show no calcium oxalate dihydrate peaks but several other chemical species. The peaks of these species appear at 1002, 1072, 1126, 1446, 1556, and 1657 cm⁻¹, these are attributed to amino acid residue (phenylalanine), nucleic acids, lipids, carotenoids, and Amide I, respectively. The fibroadenoma and ADH microcalcifications mean spectral have the same peak at 1072cm⁻¹, which belongs to the O-P-O stretch of nucleic acids, but in DCIS and IDC tissues the peak changes to 1078cm⁻¹. The ADH have more obvious peak at 1657 cm⁻¹ which assigned to different vibrational modes of the backbone and Amide I, but in DCIS and IDC tissues, the Amide I bands were disappeared. In the DCIS microcalcifications mean spectral, the peaks attributed to amino acid residue (phenylalanine) at 1003, 1031 cm⁻¹ and lipids (CH2 and CH3 bending) at 1301, 1441 cm⁻¹ show more stronger. Meanwhile, compare with DCIS, the IDC spectral shows more stronger nucleic acids peaks and weaker lipids peaks.

Conclusion: We have demonstrated the potential of SHINERS to differentiate Type II microcalcifications found in fibroadenoma, ADH, and DCIS, IDC breast tissues. The results presented in this paper provide the biochemical characteristics of the Type II microcalcifications among these tissues, and may represent a key factor responsible for mechanisms of carcinogenesis.
Title: Gene expression in synchronous primary, axillary nodal and disseminated breast cancer cells

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Body: Disseminated Tumour Cells (DTC) have been found in the bone marrow (BM) of up to 70% of patients with breast cancer metastatic to axillary lymph nodes. They are a biologically and therapeutically interesting tumour cell population. Although there is uncertainty about their relationship to cancer prognosis, DTC provide a valuable window into the processes by which primary tumour (PT) cells disseminate. DTC can be enriched from BM by immunoaffinity using antibodies directed against epithelial cell markers such as EpCAM. To better understand the changing gene expression patterns that may accompany breast cancer metastasis, we have compared the whole genome RNA expression profiles of matched PT, axillary lymph node metastases (LNM), and EpCAM-enriched cells from the BM of seven patients. Compared to PT, axillary LNM had consistently altered expression of RNAs encoding matrix metalloproteinases (MMP), growth factors, transcription factors, as well as downstream targets of Catenin-a, Tumour Necrosis Factor (TNF)-a and miR-22. Once the gene expression patterns of potentially contaminating BM cells were subtracted, compared to PT and LNM EpCAM-enriched MB cells had consistently elevated expression of RNAs encoding metabolic enzymes, ribosomal proteins, and DNA-binding factors such as YB-1. The gene expression changes we identify are candidate mediators of breast cancer metastases and represent attractive targets for further study on a cell by cell basis.
Title: Development of a fluorescent reporter system to delineate self-renewing cells in triple negative breast cancer

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Body: Background: Advanced cancers including triple-negative breast cancer (TNBC), the most aggressive breast cancer subtype, contain a self-renewing, tumorigenic cancer stem cell (CSC) population. CSCs contribute to tumor progression and therapeutic resistance. Despite an effective early response to chemotherapy, TNBC relapses with a highly heterogeneous and refractory metastatic disease enriched in CSCs. Apart from being chemo- and radio-resistant, CSCs display a high degree of multipotency, heterogeneity and plasticity. Due to the complex nature of CSCs, elucidating the characteristics of CSCs in TNBC progression continues to be a challenge. Thus, developing anti-CSC therapies to be integrated into clinical paradigms represents an immediate priority.

Rationale: A major obstacle to the identification of CSC regulatory mechanisms is the lack of experimental systems that enable the reliable enrichment of CSCs from non-CSCs for comparative analysis. While CSCs have been isolated from TNBC using CD44+/CD24- and/or ALDH+ phenotype, this enrichment paradigm requires refinement as it is not universally applicable and lacks the ability to study CSCs in real time. The limitations of these systems have excluded their application in studying the molecular heterogeneity among breast cancer tumors. CSCs are molecularly characterized based on the expression of cell surface receptors and the embryonic stem cell transcription factors NANOG, OCT4 and SOX2. NANOG, the master regulator of stem cell self-renewal, has emerged as a pro-carcinogenic factor in cancer cell lines with CSC behavior. Our previous studies have shown that silencing NANOG in cancer cells leads to reduced proliferation and self-renewal based on in vitro and in vivo experiments.

Hypothesis: We hypothesized that a Nanog promoter could be used to effectively enrich for TNBC CSCs.

Results: We generated two TNBC cell lines (MDA-MB-231 and HCC70) harboring NanogGFP reporter gene by lentiviral transductions. Increased NANOG mRNA and protein expression was observed in flow cytometry-sorted GFP+ cells compared with GFP- cells. GFP+ cells were enriched for the CSC markers CD49f and CD44+/CD24-. GFP+ cells also demonstrated an increased protein expression of the stem cell transcription factors NANOG, SOX2 and OCT4. Limiting dilution analyses revealed increased self-renewal and significantly higher stem cell frequencies in GFP+ cells. GFP+ cells demonstrated a mesenchymal phenotype with increased invasive capacity. Subcutaneous injections of GFP+ cells showed significantly higher in vivo tumor initiation and progression than GFP- cells. Flow cytometry-sorted GFP+ and GFP- cells enriched for CSCs and non-CSCs, respectively. Using this system, we performed a high-throughput flow cytometry screen and identified an additional novel CSC marker for TNBC.

Conclusion: We have developed a novel TNBC CSC reporter system using a GFP reporter driven by the Nanog promoter and identified a novel CSC marker. We have defined and validated this robust system wherein we have characterized and monitored the role of CSCs and non-CSCs in TNBC tumor initiation and progression. Using this approach, identifying CSC targets in TNBC could unravel the potential for development of innovative therapeutic strategies.
Title: Breast cancer stem-like cell activity correlates with tumour progression to metastasis but not with clinical or tumour characteristics

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Body: Introduction: Breast cancers exhibit cellular heterogeneity, containing both stem-like and more differentiated cells. The activity of cancer stem cells (CSC) is likely to be dependent on the microenvironment or niche. Using 158 patient tumour samples, correlations between niche-independent breast CSC activity and clinical and tumour characteristics were tested.

Methods: 104 early breast cancer surgical samples and 54 unrelated metastatic samples from pleural or ascitic fluid were harvested. To test CSC activity, isolated cells were grown in both primary (formation) and secondary (self-renewal) mammosphere (MS) culture. Tumour initiating activity was also tested by transplanting breast cancer fragments or cells into the sub-cutaneous flanks of NSG mice (n=84 early and n=10 metastatic).

Results: No correlation was found between MS growth, MS formation (%), MS self-renewal (%) or in vivo tumour initiation and breast cancer sub-type, grade, node status or Nottingham prognostic index. 33% of the samples that formed MS in vitro initiated tumours in vivo while only 9% that failed to form MS initiated tumour growth. Metastatic compared to early BC samples grew MS more frequently (53/54 compared to 81/104), and had a higher primary MS formation efficiency (1% vs 0.6%; P<0.001) although rates of MS self-renewal were similar. Tumour initiation in vivo was also more frequent in metastatic than early breast cancer samples (7/10 versus 25/84; P<0.02).

Conclusions: In summary, niche-independent breast CSC activity measured in vitro by MS assay and in vivo by xenograft growth is not directly correlated with standard clinical parameters. However, both in vitro and in vivo CSC activity are increased in metastatic samples. These results suggest that breast CSC activity is independent of other prognostic indicators but may predict for poor outcome tumours. Relapse free survival data are maturing and will be presented with analysis of primary tumour ALDH1 expression.
Title: Prostaglandin E receptor EP4 is a therapeutic target in breast cancer stem cells

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Body: There is an urgent need to identify mechanisms underlying the survival of cells expressing cancer stem cell or tumor-initiating properties. The cyclooxygenase-2 (COX-2) pathway is highly expressed in many breast tumors and contributes to poor outcomes. The COX-2 product prostaglandin E2 (PGE2) promotes tumor growth and metastasis by acting on the G-protein-coupled receptor EP4. We compared the expression and function of COX-2 and EP4 in mammosphere-forming and bulk populations derived from a panel of human and murine luminal and basal type tumor cells with different metastatic capacities. Expression of both EP4 and COX-2 were markedly increased in mammosphere-forming cells derived from basal and/or metastatic cells relative to the bulk population, but neither COX-2 or EP4 levels were elevated in mammospheres derived from luminal or non-metastatic cells. Breast cancer stem cells, expressing elevated EP4 are correspondingly more sensitive to inhibition with EP4 antagonists both in vitro and in limiting-dilution assays in vivo. Somewhat to our surprise, cancer stem cells remain relatively sensitive to lysis by Natural Killer cells. We have also shown that EP4 blockade protects NK cells from tumor-induced immune suppression. These studies identify EP4 as a potential therapeutic target in the general tumor cell population and show that EP4 targeting may selectively inhibit cells with tumor-initiating or stem cell properties. EP4 antagonists can directly inhibit cancer stem cells and tumor metastasis and indirectly support NK-mediated mechanisms of tumor control. We are delineating the mechanisms by which EP4 and COX-2 are upregulated in cells with stem-like properties. In addition to EP4 and COX-2, STAT3 is upregulated in stem cells. Inhibition of STAT3 reduces mammosphere-forming capacity. Our studies support a mechanism whereby COX-2/EP4 signaling induces STAT3 to support breast cancer stem cell survival by a feed-forward mechanism.
Title: Proteosome inhibitor bortezomib inhibits NFκB and effectively overcomes cancer stem cell escape triggered by Wnt inhibitor therapy in FOXC1+ basal-Like/claudin-low breast cancer

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Body: Cancer stem cells (CSCs) are considered to be an important contributing factor towards treatment failure, cancer recurrence, and mortality. CSCs are known to be more enriched in the basal-like and claudin-low subtypes of breast cancer. The Wnt/β-catenin signaling pathway is well known as a regulator of embryonic development and stem cell biology, and is prominently active in basal-like and claudin-low breast cancers. We have previously reported that transcription factor FOXC1 plays a critical role in mediating aggressive cell traits in basal-like/claudin-low breast cancer. In this study, we sought to investigate the link between Wnt signaling and FOXC1 and its potential in regulating CSC biology in basal-Like/claudin-low breast cancer. We observed that exposure of the MDA-MB-231 basal-like/claudin-low cell line (low constitutive FOXC1 expressor) to Wnt3a (a canonical Wnt signaling ligand), resulted in increased expression of FOXC1. Reciprocally, overexpression of FOXC1 in MCF10A human mammary epithelial cells led to a pronounced increase in Wnt signaling activity, strongly suggestive of a direct or indirect positive feedback loop between Wnt signaling and FOXC1. More importantly, BT549 and HS578t basal-like/claudin-low cells (high constitutive FOXC1 expressors) proved to be more sensitive to treatment with the Wnt inhibitor ICRT3 as evidenced by decreased cell viability when compared to MCF7 (luminal) or SKBR3 (HER2) breast cancer cell lines. Furthermore the decrease in cell viability appeared to be proportionate to the level of FOXC1 expression. Upon pharmacological inhibition with ICRT3 and biological inhibition with siRNA knockdown of LRP6, (a canonical Wnt signaling cell surface receptor) a decrease in FOXC1 expression level was observed in a dose and time dependent manner. This effect was particularly pronounced in mammosphere cultures enriched for BT549 cancer stem-like cells. Inhibition of Wnt signaling reduced mammosphere formation efficiency of BT549 cells, suggesting that Wnt inhibition targets cancer stem cells (CSCs) in the basal-like/claudin-low breast cancer subtype. More importantly, however, after an initial 4 day incubation period, some cells are observed to persist and later display renewed enhancement of mammosphere formation ability. Profiling of such cells interestingly revealed depletion of FOXC1+ve cells but persistence of cells displaying pronounced up regulation of stereotypical embryonic stem cell Transcription Factors OCT4, SOX2 and NANOG, strongly suggestive of a potential primitive stem cell/quiescent cell state escape mechanism. qRT-PCR based pathway activation analysis revealed marked activation of NFκB signaling in the residual cells that withstood Wnt inhibition. Simultaneous pharmacologic inhibition with Wnt inhibitor ICRT3 and the proteasome inhibitor Bortezomib (known to inhibit NFκB signaling) effectively targeted BT549 cancer stem cells in mammosphere culture and prevented the persistence/emergence of any residual cells. Taken together, our findings suggest that combination therapy approaches are likely required to effectively target breast cancer stem cells.
Title: Expression of the pluripotency transcription factor SOX2 in primary breast cancers (BCs): Correlation with clinicopathological features (CPfs) and recurrence

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Body: BACKGROUND
SOX2 is one of the pluripotency transcription factors expressed by stem cells, which plays a central role in controlling the expression of genes implicated in embryonic development and stemness maintenance. Key regulators of embryonic stem cell (ESC) identity, such as NANOG, SOX2, OCT4 and GDF3 resulted overexpressed in a variety of solid tumors with a possible role in cancer progression and prognosis. SOX2 expression has mainly been reported in basal-like BC subtype, suggesting a role in conferring a less differentiated phenotype. In our analysis we evaluated a heterogeneous group of BC tissues to determine whether the expression of ESC-regulating genes correlated with CPfs and recurrence.

METHODS
140 primary invasive BC specimens were collected from 137 female patients who underwent surgery. mRNA expression for SOX2, OCT4, NANOG, GDF3 genes was assessed by RT-PCR. Immunohistochemistry (IHC) was performed for SOX2 with mouse monoclonal antibody (1:50, Y17, Santa Cruz Biotechnology, USA). Correlations with molecular subtypes, menopausal status, grading, ER, PR, ki67 (≤ 20% and > 20%), HER2, T-size and node status were evaluated by Fisher's exact test and χ2 test. Association of ESC-genes, CPfs and DFS was estimated by univariate and multivariate Cox-regression analysis (p ≤ 0.05). Survival analysis (DFS and OS) were calculated by Kaplan-Meier curves and compared by log-rank test.

RESULTS
In 117 samples assessable by RT-PCR the genes resulted expressed as follows: NANOG=52 (44.5%), SOX2=11 (9.4%), GDF3=9 (7.2%), OCT-4=0. Correlation of mRNA gene expression with CPfs was statistically significant between NANOG and grade 2, GDF3 and node-negative status, SOX2 and higher ki67 (p=0.019, p=0.029, p= 0.035, respectively). Six out of 11 SOX2+ tumors were HER2+ (data not statistically significant); in the remaining 5 samples the fluorescence in situ hybridization was performed but no HER2 amplification was detected. At univariate analysis of DFS, SOX2 expression (HR=2.36; p=0.002), ki67 (HR= 2.19; p=0.028), T-size ≥1 (HR=2.06; p=2.011), node-status (HR=2.21; p= 0.014), ER/PR (HR=0.58/HR=0.59, p=0.065/p=0.068) resulted statistically significant. At multivariate analysis, SOX2 (HR=2.99; 95% CI 1.41-6.30; p=0.004), node-status (HR=2.44; 95%CI 1.25-4.76; p=0.009) and T-size ≥1 (HR=1.77; 95% CI:0.99-3.13; p=0.051) were independently associated with increased risk of recurrence. An earlier recurrence was observed in SOX2+ patients (median 34.9 months; 95% CI: 7.5-62.2) than SOX2- patients (median: 60.3 months; 95% CI: 32.6-88.1) (p=0.017); OS resulted shorter in SOX2+ (68.2 months 95%CI: 63.7-151.4 vs 145.3 months 95% CI: 80.5-210.2) albeit not statistically significant (p=0.104). IHC analysis showed a positive score for SOX2 protein expression in all of 11 samples with SOX2 mRNA amplification; SOX2+ protein was not detected in 20 samples randomly selected among the tissues not expressing SOX2 mRNA.

CONCLUSIONS
Our analysis confirm that ESC-regulating genes correlate with specific CPfs (grading, node-status and ki67). Notably, SOX2 resulted to be an independent prognostic factor, as it was associated with a risk of recurrence increased by 3 times, irrespective of other CPfs.
Title: Mammospheres derived from circulating epithelial tumor cells are an indicator for presence of metastasis in patient with breast cancer

Monika Pizon\textsuperscript{1}, Dorothea Zimon\textsuperscript{1}, Ulrich Pachmann\textsuperscript{1} and Katharina Pachmann\textsuperscript{1}. \textsuperscript{1}Transfusion Center, Bayreuth, Germany.

Body: Background:
A major obstacle in the successful treatment of cancer is the occurrence of metastasis. The presence of CETCs is closely related to tumor recurrence, but the mechanisms through which CETCs promote metastasis are still unclear. The aim of this study was to determine the proliferative capacity of CETCs by analyzing the frequency of mammosphere formation with subsequent phenotypic characterization of the spheres arising in breast cancer patients.

Methods:
CETCs were cultured under condition favoring growth of mammospheres from 38 patients with breast cancer, including a subpopulation of 13 patients with metastatic disease. Cell viability, stem cell marker expression and ALDH 1 activity was evaluated by fluorescence scanning microscope (Olympus Scan®R).

Results:
Sphere formation was observed in 74\% of patients with breast cancer. Patients with distant metastasis had higher numbers of mammospheres (median 13.0 vs 1.0; \(p \leq 0.001\)) compared to patients without metastasis. In multivariate analysis, a high number of mammospheres was associated with the presence of metastasis. The mammospheres area under the ROC curve was 0.99. Six or more spheres classified metastatic disease with a sensitivity and specificity of 96\% and 100\%, respectively. These results suggest that above cut-off six in the number of mammospheres is statistically highly indicator for disease progression. Analysis of surface marker expression profile of mammospheres showed that spheres cultured from CETCs had typical phenotype of cancer stem cells with a high enzymatic activity for ALDH 1 with the ALDEFLUOR assay. There was no sphere formation in a control group with 50 healthy donors.

Conclusion
This study demonstrates that a small fraction of CETCs has proliferative activity. Identifying the CETC subset with cancer stem cells properties may provide more clinically useful prognostic information and may be a new indicator for the presence of metastases.
Title: StemScreen®, an innovative platform technology for the identification of novel cancer stem cell-directed compounds

Janice Chen¹, Hai Li², Marcie A Glicksman³, Charles Karan², Christopher Brooks¹ and Eric K Rowinsky¹. ¹Stemline Therapeutics, Inc, New York, NY; ²Columbia University Medical Center, New York, NY and ³Brigham and Women's Hospital, Cambridge, MA.

Body: Cancer stem cells (CSCs) are thought to play significant roles in breast cancer initiation and progression. Conventional therapeutic agents target tumor bulk but spare CSCs, leading to tumor recurrence and relapse. Therefore, drugs that eliminate both tumor bulk and CSCs may represent the most effective treatment strategy for breast cancer. We have developed a proprietary cell-based screen called StemScreen® that permits the rapid testing and identification of novel CSC-directed compounds in a high-throughput manner. StemScreen® takes advantage of a landmark discovery that many cancer cell lines harbor stable populations of CSCs. Our initial studies utilized a breast cancer cell line transfected with a unique expression vector that is only active in the 1-5% putative CSC population. The StemScreen® platform is unique because it allows for screening of compounds within the context of the CSCs’ natural microniche environment and has advantages over traditional drug discovery methods that have been designed to identify compounds that only target tumor bulk, but not CSCs.

In an effort to increase the throughput and make the assay compatible with existing compound libraries, we miniaturized the platform to a 384-well format and optimized the line for high-content screening. A series of compound hits identified from screening these cells against smaller libraries of known active compounds as well as larger and more diverse compound libraries will be presented. We are also currently expanding this technology into other tumor types. We believe that this approach represents a major technological advance in oncology drug discovery and that this platform will be instrumental in the discovery of unique new therapies that have a dual effect on CSCs and non-CSC tumor bulk.
Title: SIRT1 inhibitors significantly reduce cancer stem cells and block epithelial mesenchymal transformation in breast cancer cells

Songlin Zhang¹, Min Li¹, Baoxiang Guan¹ and Robert Brown¹. ¹UTHSC at Houston, Houston, TX.

Body: Breast cancer stem cells are contribute to distance metastasis, breast cancer recurrence and drug resistance. SIRT1, a nicotinamide adenine dinucleotide (NAD⁺)-dependent histone deacetylase, play significant role in DNA repair, cell survival, stem cells and epigenetic modification. SIRT1 has been related to cancer stem cells in chronic myelogenous leukemia. In this study, we investigate the role of SIRT1 in breast cancer stem cells.

SIRT1 inhibitors cambinol and Ex527 are used for treatment in several breast cancer cell lines, including T47D, TB549, MDA-MB-231 and MDA-MB-468. Stem cell markers, SOX-2 and Nanog, are significantly decreased in SIRT1 inhibitor treated cancer cells by qRT-PCR and western blot in T47D cell line. The ALDH positive cells in MDA-MB-231 cell line are significant reduced from 29% to 3.2% with Adeflour assay, and the CD44 expression is significantly reduced in CD44/CD24 flow cytometry analysis. Using qRT-PCR, SIRT1 inhibitors significantly down regulate vimentin (-3.7 folds), N-cadherin and smooth muscle actin (-2.7 folds) and up regulate the gene of E-cadherin, indicating SIRT1 inhibitors can block the epithelial mesenchymal transformation (EMT) of breast cancer. SIRT1 inhibitors can significantly (40-50%) block the cancer cell invasion and migration in several triple negative breast cancer cell lines. SIRT1 inhibitors can inhibit cell proliferation on all tested breast cancer cell lines, and can induce significant cell apoptosis in T47D cells. In xenograft tumor study, SIRT1 inhibitor cambinol can significantly reduce tumor growth and inhibit tumor metastasis.

The molecular mechanism of SIRT1 inhibitors in blocking EMT and reducing cancer stem cells is likely associated with blocking the Wnt pathway. Several down stream target genes of Wnt pathway, such as cyclin D1, c-Myc and c-Jun, are significantly down regulated after using SIRT1 inhibitor cambinol, and the changes are more significant in TGF-beta stimulated cancer cells. Beta-catenin is significantly reduced including the active beta-catenin, and the decreasing beta-catenin protein may be related to the decreased Dvl proteins.

In summary, our study demonstrated that SIRT1 inhibitors can reduce breast cancer stem cell population in several cancer cell lines, block epithelial mesenchymal transformation, and inhibit breast cancer cell invasion and metastasis. SIRT1 inhibitors appear to inhibit Wnt pathway in cancer cells to block EMT and affect cancer stem cells.
Title: Inflammation promotes stem-like characteristics and increases stemness of breast epithelial cells

Jennifer Sims-Mourtada¹,², Kimberly M Arnold¹,², Lynn Opdenaker¹,³ and Daniel Flynn¹,². ¹Center for Translational Cancer Research, Helen F. Graham Cancer Center, Christiana Care Health Services, Inc, Newark, DE; ²University of Delaware, Newark, DE and ³University of Delaware, Newark, DE.

Body: Triple negative breast cancer (TNBC) accounts for approximately 15% of all breast cancers and is more likely to affect younger women, women of African American descent, and BRCA1 mutation carriers. TNBC is typically an aggressive cancer and is defined as the absence of positive staining for estrogen (ER) and progesterone (PR) receptor, and a lack of amplification of HER2. Due to its receptor status, TNBC is insensitive to the conventional targeted treatment utilized in ER/PR/HER2 positive breast cancer leading to poor prognosis and early visceral metastasis with survival rates for women who relapse within 5 years of treatment being significantly lower than hormone receptor positive breast cancer. Therefore, understanding the mechanisms that drive the formation of TNBC can aid in determining targets to develop better treatment options. We investigated differences in the tumor microenvironment between TNBC and ER positive breast cancer patient tissue samples. Hematoxylin & eosin stain of invasive ductal carcinomas revealed that TNBC patients had higher amounts of tumor infiltrating leukocytes compared to ER positive breast cancers. In premalignant breast cancer patients, ER negative ductal carcinoma in situ (DCIS) patients had significantly higher infiltrating leukocytes compared to ER positive DCIS indicating that inflammation may be a contributing factor in TNBC. Research on molecular profiling of TNBC has revealed that a subset of gene expression patterns are associated with the basal-myoeptelial cells rather than the luminal cells, however, previous evidence indicates that TNBC arises from luminal progenitors which undergo an abnormal conversion to basal-like progenitors with enhanced growth and survival promoting characteristics. We propose this conversion occurs due to the presence of inflammatory signals early in tumor development which trigger a cascade of events that leads to acquisition of a basal-like phenotype in luminal progenitor cells and upregulation of stem-like properties, resulting in the formation of basal-like breast cancer/TNBC. Treatment of non-malignant breast epithelial cells with IL-6 increased sphere-forming efficiency under stem cell growth promoting conditions and an inhibitor of the IL-6/STAT3 pathway decreased ALDH enzymatic activity as measured by Aldefluor assay. Also, treatment with IL-6 downregulated the luminal marker, epithelial specific antigen (ESA), and increased the expression of the myoepithelial marker, CD49f, on the cell surface of non-malignant breast epithelial cells, indicating an increase in the basal-like cell population and a loss of the luminal cell population. Therefore, inflammation appears to be a common feature in TNBC compared to ER positive breast cancer and the presence of an inflammatory stimulus in the breast may promote the conversion of luminal to basal-like cells, promoting the upregulation of stem-like properties and development of TNBC. These data suggest that anti-inflammatory drugs could potentially be used to inhibit these events from occurring in the development of TNBC, leading to decreased tumor growth and better treatment options.
Title: The expression of aldehyde dehydrogenase1 in cytoplasm or stroma of breast cancer is associated with clinical prognosis

Xin Guan¹, Yi Dong², Aiping Shi¹ and Zhimin Fan¹. ¹Breast Surgery, Bethune First Hospital of Jilin University, Changchun, Jilin, China and ²Breast Surgery, Jilin Tumor Hospital, Changchun, Jilin, China.

Body: Background: Previous studies have shown that ALDH1 expression in breast tumor cells was associated with poor prognosis. Whether tumor microenvironment affect prognosis of breast cancer patients is unknown by far.

Aim: We performed a research to study whether ALDH1 expression in cytoplasm or stroma of breast tumors is associated with prognosis of breast cancer patients.

Methods: As many as 160 cases with invasive carcinoma were retrieved. ALDH1 staining was respectively assessed on cytoplasmic and stroma of tumour cells. Chi-square test was used to evaluate the associations between ALDH1 expression and clinical parameters. Cox proportional hazards analysis was used for relapse-free survival(RFS).

Results: There was significant association between ALDH1 expression in stroma and Age, ER.

Association between ALDH1 expression status and clinicopathological parameters

<table>
<thead>
<tr>
<th></th>
<th>ALDH1 expression in cytoplasm</th>
<th>ALDH1 expression in stroma</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N Positive (%)</td>
<td>Negative (%)</td>
</tr>
<tr>
<td>ALL CASES</td>
<td>56 (35)</td>
<td>104 (65)</td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤50</td>
<td>94 (47.8)</td>
<td>42 (63.6)</td>
</tr>
<tr>
<td>&gt;50</td>
<td>66 (44.9)</td>
<td>42 (63.6)</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2</td>
<td>54 (30.4)</td>
<td>37 (69.6)</td>
</tr>
<tr>
<td>2-5</td>
<td>100 (50)</td>
<td>62 (52.0)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>6 (16.7)</td>
<td>5 (83.3)</td>
</tr>
<tr>
<td>Number of metastatic lymph nodes</td>
<td>0.156</td>
<td>0.156</td>
</tr>
<tr>
<td>≤3</td>
<td>80 (30.4)</td>
<td>53 (69.6)</td>
</tr>
<tr>
<td>4-9</td>
<td>57 (31.6)</td>
<td>39 (68.4)</td>
</tr>
<tr>
<td>&gt;9</td>
<td>23 (47.8)</td>
<td>12 (52.2)</td>
</tr>
<tr>
<td>ER in primary breast tumor</td>
<td>0.591</td>
<td>0.591</td>
</tr>
<tr>
<td>Positive</td>
<td>109 (34.1)</td>
<td>75 (65.9)</td>
</tr>
<tr>
<td>Negative</td>
<td>51 (43.1)</td>
<td>29 (56.9)</td>
</tr>
<tr>
<td>PR in primary breast tumor</td>
<td>0.460</td>
<td>0.460</td>
</tr>
<tr>
<td>Positive</td>
<td>112 (36.6)</td>
<td>71 (63.4)</td>
</tr>
<tr>
<td>Negative</td>
<td>48 (31.3)</td>
<td>33 (68.7)</td>
</tr>
<tr>
<td>HER-2 in primary breast tumor</td>
<td>0.460</td>
<td>0.460</td>
</tr>
<tr>
<td>Positive</td>
<td>43 (39.5)</td>
<td>26 (60.5)</td>
</tr>
<tr>
<td>Negative</td>
<td>117 (39.3)</td>
<td>78 (60.7)</td>
</tr>
</tbody>
</table>
Significance level of p<0.05 was considered to be statistically significant.

In multivariate analyses of RFS, ALDH1 expression in cytoplasm is associated with a worse prognosis. However, ALDH1 expression in stroma is not related with prognosis.

Univariate and multivariate analyses of RFS

<table>
<thead>
<tr>
<th>Characters</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Age ≤50/&gt;50</td>
<td>0.812</td>
<td>0.436-1.515</td>
</tr>
<tr>
<td>Tumor size ≤2/&gt;2</td>
<td>2.473</td>
<td>1.144-5.343</td>
</tr>
<tr>
<td>Number of metastatic lymph nodes ≤3/&gt;3</td>
<td>6.346</td>
<td>2.815-14.306</td>
</tr>
<tr>
<td>ER in primary breast tumors -/+</td>
<td>0.660</td>
<td>0.354-1.231</td>
</tr>
<tr>
<td>PgR in primary breast tumors -/+</td>
<td>0.533</td>
<td>0.288-0.988</td>
</tr>
<tr>
<td>HER-2 in primary breast tumors -/+</td>
<td>0.969</td>
<td>0.487-1.928</td>
</tr>
<tr>
<td>ALDH1 in primary breast tumors -/+</td>
<td>2.578</td>
<td>1.403-4.737</td>
</tr>
<tr>
<td>ALDH1 in primary breast stroma -/+</td>
<td>0.712</td>
<td>0.356-1.473</td>
</tr>
</tbody>
</table>

Significance level of p<0.05 was considered to be statistically significant.

Conclusions: We conclude that ALDH1 expression in stroma might suggest the presence of Cancer stem cells in tumor microenvironment. The ALDH1 expression in cytoplasmic of breast tumors rather than in stroma was an independent factor for prognosis.

Key words: Breast cancer stem cell; ALDH1; Tumor microenvironment.
Title: Maintenance of stemness of breast cancer stem-like cells by FRS2beta, a feedback inhibitor for HER, during mammary tumorigenesis

Noriko Gotoh1,2. 1Cancer Research Institute, Kanazawa University, Kanazawa City, Ishikawa, Japan and 2Institute of Medical Science, University of Tokyo, Minato-ku, Tokyo, Japan.

Body: It is important to understand the molecular mechanisms how cancer stem cells (CSC)s are maintained. We examined how HER/ErbB signaling activity is regulated in breast CSCs. We generated knockout mice of FRS2beta adaptor protein, a feedback inhibitor for HER, and crossed them with MMTV-ErbB2 mice in which overexpression of ErbB2 induces breast cancer. Tumor growth in the breast tissues of wild-type mice was much faster than those of the FRS2beta mutant mice. Sphere forming activity of tumor cells was reduced in the FRS2beta mutant mice, suggesting that FRS2beta is important for maintenance of breast CSCs. Moreover, sphere forming activity of normal mammary cells were reduced in FRS2beta mutant mice. Finally, FRS2beta is strongly expressed in CSC-enriched population in human samples. It thus appears that FRS2beta is expressed in luminal progenitor cells that are transformed into breast CSCs. Fine-tuning of HER signaling by FRS2beta may play important roles for maintenance of CSCs. Further molecular details how FRS2beta contributes to mammary tumorigenesis including maintenance of breast cancer stem-like cells will be presented.
Clinical relevance and biological properties of oligometastatic breast cancer in lung; prognostic impact of CD44+/CD24−/low cells

Rei Mimoto1, Tadashi Kobayashi1, Yoshimi Imawari1, Makiko Kamio1, Kumiko Kato1, Hiroko Nogi1, Yasuo Toriumi1, Ken Uchida1 and Hiroshi Takeyama1. 1Jikei University School of Medicine, Tokyo, Japan.

Body: Background: Metastatic breast cancer is a systemic disease. Our aim was to evaluate the clinical outcomes of pulmonary metastasectomy of recurrent breast tumors and to identify possible prognostic factors.

Methods: We reviewed data from a registry of patients with lung metastases from breast tumors who received pulmonary metastasectomy in Jikei University Hospital between 2004 and 2011. We analyzed prognostic factors for overall survival (OS) and progression free survival (PFS) after metastasectomy. We also investigated lung metastases for the prevalence of CD44+/CD24−/low tumor cells and evaluated their prognostic significance.

Results: Among 17 patients with lung metastasis of breast tumors, 5-year OS and PFS were 72% and 36%, respectively. Better OS was observed among patients with oligometastatic breast cancer (OMBC). Patients with OMBC, estrogen receptor (ER) positive cells, and disease free intervals (DFI) of >8 years had better PFS. The average prevalence of CD44+/CD24−/low tumor cells in lung metastases of breast cancer was 21%, ranging from 0 to 90%. The presence of CD44+/CD24−/low tumor cells influenced the progression after lung metastasectomy, with median PFS times of only 6 months in patients with high-prevalence of cancer-initiating cells. CD44+/CD24−/low cells with cancer-initiating properties were present in only 9% ± 12 of patients with OMBC but were found in 73% ± 21 of patients with non-OMBC.

Conclusion: Pulmonary metastasectomy may be a treatment option for OMBC patients with lung metastases. Better prognosis of OMBC may be related to low levels of cancer-initiating cells.
Title: Post transcriptionally regulating LMO2, a highly expressed gene in breast cancer stem cells, to study its invasive and metastatic properties in breast cancer

Lazaro J Mesa. Stanford University, Stanford, CA.

Body: Aggressive breast cancer intrinsically possesses many epithelial-mesenchymal transition (EMT) characteristics and cancer stem cell (CSC)-like features, suggesting that activation of EMT mechanisms generate a higher degree of invasiveness in cells with CSC-like properties. The aim of this study is to test whether LIM domain only 2 (LMO2) is implicated in the degree of invasiveness of CSC-like cells. LMO2 is an important regulator of hematopoietic stem cells and sustained expression in T-cells leads to leukemia. We found that LMO2 is expressed in mammary stem cells and in breast cancer stem cells (BCSCs). To test the functional role of LMO2 in cancerous human cell lines we designed lentiviral shRNAs to knockdown the expression of LMO2 through posttranscriptional gene silencing, a form of RNAi. shRNA sequences were verified and then used to infect breast cancer cell lines. The cells were sorted based on expression of GFP and RNA was isolated from these cells. To quantitatively demonstrate a knockdown of LMO2 we performed Real Time-PCR. We were able to generate as much as a 61 percent knockdown in our infected cells relative to those with no RNA interference. These shRNA constructs will then be used to infect patient derived xenografts to test the in vivo effects of LMO2 knockdown in breast cancer stem cells; specifically, their role in the development and propagation of the primary tumor. Further understanding how LMO2 is implicated in BCSCs will lead to development of fundamental data and knowledge as to how BCSCs develops; potentially translating to clinical treatments such as gene therapy for breast cancer patients.
Title: The impact of ALDH1 on chemo-resistance and prognosis according to intrinsic subtype in breast cancers

Kumiko Kida¹, Takashi Ishikawa², Akimitsu Yamada¹, Kazutaka Narui², Sadataka Sugae¹, Mikiko Tanabe², Yasushi Ichikawa¹ and Itaru Endo¹. ¹Yokohama City University Graduate School of Medicine, Yokohama, Kanagawa, Japan and ²Yokohama City University Medical Center, Yokohama, Kanagawa, Japan.

Body: [Background]Aldehyde dehydrogenase 1 (ALDH1) has been identified as a breast cancer stem cell marker. The clinical significance of ALDH1 as a chemo-resistant and prognostic indicator has been reported recently. However, the analysis according to each intrinsic subtype was not reported.

[Aims] To investigate the impact of ALDH1 on chemo-resistance and prognosis according to intrinsic subtypes in invasive breast cancers.

[Methods]
1) Patients and tumor specimens; A total of 653 primary breast cancer patients were enrolled in this study from 2004 to 2013 at the Yokohama City University Medical Center in Japan. We performed immunohistochemical analyses using paraffin-embedded core needle biopsy sections prior to the treatment.

2) Correlation of ALDH1 with clinicopathological factors;
Analyses were performed to investigate association of ALDH1 expression with other biomarkers and clinicopathological factors in breast cancers. Age, histologic type, tumor size, nodal status, ER/PgR/HER2 status, nuclear grade, Ki67, Topo2, p53, CK5/6 and EGFR were observed.

3) Neoadjuvant patient cohort study
234 breast cancer patients receiving neoadjuvant chemotherapy were enrolled. The correlation between ALDH1 and pathological complete response (pCR) rate was investigated in each intrinsic subtype.

4) Prognostic cohort study
We performed a Cox analysis of disease free survival and overall survival of all 653 cases according to each subtype, taking account of clinicopathological factors.

[Results]
ALDH1 expression in tumor cells was seen in 139 of 653 cases (21.3%). The ALDH1 expression correlated significantly with tumor size, clinical node metastasis, clinical staging, nuclear grade and HER2 status positively, ER and PgR status negatively. ALDH1 expression was significantly seen in HER2-positive cancers and triple negative type.

In neoadjuvant study, we analyzed 234 patients treated with neoadjuvant chemotherapy including 63 luminal type, 20 luminal-HER2 type, 45 HER2-enriched type and 106 triple negative type. The pCR rate was significantly lower in patients with ALDH1-positive cases (13.5% vs. 30.3%, p=0.003). In multivariate analysis, ALDH1 and ER are correlated with pCR rate significantly. According to the intrinsic subtypes, the correlation between pCR and ALDH1 expression was extremely significant in triple negative type (p=0.003). In HER2 positive type, ALDH1 expression had tendency with low pCR, but with no significance. In luminal type, two patients achieved pCR and both had no ALDH1 expression.

In prognostic analysis, patients with ALDH1 expression had significantly poor disease free survival (DFS; p<0.001) and overall survival (OS; p=0.044). In the multivariate Cox regression model, ALDH1 expression was an independent prognostic indicator of DFS (p=0.033), but not significant predictor of OS (p=0.124). According to each intrinsic subtypes, ALDH1 had a higher impact on prognosis of luminal type, though not significant on triple negative type.

[Conclusions]
Breast cancers with ALDH1 expression posse biologically aggressive phenotypes that tend to have a poor prognosis. Chemoresistance was significantly seen in ALDH1-positive triple negative type, on the other hand, impact on prognosis was seen in luminal type more highly than triple negative type.
**Title:** EMT activates PERK-eIF2α signaling and sensitizes cells to perturbations in endoplasmic reticulum function

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**Body:** Epithelial-to-mesenchymal transition (EMT) plays an important role in cancer progression. By undergoing an EMT, cancer cells acquire a spectrum of malignant properties, including invasiveness, multi-drug resistance and stem-like traits. Although they play an important role in tumor progression and resistance, few vulnerabilities of EMT cancer cells have been reported to date. To identify specific vulnerabilities of EMT cells, using small molecule and RNAi screens, we have discovered that induction of EMT greatly sensitizes cells to agents that perturb endoplasmic reticulum (ER) function. This unexpected sensitivity to ER stress is mainly due to the expression and secretion of large amount of extracellular matrix (ECM) proteins by cells that have undergone an EMT. In line with their increased secretory load, EMT cells display a branched ER morphology and constitutively activate the PERK-eIF2α branch of the unfolded protein response (UPR). Using a PERK-specific inhibitor, we found that PERK activation is also required for EMT cells to invade and metastasize. In human tumor tissues, EMT gene expression correlates strongly with both ECM and PERK-eIF2α genes. In summary, our findings identify a novel vulnerability of cells that have undergone an EMT, and demonstrate that the PERK branch of the UPR is required for their malignancy.
Title: IGF1R/FAK crosstalk is essential for epithelial-to-mesenchymal transition (EMT), migration, and invasion of TNBC cells

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Body: Triple negative breast cancers (TNBCs) are highly metastatic and deadly tumors that currently lack effective prevention and treatment options. Insulin-like growth factor 1 receptor (IGF1R) and focal adhesion kinase (FAK) both play a role in several developmental processes, and both IGF1R and FAK signaling pathways have been linked to a number of common pathological diseases, including breast cancers. In particular, overexpression of IGF1R and FAK are closely associated with metastatic breast tumors. However, the relationship between the IGF1R and FAK signaling cascades in metastatic TNBCs remain largely unknown. The present study aimed to investigate the interrelationship between IGF1R and FAK crosstalk in regulating the malignant properties of TNBC cells. Using stable small hairpin RNA (shRNA)-mediated IGF1R silencing and stable full-length IGF1R plasmid over-expression methods, we demonstrated that IGF1R was essential for maintaining mesenchymal morphologies of TNBC and regulating the expression of EMT markers, including vimentin, ZEB-1, Snail-1, E-cadherin, ZO-1, and claudin-1. Using colony formation, spheroid migration, and Matrigel invasion assays, we further showed that IGF1R promoted migratory and invasive TNBC phenotypes, including increased colony formation, cell migration, and invasion. Most importantly, IGF1R-mediated migration and invasion required FAK activation and could be augmented using pharmacological inhibitors of FAK. Our findings in TNBC cells demonstrate a novel role of the IGF1R/FAK signaling pathway in regulating critical processes involved in the metastatic cascade. These results may improve the current understanding of the basic molecular mechanisms of TNBC metastasis and provide a strong rationale for co-targeting of IGF1R and FAK as therapy for mesenchymal TNBCs.
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Title: Pax-5 regulates EMT and MET in breast cancer through FAK1 regulation

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Body: Metastasis accounts for 90% of deaths in breast cancers patients. Therefore, the study of genetic factors regulating cancer malignancy is a top priority to mitigate the morbidity and mortality associated to this disease. One of these factors, Pax-5, normally regulates key biological functions such as cell viability, growth, and differentiation. However, an aberrant expression of this factor results in the development and progression of cancer.

In this study, we developed breast cancer cell models with conditioned expression of the Pax-5 to evaluate signaling pathways relevant to breast metastasis and cancer progression. We found that Pax-5 extinguishes several aspects of cancer aggressivity such as: proliferation, spheroid formation, migration and invasion. At the molecular level, we found that Pax-5 modulates cancer malignancy through the regulation of various components of the epithelial to mesenchymal transitioning (EMT) process in addition to key signaling targets such as: NFκB and the Focal Adhesion Kinase 1 (FAK1). We also demonstrate that Pax-5 decreases FAK1 level trough up-regulation of miR-135b, a direct repressor of FAK1 expression. Altogether, our findings suggest that the presence of the Pax-5 lead to less aggressive breast cancers by promoting mesenchymal to Epithelial transitioning (MET). These findings bring light to molecular mechanisms driving breast cancer malignancy and benefit our quest in the development of diagnostic and therapeutic strategies against breast cancer progression.
Title: Treatment of metastatic breast cancer using two nanoparticles combined with siRNA targeting Twist1 to inhibit EMT

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Body: Breast cancer is the 2nd leading cause of cancer related deaths among women in the US with over 240,000 diagnoses and 40,000 deaths expected in 2014. Among the more serious and deadly forms of breast cancer are the Triple Negative Breast Cancers (TNBC) (ER-, PR-, HER2-). Mortality rates among patients rise dramatically when these cancers spread beyond the primary tumor site. Therefore reduction of tumor cell dispersion is a key component to minimizing mortality rates. Epithelial-Mesenchymal Transition (EMT) is the process by which cancer cells downregulate proteins associated with cell to cell adhesion (e.g. E-cadherin) resulting in cells that are able to detach from neighboring cancer cells, invade adjacent tissue, and eventually enter the circulatory system or lymphatics. The process of EMT is tightly regulated by the transcription factor Twist1, which is often overexpressed in breast cancer. Therefore, Twist1 serves as an excellent therapeutic target whose downregulation would result in fewer metastatic cancer cells and correspondingly reduce cancer mortality.

Our lab has designed and tested (in vitro) two siRNA based therapeutics that target Twist1 in a TNBC cell line (SUM 1315). These siRNA molecules have been strategically designed and modified to make them resistant to degradation, enhance silencing effects and diminish their immunogenicity. To overcome the inherent problems with delivery of siRNA (both in vitro and in vivo) we have been testing two nanoparticle delivery systems. Recent advances in nanotechnology have led to the development of a variety of nanoparticles that provide valuable tools for cancer therapy. We are testing two different types of nanoparticles in this study: The first is a Polyamidoamine (PAMAM) dendrimer (YTZ3-15) which is a truncated 3rd generation dendrimer which has been modified with a lipophilic tail to enhance cellular uptake. The second is a mesoporous silica nanoparticle (MSNs) which is able to not only deliver siRNA, but is also contains pores which allow simultaneously delivering chemotherapies such as doxorubicin. This novel siRNA gene silencing and nanotechnology-based therapy should allow us to exert more precise temporal control during breast cancer treatment.

When complexed with either nanoparticle we have found significant Twist1 knock down in vitro as well as reduction of Twist1 target genes that are associated with EMT. We have also shown that Twist1 silencing reduces migration and invasion of SUM 1315 breast cancer cells in vitro Recently we began testing these siRNA+Nanoparticle complexes in an orthotopic murine model using Firefly Luciferase expressing SUM 1315 cells in immunocompromised mice (NSG). The results of this in vivo research have shown that there is significant uptake of the siRNA+Nanoparticle complexes in the tumors when compared to other organs. Results of these studies are significant because TNBC is particularly drug resistant and metastatic; and superior therapies are urgently needed to effectively treat patients with these breast cancers. This approach paves the way for TNBC treatment that incorporates Twist1 knock down (resulting in renewed chemosensitivity) and simultaneous delivery of a chemotherapy reagent.
Title: A potential role for Janus protein tyrosine kinases in the regulation of epithelial-mesenchymal transition in a model of epidermal growth factor induced breast cancer epithelial-mesenchymal transition

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Body: Background. Epithelial-mesenchymal transition (EMT), a process whereby tumorigenic epithelial cells acquire an invasive and migratory phenotype, is an important event in the invasion-metastasis cascade. As such, intracellular signaling pathways involved in the regulation of EMT represent potential therapeutic targets in the treatment and prevention of invasive cancer subtypes. The calcium ion, a highly versatile intracellular messenger, plays an important role in processes important in tumorigenesis including invasion and metastasis, and altered calcium signaling has been identified in various cancers. We recently identified that activation of signal transducer and activator of transcription 3 (STAT3) and expression of specific EMT markers in the MDA-MB-468 cell line model of epidermal growth factor (EGF) induced EMT display some calcium dependence. While the calcium permeable ion channel TRPM7 was shown to partially regulate this STAT3 activation and vimentin expression, the precise mechanisms of their regulation are not yet fully understood. The aim of this research was to investigate the upstream intracellular signaling pathway involved in EGF stimulated STAT3 activation and the subsequent induction of EMT in this model.

Methods. MDA-MB-468 basal-like breast cancer cells were pre-treated for 1 hr with the Janus protein tyrosine kinase (JAK) inhibitor, JAK inhibitor I (1 and 10 µM), or the Src family tyrosine kinase inhibitor, PP2 (0.1, 1 and 10 µM), followed by stimulation with EGF (50 ng/mL) for 10 or 20 min, and 24 h to assess effects on STAT3 activation and/or EMT marker expression, respectively. Total cellular protein was isolated following inhibitor treatment ± EGF stimulation, and the level of phosphorylated STAT3 (10 or 20 min) or vimentin protein expression (24 h), was analyzed using Western blotting. Cellular RNA was isolated following inhibitor treatment ± EGF stimulation and levels of vimentin mRNA (24 h) were assessed using real time RT-PCR.

Results. Treatment of MDA-MB-468 breast cancer cells with JAK inhibitor I resulted in a significant decrease in EGF stimulated STAT3 phosphorylation, while inhibition of Src family tyrosine kinases with PP2 also significantly decreased EGF stimulated STAT3 phosphorylation. In addition to its effects on STAT3 phosphorylation, pre-treatment of MDA-MB-468 cells with JAK inhibitor I also appeared to decrease EGF-induced vimentin protein and mRNA expression, indicating a potential role for Janus protein tyrosine kinases in the induction of EMT in this model.

Conclusions. Janus protein tyrosine kinase signaling appears to play a role in the regulation of STAT3 activation, and the induction of the EMT marker vimentin in the MDA-MB-468 cell line model of EGF-induced EMT. Future studies will focus on investigating the specific JAK family member(s) involved in the EGF-STAT3 signaling pathway, as well as the nexus between calcium and identified regulators of EGF stimulated STAT3 activation, and EMT marker expression, in this model of breast cancer EMT.
Title: Niclosamid overcomes epithelial-mesenchymal transition of lapatinib resistance through inhibiting Src activation in HER2 positive breast cancer

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Body: Background: The Src pathway is known to regulate tumor metastasis and plays a role in epithelial-mesenchymal transition (EMT). Lapatinib, although, has improved clinical outcome for HER2 positive breast cancer patients, acquired resistance to lapatinib remains an important reason influencing its clinical efficacy. Our previous research shows lapatinib acquired resistance HER2 positive breast cancer cell lines, SKBR3-R and BT474-R have EMT phenomenon and multiple pathway activated. Niclosamide is an FDA-approved antihelminthic agent, which is found has cytotoxicity effect on tumor stem cells recently. We wished to determine the effect of niclosamide on EMT and lapatinib resistance cells and investigate niclosamide as a potential therapeutic agent for HER2 positive breast cancer.

Methods: SKBR3 and BT474, two HER2 positive breast cancer cell lines, were continuously exposed to increasing doses of lapatinib to establish two stable cell lines resistant to lapatinib, SKBR3-R and BT474-R. Cell proliferation was determined by CCK8 assay. Protein expression was determined by western blotting. Invasion ability was analyzed by transwell assay. FITC staining flow cytometry (FCM) was conducted to observe the percentage of apoptosis.

Results: Both two HER2 positive lapatinib resistant cell lines, SKBR3-R and BT474-R had EMT phenomenon. Niclosamide had a stronger inhibition effect on lapatinib resistant cell lines than parental cell lines, and induced more apoptosis by FCM. Western-blot showed niclosamide could reverse the EMT phenomenon of SKBR3-R and BT474-R with E-cadherin up-regulated and snail, vimentin down-regulated at the concentration of 0.5-1µM. A significant reduction of Src signaling was also confirmed, as well as the downstream Akt and Erk pathway. After adding niclosimide for 48 hours, SKBR3-R and BT474-R’s capability of invasion were inhibited.

Conclusion: Our results suggested that niclosamide had a strong cytotoxic effect on HER2 positive lapatinib resistant breast cancer cell lines. The EMT induced by lapatinib resistance could be reversed by niclosamide, associated with the inhibition of Src pathway activation, as well as the downstream pathways. Niclosamide treatment produced reduced levels of invasion, serving as a novel therapeutic way for lapatinib-resistant breast cancer patients.
Title: Prostaglandin E receptor 2 (EP2) regulates breast cancer stem-cell like property and promotes epithelial-mesenchymal transition

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Body: Background: Presence of cancer stem-like cells (CSCs) is the main obstacle for poor treatment response and mortality in breast cancer patients. Prostaglandin E receptors have been reported to play a role in epithelial-mesenchymal transition (EMT) and metastasis, however, the contribution on cancer stem cell compartment remains unstudied.

Methods: Human xenograft breast cancer model was used to study the expression of EP receptors during cancer development. Construction of stable EP2-expression cells was used to study tumorigenesis and characterization of EP2 receptor. Functional role of EP2 receptor on cell proliferation, flow cyometry, invasion and EMT gene expression array were performed in transfected cells. Expression of EP2 receptor was compared in primary tumor tissues by immunostaining and real-time PCR.

Results: EP2 receptor was predominantly expressed in animal tissues during cancer development, as well as in human primary tumor tissues. In mouse xenograft model, MB-231-EP2 clone developed a more aggressive tumor with a larger tumor size and showed a significant increase in cancer stem cell marker aldehyde hydrogenase (ALDH1) expression. In vitro study, MB-231-EP2 clone increased colony formation capacity and S-phase entry by the regulation of E-cadherin, TWIST1 and ALDH1. Importantly, we found that Twist1 expression level was higher in breast cancer patients than healthy controls and was associated with ALDH1 expression.

Conclusions: These findings implicated that EP2 receptor was crucial to nurture CSC phenotype and promote tumorigenesis in breast cancer. Blocking of EP2 might be a potential therapeutic strategy to improve treatment response for breast cancer patients.
Interaction of mesenchymal stem cell with breast cancer lineage cells and evaluation of their biological behavior

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Body: Breast carcinoma is a highly prevalent and incident disease. The interaction between tumor stroma and malignant epithelial cells has been reported as a mechanism of resistance to cytotoxic treatment. Mesenchymal cells originating from the tumor stroma create a favorable microenvironment for cancer stem cells (CTTs) maintenance. CTTs are able to repopulate the host with tumor cells of the same origin on distant sites. It is postulated that this interaction modulates the ability of tumor to invade and disseminate. The aim of this study is to analyze the association MCF-7 cell line with human mesenchymal stem cells (MSC), and evaluate the biological behavior and phenotypic changes. MSC cells derived from Wharton's jelly were co-cultured with MCF-7. Cell migration assay: MSC cells were seeded into 12 well plates and co-cultured with MCF-7 cells in the proportions 1% MSC + 99% MCF-7, 10% MSC + 90% MCF-7, 30% MSC + 70% MCF-7 and 100% MCF-7 in 5x10⁴ total cells. After 5 days of culture, two lines cross were traced on the bottom of the well. An inverted microscope with 10X objective was used to photograph at time 0 hours, 24 hours, 48 hours and 120 hours until confluence. We analyzed the phenotypic changes and mean migration time of cell culture and co-culture. The confluence of the cell layer of the area of migration occurred after 120 hours only in co-culture of MSC 30% and 70% MCF-7 (163.66Åμm to 0 μm). Flow cytometry: MSC cells were seeded into 6 well plates and co-cultured with MCF-7 cells in the same proportions as described above in 1x10⁵ total cells, and maintained at 37° C with 5% CO² for 5 days. Were quantified cells with CD44+/CD24- profile with anti-CD44 antibody (APC) and anti-CD24 (PE). The ANOVA with Tukey's post-test were used for statistical analysis. Data were presented as mean ± standard deviation, p< 0.05. There was no significant difference in CD44+/CD24- in the comparison groups: MSC vs. cell co-cultures; cell co-cultures vs. cell co-cultures; MCF-7 vs. 1% MSC+ 99% MCF-7; MCF-7 vs. 10% MSC + 90% MCF-7. We observed a significant increase in CD44+/CD24- population comparing MCF-7 vs. co-culture of 30% MSC + 70% MCF-7 (95% CI of diff, -55.56 to -27.71). Conclusions: The co-culture with MSCs an MCF-7 is associated with an increase in cell migration and, the is a increase in CD44+/CD24- cells. These findings suggest that the interaction of mesenchymal stem cells with MCF-7 may be is able to acquire potential metastatic profile.
Autoimmunity in breast carcinogenesis. A new paradigm

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Body: The B cell response in breast cancer [BC] may have a tumor-promoting effect. The objective of this work was to demonstrate that TAAs and cytokines inflict autoimmune damage to the breast creating a chronic inflammatory milieu that promotes BC progression

Methods: The DCIS.com nude mouse xenograft model of BC was treated with rituximab and dexamethasone. Immunoscreening of a cDNA library, PCR, and sequence determination. Immunohistochemistry [IHC] for inflammatory cytokines, immunoglobulins and complement and the TUNEL assay.

Results: Deposition of immunoglobulins and complement was shown on breast epithelial and stromal cells and the involvement of IL-4, IL-6, IL-17, TNF-alpha and NFkB was demonstrated by IHC staining. We identified autoantibodies recognizing multiple human breast epithelial and stromal antigens and the Fc and V regions of immunoglobulins. Rituximab and dexamethasone treatment markedly modified the macroscopic and microscopic appearance of BC and the pattern of cytokine deposition, induced increase in vascularity, marked stromal hypertrophy and massive debulking of the tumor by drastically reducing the tumor mass through apoptosis.

Conclusions: These results suggest that autoantibody- and cytokine-mediated autoimmune damage, triggered by TAAs creates a chronic inflammatory milieu with generation of pro-inflammatory and tumor promoting signals supporting BC progression. The dramatic results of the treatment of the xenograft model with rituximab and dexamethasone illustrate the potential of treating BC as a chronic rheumatic autoimmune disease. The demonstration of a major role of autoimmunity in BC progression may have a transformative impact on prevention, diagnosis and treatment of BC.
Title: Autoimmunity to centrosomal and proteasome proteins in breast cancer. A link to chromosome instability?

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Body: BACKGROUND. Perturbation of centrosomal or centrosome-associated proteins has been observed in nearly all human solid tumors and has been implicated in the origin of chromosomal instability. Ubiquitin-proteasome degradation is highly dependent on the organizing capabilities of the centrosomes. Here we report that autoantibodies in breast cancer [BC] sera target centrosomal proteins as well as important proteins involved in proteasome protein degradation.

METHODS. We immunoscreened a T7 cDNA library of BC proteins and the association of the cloned autoantigens with BC was studied by autoantigen microarray analysis. We used immunohistochemistry [IHC] to investigate the expression of the centrosome and centrosome-associated autoantigens identified.

RESULTS. Immunoscreening with BC sera led to the identification of autoantibodies recognizing epitopes developing in a family of proteins located on the centrosomes such as NIMA-related kinase 7, dynein heavy chain domain 3, peri-centriolar material-1, isomorph CRA, and stathmin-1, the ubiquitin-conjugating enzyme E2, the proteasome 26S subunit and the SUMO/sentrin peptidase. Antibody reactivity to these proteins which are associated with centrosome assembly, microtubule function and protein degradation were highly associated with the diagnosis of BC. IHC staining of paraffin-embedded BC sections with specific antibodies showed that aurora and stathmin-1 and other centrosome antigens are expressed in BC.

CONCLUSIONS. The discovery of autoantibodies targeting important centrosome and proteasome proteins associated with BC suggests that this immune reactivity could be related to autoimmunity developing in BC. Our findings indicate that these autoantibodies might be biomarkers of early BC and suggest the possibility of a link with chromosomal instability in BC.
Title: The association between primary breast cancer and thyroid cancer: A meta-analysis

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Body: Background: Previous studies have suggested an aetiological link between breast cancer and thyroid cancer, however, there has never been a formal meta-analysis which collates the existing evidence supporting the hypothesis that breast cancer or thyroid cancer predispose an individual to developing the other.

Method: A systematic search was carried out using the electronic databases Pubmed and Medline. We searched for articles containing epidemiological evidence of breast cancer following thyroid cancer and vice versa. Additionally, we searched for articles that included epidemiological data involving the incidence of all second primary malignancies following both breast cancer and thyroid cancer, and compared the data sets.

Results: The meta-analysis performed in a total of 18 studies, showed that there is a significantly increased risk of developing thyroid cancer as a second primary malignancy of breast cancer (RR=1.59, 95% CI: 1.28-1.99; I²=79.99, p<0.001). Additionally, there was marginally increased risk of developing breast cancer as a second primary malignancy of thyroid cancer (RR=1.24, 95% CI: 1.18-1.30; I²=24.67, p=0.18), compared to the general risk of developing a second primary malignancy following thyroid cancer.

Conclusion: The findings of this meta-analysis suggest that there is less likely to be a common aetiological factor of the two cancers, but rather a specific aspect of the pathophysiology of breast cancer which pre-disposes individuals to develop thyroid cancer. Elucidation of the common mechanisms between breast cancer and thyroid cancer will have important implications in both diagnostic and therapeutic management of these cancers. Benefit of thyroid ultrasound screening after breast cancer surgery needs to be assessed.
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Title: MBC alliance: Coordinating metastatic research from lab bench to clinical trials

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Body: The Metastatic Breast Cancer (MBC) Alliance[1] consists of non-profit advocacy, funding organizations and industry partners who seek to transform and improve the lives of women and men living with MBC. There is no cure for MBC and metastasis is the cause of virtually all breast cancer deaths. One objective of the Alliance is to understand the MBC research landscape from basic lab research through translational and clinical trials, to epidemiology, quality of life and patient reported outcomes. The MBC Alliance partnered with the International Cancer Research Partnership (ICRP[2]), an alliance of governmental and charitable organizations from the USA, Canada, Europe, Australia and Japan that fund regional, national and international cancer research grants and awards. The ICRP database of member’s funded grants contains >60,000 grants since 2000, from >80 member organizations, totalling over $14 billion USD. Each project is coded to a Common Scientific Outline (CSO), a classification system of broad areas of cancer research. Objective: To review the last ten years of breast cancer research grant funding related to metastasis and clinical trials to identify the most promising molecular targets, pathways and therapeutics in development for MBC. Methods: ICRP partner-funded grants related to MBC were queried from the ICRP database and clinical trials were queried from Clinicaltrials.gov and breastcancertrials.org. We also conducted interviews with 75 experts including advocates, scientists, clinicians, and leaders of professional societies and cooperative groups. The database searches resulted in a pool of 150 open clinical trials for MBC and 17,985 breast cancer-relevant grant awards. Relevant grant awards and clinical trials were then coded with the assistance of keywords and manual review, to one or more, of 6 categories relevant to the hallmarks of cancer (Hanahan & Weinberg[4]) and metastasis (Steeg[5]). Results: The ICRP database contained 2,250 unique awards related to MBC. The awards are predominantly basic research (70%) and translational (24%) and that profile has not changed significantly over a 5-year period 2008-2012. Awards active in 2012/2013 show that the main areas of focus are: i) early steps to invasion (10%) and, ii) metastatic colonization (15%). Data from 130 trials have been assigned to the Hanahan/Weinberg framework: sustaining proliferative signal, resisting cell death, enabling replicative mortality, genome instability and mutation, tumor promoting inflammation, activating invasion and metastasis, avoiding immune destruction, and other categories like cancer stem cells. Within these categories, the molecular targets of the drugs being used in the trial were further subdivided (e.g., PI3, RAF, CDK). Of the trials analyzed, 71 were Ph I or PhI/II, 47 Ph II and 11 Ph III. Conclusion: Using publicly available databases we were able to develop a comprehensive list of molecular targets, pathways and therapeutics for MBC that will enable improved coordination of MBC research. [1] https://www.mbcalliance.org/; [2] https://www.icrpartnership.org/index.cfm; [3] CSO 1.4 (Biology of progression) and keywords associated with metastasis.; [4] Hanahan D and Weinberg RA, Cell 2011;144:646-674.; [5] Steeg PS. Nature Medicine 2006;12(8)895-904.
Body: The International Cancer Research Partnership (ICRP[1]) is an alliance of governmental and charitable organizations from the USA, Canada, Europe, Australia and Japan funding regional, national and international cancer research grants and awards. One key activity of the partnership is a database of information about member’s funded grant projects (N>60,000 grants, from 80 members, totalling over $14 billion USD). Each project is coded to a Common Scientific Outline (CSO), a classification system of broad areas of cancer research. Breast cancer is the most common cancer in women worldwide, however, only about 5-10% of breast cancer is attributable to genetic predisposition,[2] and about one third of cases are attributable to known genetic or other risk factors. In 2013, the Interagency Breast Cancer and Environmental Research Coordinating Committee (IBCERCC)[3] recommended that funding organizations plan strategically to accelerate the pace of scientific research on breast cancer and the environment. Thus, the ICRP has developed a mechanism to track activity and trends in research into environmental influences on breast cancer, to provide a baseline for future assessment of progress. Methods: ICRP-funded grants related to environmental influences on breast cancer were queried from the ICRP database. We focused on three time points: awards that were active in 2006, 2008 or 2010. The search resulted in a pool of 11983 breast cancer-relevant awards that was narrowed further to 1107 awards of relevance using a combination of keyword searches and specific Common Scientific Outline (CSO) codes.[4] Relevant awards were then coded with the assistance of keywords and manual review, to one or more, of 6 categories of environmental research: Behavior-Lifestyle, Behavior-Tobacco exposure, Chemicals-Chemical pollutants, Chemicals-Exogenous hormones, General Infection-Microorganisms and Radiation. Results: Between 2006 and 2010, the numbers of active awards declined and funding levels also fell. Most of the funded research is focused on behavioral/lifestyle factors in breast cancer (e.g., diet, alcohol intake, and shift work patterns). Further analysis of the Behavioral/Lifestyle category reveals that the major area of activity is in the role of nutrition/alcohol in cancer, closely followed by the contribution of obesity and reproductive factors (age of menarche, parity etc.). Conclusion: We were able to utilize a CSO ‘filter’ to identify trends in funded grant projects related to the environment and breast cancer. The decline in numbers and research funding between 2006 and 2010 is concerning. As breast cancer incidence continues to increase, research efforts to understand the causes of increased incidence are essential. Further research investment in these areas may be required.

[1] https://www.icrpartnership.org/index.cfm
[4] CSO areas 2.1, 2.3, 1.2, 2.4, 6.2 (Etiology, Basic Biology of cancer initiation, and surveillance).
Got patient advocates? The value of patient advocate participation in a large research study to develop personalized risk-based breast cancer screening strategies

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Body: Background: Over the last several years there has been confusion among women about breast cancer screening and patient advocates are increasingly used to help women understand the changes. In 2009, the U.S. Preventive Task Force (USPSTF) recommended that women under the age of 50 do not need routine screening. New state laws require breast density results to be given to women and their providers for making their screening choices. Women are not sure what to do with this information and are offered little guidance to personalize their screening recommendations. The goal of this study is to develop a risk model for improved personalized breast cancer screening recommendations. Patient advocates were incorporated throughout the design, recruitment, analysis, and dissemination phases of the study.

Methods: The research team included three patient advocates who participated as full members in the bi-weekly and quarterly team meetings throughout the duration of the study. All advocates were breast cancer survivors. The primary components of the study included focus groups to understand women’s knowledge and views on breast density as well as personalized screening, a telephone survey to gain a broader view on these topics, and recruitment to a case:control study to build a breast cancer risk model that incorporates an automated measure of breast density.

Results: Enrollment was completed over one year with 3,445 women; 839 cases and 2,606 controls. Study design and resulting recruitment strategies were reviewed early with regular feedback by the patient advocates. At the advice of the advocates, Facebook was chosen as primary social media, resulting in nearly 200 posts (stories) and 1583 likes for the project. Many of the posts were generated by or featured advocates. Regarding the focus groups, the advocates developed the questions. Women were informed about the study by the advocates and educated about breast density. The advocates were key in using the focus groups to find the right language for enrollment materials, obtain their perception of the importance of the study, and understand their views regarding a new model for personalized screening for women. The advocates were likewise key in developing questions for and analyzing results of the telephone survey. In the analysis phase, the advocates assisted the team in understanding the results of the risk questionnaires. For example, most women did not know the type of breast cancer that they had been diagnosed with or even if it was invasive. The advocates confirmed how and why even highly educated women would not necessarily retain this information. Finally, the advocates will have a strong role in the eventual dissemination of the study findings to women.

Conclusions: The investigators have developed a breast cancer risk model that includes an automated measurement of breast density, with the goal of personalizing screening for women. The inclusion of patient advocates throughout all phases of the study improved knowledge and insight of the investigating team. Their role extended beyond community engagement and development of study materials. The advocates became integral members of the study team.
Title: Measurements of advocate participation in and contributions to the Mayo Clinic breast cancer SPORE

Cynthia Chauhan¹, Wayland Eppard¹, Lori Denison¹, Debra Gearhart¹, Lawanna Holmes¹, Ruth Kraft¹, Linda Miller¹, Mary A Sitta¹, Mary L Smith¹, James Ingle¹ and Matthew Goetz¹. ¹Mayo Clinic, Rochester, MN.

Body: [Background] Patient advocates bring a unique, valuable perspective and presence to SPOREs. However, although patient advocates are recommended members of SPORE grants, guidelines are not given on the role of advocates or on measurement of the performance of advocates. [Methods] The Mayo Clinic Breast Cancer SPORE Patient Advocate Advisory Committee has developed a participation measurement tool. This tool will underpin and augment evaluations of the individual advocate contributions by the program PI and the SPORE project leaders. The Advocate Advisory Committee Chair will review the evaluations with the individual advocates. Annually, the advocate committee will review the performance of the group by the standard of the metrics. [Results] Advocates kept a log of their SPORE activities for four months then worked with a statistician to discern patterns of measurable, evaluable behaviors. The determined evaluable areas are training, scientific activity, meeting attendance, communication. Currently, those metrics are being tracked. Advocates developed the metrics together and report activities at the monthly advocate meetings. Currently, activities are simply tracked and categorized. No minimum standards are set. This will be the third step in the development of the metrics. [Conclusions] This approach is the first step in the development of meaningful evaluation of advocate SPORE activities. It quantifies the participation of advocates in the SPORE and provides the basis for developing a qualitative performance evaluation. The SPORE PI and the Committee Chair will use the evaluations to interpret the involvement of advocates in SPORE research and activities and to plan interventions to strengthen the advocate contributions to the SPORE.
Title: ICRP analysis: Obesity research in breast cancer

Kari Wojtanik¹, Rhonda Aizenberg², Susan Higginbotham³ and Lynne Davies⁴. ¹Susan G. Komen, Dallas, TX; ²Pancreatic Action Cancer Network; ³American Institute for Cancer Research and ⁴International Cancer Research Partnership.

Body: The International Cancer Research Partnership (ICRP) is an alliance of governmental and charitable organizations from the USA, Canada, Europe, Australia and Japan, funding regional, national and international cancer research grants and awards. One key activity of the partnership is the ICRP database of information about member’s funded grant projects (N>60,000 grants, from 80 members, totaling $13.6 billion USD). Each project is coded to a Common Scientific Outline (CSO) classification, a classification system of broad areas of cancer research. Obesity has been associated with an increased risk of developing several cancer types, including breast cancer. Worldwide, obesity rates have nearly doubled since 1980 (WHO), and there is significant concern that rising rates of obesity will result in additional obesity-related cancer incidence. Breast cancer is the most common cancer in women worldwide and its incidence has risen in most countries in the last 30 years.¹ In addition, there is convincing research evidence that body fatness is linked to breast cancer incidence (postmenopause).² With this in mind, the ICRP has analyzed obesity-related breast cancer research in its portfolio over three time periods: 2006, 2008 and 2010.

Methods: Using a combination of keyword searches and manual review, a total of over 1040 awards over the period 2006-2010 were found in the ICRP portfolio that were related to obesity and cancer. Of these, 353 awards were considered to be relevant to breast cancer (relevance ≥25%). These were assessed by Common Scientific Outline (CSO) areas.³ Results: The numbers of obesity-relevant awards and research investment were higher for breast cancer than for any other cancer type in the ICRP portfolio from 2006 to 2010. Research was being conducted across all CSO areas, from basic biology, etiology, prevention, early diagnosis/prognosis to treatment and cancer survivorship. Between 2006 and 2010, there was a slight decrease in etiology research (CSO2), and an increase in research into cancer survivorship (CSO6). It is notable that training awards are increasing, indicating that the research organizations contributing data to this analysis consider workforce training to be a priority area.

Conclusion: We were able to use the ICRP database to identify trends in funded grant projects related to obesity research and breast cancer. Despite increased numbers of awards, the overall stasis in research funding over this period, and the decline in investment in etiology is concerning. As breast cancer incidence continues to increase, research efforts to understand the causes of increased incidence are essential. Further research investment in these areas may be required.

¹ http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx (accessed 7th January 2014)
³ https://www.icrpartnership.org/CSO.cfm
Title: onlineDeCISion.org: An interactive web-based clinical decision aid for DCIS treatment

Elissa M Ozanne¹, Natasha K Stout², Katharine Schneider³, Djøra Soeteman⁴, Deborah Schrag², Michael Fordis³ and Rinaa S Punglia². ¹Dartmouth Institute for Health Policy, Hanover, NH; ²Harvard Medical School; ³Baylor College of Medicine, Houston, TX and ⁴Tufts Medical Center.

Body: Purpose: Treatment decisions regarding Ductal Carcinoma in Situ (DCIS) are complex, and patients often have inaccurate and incomplete understanding of the risks and benefits they face. Our objective was to create a web-based decision aid (onlineDeCISion.org) that can be used in clinical practice to guide both clinicians and their patients with these decisions.

Methods: We developed a web-based clinical decision aid to provide tailored information about DCIS treatment choices including an individual patient’s risk of recurrence, likelihood of long-term breast preservation and survival outcomes following up to 6 different treatment strategies for DCIS (lumpectomy, lumpectomy with radiation, lumpectomy with tamoxifen, lumpectomy with radiation and tamoxifen, and mastectomy with or without breast reconstruction). The decision aid is populated by our previously developed simulation model of DCIS outcomes. A theoretical framework and best-practices for web-based decision tools guided the development of the decision aid including semi-structured interviews and usability testing with a diverse group of multidisciplinary clinicians and patient advocates.

Results: The decision aid was designed to include these key features: 1) descriptions of treatment options; 2) ability to input patient health-adjusted age; 3) tailored likelihood of time-specific (10-year and lifetime) recurrence and survival outcomes; and 4) projections of downstream effects of each treatment. The decision aid provides default recurrence risks based on clinical trial data but allows clinicians to customize 10-year DCIS and invasive recurrence risks to retain flexibility to display expected outcomes for individual patients. These estimates can be based on the patient’s actual age, or age adjusted for health status, allowing for a more realistic expectation of the benefits each treatment holds.

Conclusion: Our web-based decision aid displays tailored outcomes following different treatment strategies for DCIS, allowing patients to be better informed about the tradeoffs of treatments available to them and select treatments consonant with their personal preferences, improving the quality of decision making for DCIS. The interactive design features allow users of the decision aid the ability to address uncertainty around risks of recurrence and comorbidity risks and facilitate the use of the decision aid across diverse populations. While the decision aid warrants further evaluation, the results of our study promise to improve decision making in patients with DCIS.
Body: Background
App. 200 new breast cancer patients are treated each year with chemotherapy at the outpatient ward at Roskilde Hospital in the Region of Zealand. The adverse effect of the treatment determines whether the patients are able to receive the planned dose. Studies indicate that the easier access patients have to accurate and relevant information about their disease, the better they manage. Other studies indicate that the better the patients handle the adverse reactions to chemotherapy, the more they comply to treatment as planned. The patients are the best to report the experience of their side effects, so it was an obvious choice to get the patients to self-report adverse effects.

Aim
The primary aim of the study was to create an online portal that could provide patients with better access to relevant guidance and accurate information about self assessed side effects

A secondary aim was
- to provide the patients with relevant information at the right time in order to increase their empowerment to act and comply with the treatment.
- the development of a database based on all the registrations done by the patients for further research and developmental projects.

Methods
We created, as part of a national project, an online portal, where the patients can make real time records of their side effects. The adverse effects were graded according to CTCAE standards. According to the grading the patients get practical online advice on how to act. The portal enabled the patients to get a visual overview of the time-line of their own adverse effects, and a possibility to compare the timelines of adverse effects with other similar patients. The patients were interviewed by telephone as to their experience with the use of the portal

Results
55 pt were included, 35 pt gave feed-back to the portal. Median age 61 år. Average IT kompetence level was 3,9 (out of 5): “experienced user and adequate in surfing on the internet”

Results
28 out of 35 patienter experienced benefit from the advices and instructions given on the portal. It was after the first series of treatment and in connection with newly experienced side effects that the advices were experienced as most valuable. It was appreciated that the portal could give “round the hour” advices. It was appreciated that the advices were practical instructed the patients as to what they could do themselves.

Conclusion
There was a patient demand for an around the clock realtime support to the experienced adverse events.
- It is of great importance that the portal is easy to access and intuitive in use.
- It is essential to find the right balance between secure login and easy access for the patients.
- Mobile access to the portal would bring the help closer to the patients and generate more data for the database.
Decision-making surrounding adjuvant chemotherapy in young women with early stage breast cancer

Shoshana M Rosenberg¹, Karen Sepucha², Kathryn J Ruddy³, Lidia Schapira², Steven Come⁴, Virginia Borges⁵, Evan Morgan¹, Nancy U Lin¹, Shari Gelber¹, Rulla M Tamimì⁶ and Ann H Partridge¹. ¹Dana-Farber Cancer Institute, Boston, MA; ²Massachusetts General Hospital, Boston, MA; ³Mayo Clinic, Rochester, MN; ⁴Beth Israel Deaconess Medical Center, Boston, MA; ⁵University of Colorado, Aurora, CO and ⁶Brigham and Women's Hospital, Boston, MA.

Background: There is an increasing recognition that many young women with breast cancer will have favorable outcomes without chemotherapy. We sought to characterize decision-making surrounding adjuvant chemotherapy treatment (CT) in this population for whom chemotherapy has historically been a standard of care.

Methods: As part of an ongoing, multi-center, prospective cohort of young women diagnosed with breast cancer at age 40 and younger, we identified 657 women with Stage I-III breast cancer. Participants were asked to complete surveys by mail that included questions about socio-demographics, decision-making, and treatment history within the first year following diagnosis. Tumor characteristics were ascertained via pathology and medical record review. We used Chi-square tests to compare: decisional involvement (patient-driven vs. shared vs. physician-driven), degree of confidence, and feeling informed about the CT decision (the latter two measured on a 0-10 scale, categorized as follows: 0-5=low; moderate=6-8; 9-10=high) between women who did and did not receive CT. To explore clinical appropriateness of the CT decision, we used logistic regression to assess the relationship between tumor characteristics and non-receipt of CT among women with Stage I/II disease.

Results: Among women with Stage I (n=250), II (n=312), and III (n=95), disease, 66%, 95%, and 100%, received CT, respectively. A greater proportion of women who had CT were highly confident with their decision compared with women who did not have CT (80% vs. 60%, p<0.0001); women who did not have CT were more likely to report a low level of feeling informed about the CT decision compared to women who received CT (20% vs. 5%, p<0.0001). Women who did not have CT were also more likely to report the final CT decision as made by their doctor (49% vs. 28%) and less likely to report a shared decision (33% vs. 59%, p<0.0001). Non-receipt of CT in women with Stage I/II disease (n=546) was associated within having node negative disease, T1 (vs. T2 or larger), Her2- negative, and hormone receptor positive tumors.

Conclusion: Although non-receipt of CT would be expected to be viewed favorably by patients and doctors, we found that women who received CT felt more confident and better informed than those who received no CT. Given that women who did not have CT were also less likely to perceive the CT decision as shared, improved communication together with better decisional support may be beneficial, especially for women who do not receive adjuvant chemotherapy.
Title: Characterizing the metastatic breast cancer patient experience around preparing for a treatment decision

Joanne Buzaglo¹, Melissa Miller¹, Anne Morris¹, Allison Harvey¹ and Mitch Golant¹. ¹Cancer Support Community, Philadelphia, PA.

Body: Background: An estimated 155,000 people are living with metastatic breast cancer (MBC) in the US. With new developments in treatment, people are living longer with MBC and are confronted with more complex treatment decisions. Patient-provider communication is typically inadequate and patients are not fully prepared for communicating effectively with their doctor.

Methods: Since March 2013, the Cancer Support Community has registered 909 people living with MBC to the Cancer Experience Registry, an online initiative designed to learn and raise awareness about the psychosocial impact of cancer. 572 registrants responded to questions about their experience with making treatment decisions. This sample was 99% female, 91% Caucasian, and 69% with a college degree and median age 56. Median time since MBC diagnosis was 3 years.

Results: Before making a treatment decision, nearly all (91%) reported receiving information about their cancer type; 76% received information about their treatment choices. Only 41% indicated they received information about clinical trials prior to making a treatment decision. Just over half reported they had quite a bit of knowledge about their treatment options. However, 22% had little or no knowledge about their treatment options. Thirty-eight percent received treatment decision support prior to making a treatment decision; 45% would have liked more support. Twelve percent had little or no involvement in their treatment decision-making process. Nearly one-third (29%) did not feel they had a treatment choice and 28% reported they did not have enough time to make a treatment decision. Those who wrote down a list of questions prior to their first visit to discuss treatment options with their health care provider felt significantly more prepared to discuss their treatment options (p<0.001). About two-thirds of MBC registrants were satisfied with various aspects of the treatment decision-making process: outcome of the treatment(s) received (70%); doctor’s explanation of the benefits of each option (67%); how they arrived at a decision (66%); how much they participated in making the decision (64%); and their doctor’s explanation of the risks and side effects (64%). Sixty-nine percent thought it would be important to get help with gathering information, and 68% with developing a written list of questions before their meetings with cancer specialists; only 47% thought it important to obtain audio-recordings of appointments.

Conclusion: Although over two thirds of these women were satisfied with various aspects of treatment decision making including their communication and interaction with their doctor around the decision, nearly 30% of women thought that they had no choice or felt rushed in making a decision. Those women who prepared a list of questions prior to a consultation with the doctor were significantly more prepared in making an appropriate decision. While a small majority of patients report being knowledgeable about treatment options, a significant proportion report not having enough knowledge or support to fully engage in a treatment decision. Further efforts are needed to address gaps in the delivery of decision support to MBC patients.
2014 San Antonio Breast Cancer Symposium

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**Title:** eHealth in modern breast cancer treatment: New possibilities in communication between patients, doctors and nursing staff

Rachel Wuerstlein¹, Thomas Kirkovits¹, Caroline Drewes¹, Daniel Schiltz¹, Ingo Bauerfeind², Renate Haidinger³, Kerstin Paradies⁴, Ursula Goldmann-Posch⁵, Timo Schinkoethe¹ and Nadia Harbeck¹. ¹Brustzentrum am Klinikum der Universitaet Muenchen, CCC of LMU, Munich, Bavaria, Germany; ²Gynaekologie und Frauenheilkunde am Klinikum Landshut, Landshut, Bavaria, Germany; ³Brustkrebs Deutschland e.V., Hohenbrunn/Munich, Bavaria, Germany; ⁴KOK – Konferenz onkologischer Kranken- und Kinderkrankenpflege, Hamburg, Germany and ⁵Mamazone – Frauen und Forschung gegen Brustkrebs e.V., Augsburg, Bavaria, Germany.

**Body:**

**Introduction:**
Lack of compliance and adherence in oral and s.c. treatment of breast cancer (BC) are huge problems leading to significant impacts in morbidity and mortality. During long term treatment, constant patient contact can't be secured and possible side effects not be treated adequately. Where conventional mailing systems failed, as reported in the PACT program, eHealth could be a possible solution to increase adherence among patients and to ameliorate the communication between patients, oncologist and nurses.

The objective of this study is to investigate the actual internet usage habits and property of new media among BC patients, their oncologists and the nursing staff to find new possible ways to improve compliance and adherence in long term treatment.

**Methods:**
By using 3 different questionnaires (33 items), the actual usage of internet and modern media among BC patients and their healthcare professionals (oncologists and nursing staff) is surveyed. Also, the equipment of media (computer, smartphone, etc.) in private as well as in business use is investigated. Huge care and attention is given to possible future eHealth systems for additional patient support. The collected data also includes age, sex, workplace and, in case of medical professionals, their emphasis.

Patients completed the questionnaire at two patient conventions and before consultations, oncologists and nurses were asked to answer the questionnaire at several local BC meetings.

**Results:**
631 patients, 120 oncologists and 96 nurses completed the questionnaire in 2013.

The internet usage in general and for health related issues is very high among all three subgroups (patients: 93% and 77%, respectively; oncologists: 100% and 98%; nurses: 92% and 93%). Among patients, even above age 60, 51% report to use the internet every day. Taking a look at participant's equipment of new media, the property of a personal computer is very high (78%; 99%; 95%).

Medical professionals as well as the majority of patients can imagine getting additional support during long term therapy using eHealth technologies (e.g. for monitoring of and interventions concerning side effects) (see table).

**Discussion:**
This survey, which is the first BC specific study representing internet usage habits among BC patients and their medical professionals, shows high acceptance of new interactive ways of communication between patients, doctors and nurses who are all taking part in treatment of BC. Introducing eHealth may help increase compliance and improve and individualize the doctor-patient-relationship which will possibly lead to decreased mortality and higher patient and staff satisfaction.

**Table 1**

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Doctors</th>
<th>Nursing Staff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of participants</td>
<td>631</td>
<td>120</td>
<td>96</td>
</tr>
<tr>
<td>Internet usage in general</td>
<td>92.7% (495/534)</td>
<td>100% (119/119)</td>
<td>91.7% (88/96)</td>
</tr>
<tr>
<td>Property of media</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PC</td>
<td>77.7% (490/631)</td>
<td>99.2% (119/120)</td>
<td>94.8% (91/96)</td>
</tr>
<tr>
<td>Category</td>
<td>Smartphone (%)</td>
<td>Wish (%)</td>
<td>Registration (%)</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----------------</td>
<td>----------</td>
<td>------------------</td>
</tr>
<tr>
<td>Wish for additional patient support:</td>
<td></td>
<td></td>
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<tr>
<td>via Internet</td>
<td>25.5% (161/631)</td>
<td>70.8% (85/120)</td>
<td>45.8% (44/96)</td>
</tr>
<tr>
<td>via Smartphone</td>
<td>53.8% (276/513)</td>
<td>66.4% (79/119)</td>
<td>56.8% (54/95)</td>
</tr>
<tr>
<td>Registration of side effects via electronic devices</td>
<td>24.2% (119/492)</td>
<td>51.3% (60/117)</td>
<td>31.1% (28/90)</td>
</tr>
<tr>
<td>Registration of side effects via electronic devices</td>
<td>41.1% (204/497)</td>
<td>71.2% (84/118)</td>
<td>56.1% (51/91)</td>
</tr>
</tbody>
</table>
Title: Group visits to provide gynecologic care for women affected by breast cancer

Sally R Greenwald¹, Sarah Watson¹, Tami S Rowen¹ and Mindy Goldman¹. ¹University of California, San Francisco, CA.

Body: Background
Advancements in treatment have resulted in a growing number of women now living with or affected by breast cancer. This patient population has complex and unique gynecologic needs. Two thirds of breast cancers are hormone positive and these patients will often be given Tamoxifen or aromatase inhibitors which have specific gynecologic effects. Additionally, oophorectomy may be done as part of breast cancer treatment. Chemotherapy may induce menopause with the need for safe and effective treatments for menopausal symptoms. Gynecologists have a very important role in providing care to women affected by breast cancer. Group visits are increasingly used to provide education and care in the setting of chronic disease, including breast cancer. There is limited data regarding the use of this type of healthcare delivery in providing gynecologic focused care to this complex and growing patient population.

Methods
This is a cross-sectional study measuring patient expectations and satisfaction with the practice of group visits as a model for providing gynecological health information to women affected by breast cancer. From July 2010 through February 2014 we surveyed women who participated in a two hour new patient visit that involved a one hour information session followed by an approximately 30 minute individual exam with a gynecologist. The goals were to measure the role of a small group format and an individual’s sense of satisfaction with their gynecology clinic experience. Demographic data was collected to determine if there were any correlations with satisfaction and women’s age, stage of disease, hormone receptor and menopausal status.

Results
A total of 104 women participated during the study period. Over 80% of participants in group visits felt that their expectations were met during the visit. Eighty five percent felt they had a better knowledge of treatments for gynecologic side effects from cancer treatments. When assessing disease and demographic factors that might be associated with satisfaction, we found that women with hormone receptor positive breast cancer and those with treatment induced menopause were more likely to prefer group visits than women with hormone receptor negative breast cancer or natural menopause. Women with stage 3-4 disease preferred the group education visit experience more than women with stage 1-2 disease.

Conclusion
There is a need to provide comprehensive gynecologic care and education to the expanding population of women impacted by breast cancer. Our data suggests that group visits are an effective and well-received strategy for providing gynecologic care to this niche patient practice. Incorporating a group visit model into a practice is a cost-effective educational modality to providing sources of information and support. Based on our observed differences in satisfaction and preference for group visits by stage of disease, menopause status and hormone receptivity, we recommend investigations grouping similar patients to further improve unique patient education and satisfaction.
Title: Patient experience of the introduction of an enhanced recovery programme for breast cancer surgery

Alistair RM Macey¹, Jayne I McGIvern¹, Juliette Murray¹ and Alison K Lannigan¹. ¹Wishaw General Hospital, Wishaw, Lanarkshire, United Kingdom.

Body: Introduction
The introduction of an enhanced recovery programme for breast cancer surgery in our institution has provided an opportunity to redesign the service and the way in which patients receive pre-operative information. It has been a challenge to provide the same amount of information and support for patients with a shorter period of inpatient stay. The enhanced recovery programme has been shown to reduce inpatient stay by 50% with no increase in complications¹. Almost all patients having breast conservation and sentinel node biopsy are day cases and those who have a drain in following mastectomy or axillary node clearance typically go home the day following surgery.

Aim
To audit patient experience, outcome and satisfaction following the introduction of an enhanced recovery programme.

Methods
An enhanced recovery programme was first introduced in our institution in Sep 2012. Between December 2012 and December 2013 80 patients having breast cancer surgery in Wishaw were sent a detailed questionnaire asking them about all aspects of their treatment. Two patients were excluded from the questionnaire one because of learning difficulties and the other because of acute psychiatric illness. There was a 89% response rate with 71 responses having been received to date. All patients reported that they were given enough information at the time of their diagnosis. 97% were supported at this time by a breast care nurse and found this helpful. 84% were given a written record of their proposed treatment and 94% of patients had a further pre op consultation with a breast care nurse to discuss treatment which was felt to be helpful. At this appointment half of patients were able to meet a physiotherapist and go over post-operative arm exercises. 86% of patients felt that the length of stay was just right and 8% of patients felt it was not long enough. 70% of a patients were discharged in the afternoon, between 12.00 and 17.00, 9% in the morning and 21% after 17,00. A third of patients had a drain in situ at the time of discharge and 90% reported no concerns at all about going home with a drain. Of the 40% of patients that did need to get in touch with a post op question prior to clinic three quarters contacted their breast care nurse and all reported being satisfied with the management of their query or problem. Overall 79% of patients reported being very satisfied and a further 14% satisfied with the communication and management of their diagnosis and surgery.

Conclusion
An enhanced recovery programme can be introduced to reduce inpatient stay while still offering enough support and information for breast cancer patients around the time of surgery.

Reference
¹ Does the implementation of an Enhanced Recovery Programme impact on post-operative outcomes in populations with significant comorbidity and social deprivation?
E.V. Woon¹, P.L. Wong¹, J. Murray¹, A. Lannigan¹.
¹Wishaw General Hospital, General Surgery, Glasgow Scotland, United Kingdom.
Title: Understanding potential gaps in treatment discussions between caregivers/patients with metastatic breast cancer and oncologists

Musa Mayer¹, Helen L Coons², Stephen Jones³ and Deana Percassi⁴. ¹AdvancedBC.org, New York, NY; ²Women's Mental Health Associates, Denver, CO; ³Molecular Health, The Woodlands, TX and ⁴Nielsen Consumer Insights (formerly Harris Interactive, Inc.), Rochester, NY.

Body: About 5% of newly diagnosed cases of breast cancer in the US involve cancer that has metastasized to distant parts of the body, and about 20%-30% of patients diagnosed with early-stage breast cancer later experience a distant metastasis. The 2013 Global Count Us, Know Us, Join Us survey found that 53% of US women with metastatic breast cancer (MBC) surveyed wished they had more time to discuss their needs during healthcare visits, and 60% believed their cancer treatment options are limited.

To better understand unmet patient emotional, informational, and care needs and potential communication gaps in discourse with physicians, the Make Your Dialogue Count survey was developed for (1) women ≥21 years of age with MBC; (2) caregivers of women with MBC who are ≥21 years of age; or (3) licensed US medical oncologists who treat ≥5 women with MBC per month.

Surveys were conducted online, by paper, and by telephone in the US from June through August 2014. Respondents to one survey were not necessarily related to or associated with respondents to another survey. Patient and caregiver data were unweighted. Oncologist data were weighted by geographic region and years in practice by sex to align with actual proportions in the population.

The survey was completed by 359 patients, 234 caregivers, and 252 oncologists. Patients/caregivers were mainly white (81%/73%) or hispanic (8%/18%). Most oncologists practiced general oncology (74%), for a mean of 18 years.

Patients/caregivers lacked some basic disease knowledge: 20%/29% did not know the HER2 status, 16%/20% did not know the hormone receptor status, and 28%/32% reported being told by oncologists that MBC was possibly curable. At initial diagnosis of MBC, all groups felt it was important to discuss expectations for treatment efficacy and side effects and long-term plans and goals, but patients/caregivers reported that these topics were not discussed often enough. When patients were asked what emotions they felt, and oncologists what emotions they observed in their patients at the time of initial diagnosis of MBC, surveyed patients were less scared, anxious, distressed, and worried, but also less committed, determined, hopeful, and confident than oncologists observed their patients to be. Most (71%) patients had ≥1 treatment change (median of 3 changes among those with treatment changes). Patients at the time of treatment change were less scared and distressed and more hopeful than they had been at the time of initial diagnosis of MBC, suggesting adaptation to and/or acceptance of disease status over time. Patients said they were committed to proactively managing side effects (96%) and wanted more information on side effect prevention and minimization (73%), yet only 57% openly discussed side effects with their oncologists. Still, 46% of patients wished their healthcare teams did more to help them manage side effects.

The Make Your Dialogue Count survey identified gaps in the discourse between patients/caregivers and oncologists that directly affect disease management. Closing these gaps and understanding the needs of patients throughout their journey are necessary to establish effective patient/caregiver-oncologist relationships and provide patients with the care they need.
Title: Treatment of metastatic breast cancer patients by community health practitioners: Practice pattern and competence assessments

Sara R Fagerlie¹, Alison Heintz¹ and Maureen Haas¹. ¹Educational Concepts Group, LLC, Atlanta, GA.

Background: The complexity of current treatment and management for patients with metastatic breast cancer continues to rise. The volume and pace of scientific advances make it challenging for the community practitioner to stay abreast of optimal patient care. Education is key in disseminating critical information to practitioners and allows professional reflection of appropriate therapeutic decision making and peer discussion. Understanding the base knowledge, competence, and current practice patterns of the community practitioner is critical to identification community needs and implementation of education that impacts patient care.

Methods: During 2013 and 2014, educational outcomes assessments were gathered during 2 education programs consisting of 47 live independent continuing medical education (CME) activities held within community practices across the USA. Participants were asked a series of case-based questions via an audience response system to assess baseline knowledge, competence, and identify practice patterns. Assessments were repeated following the 1-hour CME certified activity. Long-term assessment was conducted electronically 6-weeks following the educational initiative.

Results: The programs educated 958 practitioners, including 515 physicians. Practice patterns for frontline treatment varied at baseline. Education resulted in treatment more highly aligned with practice guidelines and recent advances. At baseline 75% of practitioners and 30% of the physician target audience were unable to identify appropriate next steps in a borderline HER2+ patient. The education reduced the gap by 25%. 6-weeks following the activity 100% of the assessed physician target audience agreed or strongly agreed that the education influenced their interpretation of HER2 testing. Practice gaps in practitioners ability to identify, recall, and interpret practice changing clinical trials were also identified. Newness of treatment data, lack of reimbursement, and treatment side effects were the most common barriers identified in applying the education into clinical practice.

Conclusions: The results highlight the diversity of current clinical practice in the community practice setting for patients with metastatic breast cancer and the positive impact of focused education. Knowledge and competence practice gaps were identified to pinpoint specific areas of benefit for the community practice setting in the treatment of patients with metastatic breast cancer.
Title: Managing breast and ovarian cancer risk: A novel approach to teaching residents comprehensive risk reduction and management strategies

Deborah S Lindner1. 1Northwestern University Feinberg School of Medicine, Chicago, IL.

Body: Introduction: Awareness about genetic predisposition to breast and ovarian cancer and access to genetic testing has increased dramatically over the last decade, particularly with the increase in direct to consumer marketing of genetic testing. While professional organizations have published bulletins addressing this topic, a working knowledge of how to identify and manage high risk patients is lacking among many physicians. The goal of this pilot study was to assess knowledge deficits in breast and ovarian cancer risk assessment and management among primary care residents and to determine whether a novel approach to teaching this subject will help bridge the perceived knowledge gap.

Methods: A novel case based learning module was developed by a multidisciplinary team of Breast Surgeons, Gynecologic Oncologists, Obstetrician/Gynecologists, Psychologists and Genetic Counselors as a practical approach to assessing risk level based on personal and family history and applying standard recommendations for screening and risk reduction. This approach is unique, as lectures in an academic setting often focus on management of high risk patients, but not on risk stratification and risk reduction in all comers. Surveys were completed by residents after the workshop to assess perceived knowledge before and after the presentation.

Results: 106 residents from Internal Medicine, Obstetrics and Gynecology and Family Medicine residency programs were surveyed after participating in the lecture and case based learning module. 87 (82%) responded that the information presented was new to them. 101 (95%) responded that the training increased their knowledge about the options available to young women for risk reduction and early detection of breast and ovarian cancer. 105 (99%) responded that after attending the training, they understood how to identify patients that are at an increased risk for developing breast and ovarian cancer and should be referred for genetic counseling and testing. 100 (94%) indicated they would incorporate material in the training into their everyday practice.

Discussion: Resident surveys confirmed there is a knowledge gap among trainees in breast and ovarian cancer risk assessment and management. While professional organizations have created guidelines for patient management, most trainees are either unaware of recommendations, or feel the information is not being presented in a way that allows them to use it practically in patient management. The novel educational workshop presented here increased perceived knowledge among residents, allowed them to correctly identify high risk patients, and increased their working knowledge of risk reduction strategies among high and low risk patients. Recent studies have demonstrated poor coordination of care and lack of followup for the majority of high risk patients who do not opt for risk reducing surgery within the first few months after diagnosis of a gene mutation. The need for more comprehensive education among emerging medical professionals in this area is clear. Using a practical, risk stratification approach to identify and manage high risk patients is successful in bridging the knowledge gap among residents in primary care specialties.
Title: Multidisciplinary breast cancer care registry and quality control system in the Netherlands: The NABON breast cancer audit

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Body: Background
Previous quality assessments in oncology focused on surgical issues such as number of annual operations per hospital. However, high-quality of care depends on an excellent interplay between all disciplines involved in cancer care. The NABON (National Breast Cancer Organisation of the Netherlands) has, therefore, developed a multidisciplinary set of 30 quality indicators to check and improve breast cancer care. Health insurers have recently decided to use this information for purchasing.

Methods
The NABON Breast Cancer Audit (NBCA) started in 2011. Data on all newly diagnosed patients with invasive breast cancer in the Netherlands are collected by the Netherlands Cancer Registry (n=61 hospitals) or by the physicians themselves (n=31 hospitals). Data capture is facilitated using a web-based portal and feedback to participating hospitals on their own data is being done every week. Since 2012 all Dutch hospitals participate. A set of quality indicators on process and outcome was selected following established clinical guidelines and is being supervised by a multidisciplinary steering committee.

Results
Data of all 41,958 breast cancer patients treated between 2011 and 2013 were collected. In 2013, 94% of patients were discussed in the multidisciplinary team prior to first treatment and 98% after surgery. BI-RADS score was used in 98% of radiological reports. After neo-adjuvant chemotherapy, 7.6% of patients had positive specimen margins following first breast conserving surgery compared to 5.0% and 20% of patients following primary breast conserving surgery for invasive cancer and ductal carcinoma in situ, respectively. Eighteen percent and 36% of patients underwent immediate breast reconstruction after mastectomy for invasive cancer and ductal carcinoma in situ, respectively. Pathological analysis showed 12% of patients had HER2 positive and 85% had ER positive disease. Neo-adjuvant or adjuvant systemic therapy was given to 63% of patients and neo-adjuvant or adjuvant chemotherapy to 39% of patients. Time between diagnosis and first treatment was generally short: 49% to 85% underwent primary surgery with and without immediate reconstruction, respectively, and 76% underwent neo-adjuvant chemotherapy within 5 weeks from first biopsy. At the conference we will show that variation between hospitals was not related to annual surgical volume per hospital.

Conclusion
The NBCA is a unique national system to provide and confirm quality assessment in breast cancer and to drive improvements in quality of multidisciplinary breast cancer care. Present results show an overall high quality of care in the Netherlands and provide insight in items of improvement.
Title: Education and information preferences for women with triple-negative breast cancer: Should personal or medical demographic variables impact program tailoring?

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Body: Purpose: To determine interest in tailored breast cancer education and information among women diagnosed with breast cancer based on personal and medical demographics, including subtype of breast cancer, cancer stage, age, living situation (single, married, children, student, retired), race/ethnicity, or sexual orientation.

Respondents and Methods: An 80-question online survey was designed to identify education, information, and support needs among women diagnosed with breast cancer after 2006. Respondents self-identified their breast cancer subtype and selected up to 3 options from a list of preferences about ways in which education and information needs should be tailored. Options were "breast cancer type," "age," "stage," "living situation," "race/ethnicity," "sexual orientation," "no preference," and "other." The responses of 656 women diagnosed with triple-negative breast cancer (TNBC) (25.1%) were compared to those of 1,954 women with other subtypes of breast cancer (74.9%). Logistic regression was used to assess differences in tailoring preferences between TNBC and non-TNBC women.

Results: Compared to non-TNBC women, TNBC women had a significantly stronger preference for information tailored to breast cancer subtype (71% vs. 49%, p<0.001) and race/ethnicity (5% vs. 2%, p=0.002) and significantly lesser preference for tailoring based on cancer stage (43% vs. 47%, p=0.004) and living situation (15% vs. 25%, p<0.001). The difference in preference for racial/ethnic tailoring between TNBC and non-TNBC participants may be due to the overall low proportion of non-white respondents to the survey (18.4%) and the higher proportion of non-whites in the TNBC group (18% vs. 11%, p <0.001). There was a significantly different pattern of preference by age group between TNBC and non-TNBC women. TNBC women under 40 had a stronger preference for tailoring to their age group than younger non-TNBC women (age <30, 89% vs. 77%, age 30-39, 74% vs. 62% p=0.02). Across women of all breast cancer subtypes, cancer stage played a role in tailoring preference. Women with higher stage (stage >=2) breast cancer had a stronger preference for tailoring materials based on cancer stage (p<0.001); this pattern did not differ by TNBC status.

Conclusion: Education and information tailoring preferences show marked differences by breast cancer subtype. Women with TNBC strongly prefer education and information be tailored to their breast cancer subtype and their race/ethnicity, but are less interested in cancer stage or living situation-specific tailoring. Additionally, younger age influenced preferences in women with TNBC. Across all women, advancing cancer stage influenced preference toward stage-tailored materials. Healthcare providers, cancer centers, and breast cancer organizations should consider developing education and information tailored to the needs of TNBC patients and survivors.
Title: Improving completeness of client-level data collection in the Avon Breast Health Outreach Program through electronic tablet technology

Lindsay Senter¹, Marvin R Aliaga¹, Kelly M Opdyke¹, Kathryn Gates-Ferris¹ and Marc Hurlbert². ¹Cicatelli Associates Inc (CAI), New York, NY and ²Avon Foundation for Women, New York, NY.

Body: Background: The Avon Breast Health Outreach Program (BHOP) supports community-based organizations to provide breast cancer education and outreach, and navigate low income and uninsured women to breast cancer screening and treatment. BHOP organizations collect demographic and health information on clients through a standardized interview using the Clinical Intake Form (CIF). Research demonstrates that transitioning away from paper completion to audio-computer assisted self-interview allows for increased rates of survey completion. Given the advancement of tablet technology, there is significant opportunity for organizations to adopt electronic data collection of clinical information to improve data quality.

Objective: This presentation compares rates of CIF completion collected among a low income, diverse client population via two modalities; self-administered paper-based interviews (SAPI) versus electronic collection using iPads.

Methods: The CIF is organized into three sections: demographics [12 items], breast health [9 items], and access/use of health services [5 items]. In 2013, 26 BHOP grantees adopted and utilized iPads to administer the CIF with the iSurvey application, after receiving training and technical assistance on tablet usage. Data were restricted to women >=18 years, who self-administered the CIF, English-only, collected between March and December 2013, and among only those grantees who employed both modalities. The BHOP dataset was analyzed to compare CIF completeness across the two modalities. ‘Completeness’ is defined as having a response to every question in the CIF; ‘missing’ is defined as any question that was not responded to or was left blank. Percent of completeness and mean number of missing responses in the CIF overall and by section were analyzed by modality. Odds Ratios (OR) on completeness by modality were also calculated controlling for race, income, education, place of birth and residence.

Results: There were 8,004 CIFs analyzed; 2,144 via iPad (27%) and 5,860 via SAPI (73%). Among the iPad mode, 84% of the CIFs had complete data, compared to 47% in the SAPI. The mean number of missing values across the entire CIF was 0.2 via iPad, compared to 1.3 with the SAPI. SAPI variables had ten or more times as much missing data as the iPad variables (e.g., "self-reported breast symptoms" item had 7% missing data in SAPI vs. 1% in iPad). Adjusting for key demographic characteristics, clients were seven times more likely to complete the entire CIF via the iPad as compared to the SAPI [Overall OR= 7.3(6.0-9.0)].

Conclusions: Given the significantly higher rates of CIF completion using iPads, there are important implications for health care organizations to adopt and utilize electronic collection of their health interview data. Future research should study iPads ability to handle high patient volume in less time and with more accuracy compared to SAPI.

Discussion: Tablets are a relatively inexpensive option for organizations dedicated to assuring complete data collection. While administrative barriers arise with new technology, the significantly positive aspects of tablet data collection of increased quality and completeness overshadow such burdens.
Title: Perceptions about cancer clinical trials among metastatic breast cancer patients: Findings from a patient powered registry

Anne Morris¹, Melissa Miller¹, Allison Harvey¹, Mitch Golant¹ and Joanne Buzaglo¹. ¹Cancer Support Community, Philadelphia, PA.

Body: Background: An estimated 155,000 people are living with metastatic breast cancer (MBC) in the US. With new developments in treatment, people are living longer with MBC. Advances in treatment are handicapped by patients’ limited (3-5%) participation in cancer clinical trials (CCTs). While much effort has been placed on better training physicians and health care providers in talking to their patients about the appropriateness of such participation, barriers remain.

Methods: Since March 2013, the Cancer Support Community has registered 909 people living with MBC to the Cancer Experience Registry, an online initiative designed to learn and raise awareness about the psychosocial impact of cancer. 557 registrants responded to questions about their beliefs, attitudes and experience with CCTs. This sample was 99% female, 91% Caucasian, and 69% with a college degree and median age 56. Median time since MBC diagnosis was 3 years.

Results: Only half of registrants reported that a member of their health care team spoke to them about participating in CCTs. Nearly one-quarter (23%) report that they took part in a CCT. 48% considered a CCT as a treatment option for their MBC and 34% did not know if a CCT was available to them. 67% are uncomfortable with being randomly assigned to a treatment. 18% do not trust the medical establishment and fear they will be used as a “guinea pig” for research. 63% fear receiving a placebo in a CCT; however, only 6% report that they don’t understand what clinical trials are. The table below shows differences in beliefs between those who report participating in a CCT and those who have not participated in one.

<table>
<thead>
<tr>
<th>Statement</th>
<th>percent agree or strongly agree if did NON take part in CCT n=419</th>
<th>percent agree or strongly agree if DID take part in CCT n=126</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>I am uncomfortable with being randomly assigned to a treatment</td>
<td>67</td>
<td>60</td>
<td>0.001&lt;</td>
</tr>
<tr>
<td>I do not trust the medical establishment and fear I will be used as a guinea pig for research</td>
<td>18</td>
<td>8</td>
<td>0.01</td>
</tr>
<tr>
<td>I fear receiving a placebo in a clinical trial</td>
<td>63</td>
<td>52</td>
<td>0.001&lt;</td>
</tr>
<tr>
<td>I do not understand what clinical trials are</td>
<td>6</td>
<td>2</td>
<td>0.001&lt;</td>
</tr>
<tr>
<td>I fear side effects that might come with treatment on a clinical trial</td>
<td>54</td>
<td>43</td>
<td>0.001&lt;</td>
</tr>
<tr>
<td>I would be unable to fulfill trial requirements due to logistical barriers such as transportation</td>
<td>22</td>
<td>13</td>
<td>0.001&lt;</td>
</tr>
<tr>
<td>There are no clinical trials available in my community</td>
<td>22</td>
<td>12</td>
<td>0.001&lt;</td>
</tr>
<tr>
<td>My health insurance would not cover it</td>
<td>27</td>
<td>18</td>
<td>0.001&lt;</td>
</tr>
</tbody>
</table>

Conclusion: This population of MBC patients reports a range of experiences and often conflicting beliefs about CCTs. While this survey is limited to self-report data, there is persistent suspicion among these women about participating in CCTs. Although these women report that they understand what a CCT is, they fear receiving a placebo and being randomized to different treatment arms without at least the standard of care. These misconceptions persist even among people who have participated in a CCT.
These findings highlight the need for treatment decision counseling that educates patients about all their treatment options including CCTs and for training of health care providers to better inform patients about CCTs.
Title: The financial costs of metastatic breast cancer and the decisions patients make to cope with costs: Findings from the Cancer Experience Registry

Joanne Buzaglo¹, Anne Morris¹, Melissa Miller¹, Allison Harvey¹ and Mitch Golant¹. ¹Cancer Support Community, Philadelphia, PA.

Body: Background: An estimated 155,000 people are living with metastatic breast cancer (MBC) in the US. With new developments in treatment, people are living longer with MBC and have to manage greater financial burden related to care, including copays and out of pocket costs.

Methods: Since March 2013, the Cancer Support Community has registered 909 people living with MBC to the Cancer Experience Registry, an online initiative designed to raise awareness about the psychosocial impact of cancer. 496 registrants responded to questions about the financial cost of MBC. This sample was 99% female, 91% Caucasian, and 69% with a college degree and median age 56. Median time since MBC diagnosis was 3 years.

Results: 38% of registrants report being seriously or very seriously concerned about health insurance or money worries and 46% reported currently experiencing intrusive ideation about the financial cost of care. Registrants reported experiencing a significant burden from MBC related expenses, as shown in the table below.

<table>
<thead>
<tr>
<th>Expense</th>
<th>n</th>
<th>≥3 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>copays for medical treatments (e.g., surgery, chemotherapy, radiation, etc.)</td>
<td>492</td>
<td>46.1</td>
</tr>
<tr>
<td>Prescription drugs and over the counter medication (including co-pays)</td>
<td>492</td>
<td>40.9</td>
</tr>
<tr>
<td>Copays for medical appointments/visits</td>
<td>492</td>
<td>39.3</td>
</tr>
<tr>
<td>Diagnostics or treatment not covered by insurance</td>
<td>492</td>
<td>39.7</td>
</tr>
<tr>
<td>Complementary medicine or alternative therapy (e.g., vitamins, homeopathy)</td>
<td>491</td>
<td>37.9</td>
</tr>
<tr>
<td>Travel (parking, gas)</td>
<td>489</td>
<td>20.6</td>
</tr>
<tr>
<td>Medical supplies</td>
<td>489</td>
<td>21.3</td>
</tr>
<tr>
<td>Late fees on bills</td>
<td>492</td>
<td>19.6</td>
</tr>
<tr>
<td>Counseling or therapy</td>
<td>488</td>
<td>19.9</td>
</tr>
</tbody>
</table>

Because of these expenses, registrants have: foregone vacations, celebrations, and social events (53%); sold property (12%); refinanced their house (13%); filed for bankruptcy (5%); downsized their living accommodations (16%); liquidated their assets (19%); depleted their savings (40%); borrowed against or used money from a retirement plan (27%); cut their grocery expenses (42%); applied for or used public assistance (14%); chosen a treatment that is not as effective but costs less (9%); asked their doctor if there was a less expensive treatment (19%); tried to negotiate payments with credit companies (24%); negotiated with service providers to reduce costs (22%); used pharmaceutical assistance programs (26%); accepted money from friends or family (39%); and cashed in a life insurance policy early (8%), among others. In order to reduce the cost of treating MBC, registrants often or always postpone seeking psychological counseling or support (20%) and delay follow up on recommendations for complementary treatment such as physical or occupational therapy and nutrition counseling (13%).

Conclusion: MBC places a significant financial burden on patients, which can result in patients taking measures that can significantly impact their quality of life. Future implications for research include the development and evaluation of interventions designed to enhance doctor-patient communication and support (e.g., financial counseling) to ensure that the financial cost of MBC does not negatively impact the patient’s quality of life, course of cancer care, and health outcomes.
Introduction:
Breast cancer survivors benefit from supportive, professionally facilitated group discussions to assist their individual recovery from treatment and place their cancer in a life perspective. We have developed a multi-session group discussion to aid survivors in this path of recovery.

Materials and Methods:
The Pink Ribbon Survivors Network (www.PinkRibbonSurvivorsNetwork.org) has developed an online library for breast cancer survivors titled, "The Curriculum for Recovery." This library contains professionally vetted online articles in 19 categories related to survivorship issues facing breast cancer survivors. The library houses over 400 articles, in total. We have developed a 5 session program of discussion for breast cancer survivors integrating this library resource as a basis for discussion material. The 5 sessions are titled: 1.) Physical Recovery and New Adjustments, 2.) Nurturing Yourself: The Importance of Self-Care 3.) Fostering Positive Relationships, 4.) Understanding your Emotions: Doubt and Hope in Survivorship, and 5.) Prioritizing, Leaving Your Legacy, Moving Forward.

Each session draws from specific categories of the Curriculum for Recovery Library. Participants are asked to read specific articles from the online library in preparation for group discussion. The 5 sessions lead the participants from the issue of immediate physical recovery to gaining a philosophic life perspective on the impact of breast cancer in their lives.

Results:
Website usage: Since its inception in July, 2012, the website's resources have been accessed from 2300 cities in 128 countries. Its libraries have been viewed 9,000 times, tallying over 30,000 page views. The Survivors' online library hosts information in 19 different categories. Separate online libraries specialize in clinical literature for cancer care professionals and primary care professionals.

During registration for this group, participants will be asked about coping issues, and their goals in this group participation. Feedback after each session will be obtained. We intend to develop a manual for professional facilitators to use in order to be able to facilitate similar groups in their center. This manual will be available online within our website's libraries.

Discussion:
Studies have shown that breast cancer survivors benefit from group participation for support, education and discussion during their breast cancer recovery. We have developed a multi-session group discussion program that can be replicated free of charge at any location, utilizing the online resource noted here, along with the comprehensive facilitator's guidelines. This program is designed to bring survivors from initial issues of early recovery to gaining a broad perspective of how breast cancer has affected the individual's life and her future. This program is well represented in a brief live presentation at the San Antonio Breast Cancer Symposium that will be illustrative of its value to breast cancer survivors, and the ease of replication at other locations.
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-12-05
Average Grade: 4.83

Title: Psychological status of early breast cancer (EBC) patients (pts) after surgery and before the starting of aromatase Inhibitors (AIs). Results of a single-institution, prospective study

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Body: BACKGROUND
AIs are the milestone adjuvant treatment for postmenopausal early HR+ BC pts. Non-steroidal AIs induce a significant oestrogen deprivation, responsible in approximately 40% of the pts for muscle-skeletal adverse events (MSKs) and for discontinuation in 25%. The present study aims to evaluate the impact of the diagnosis communication and of the need for AI therapy on the future development of MSKs events in a prospective cohort of EBC pts.

PATIENTS AND METHODS
The analysis of patients’ psychological attitude was conducted by using 3 different tests: SCL 90-r for psychological symptoms, SF-36 for the self-perception of the health status and COPE-NVI for evaluation of coping. After a full explanation of the AIs potential adverse events by the oncologist, the questionnaires were self-administered with the support of a dedicated psychologist at the moment of the first visit (T0). For the future correlation between baseline psychological attitude and the development of AI-related events, the presence and the intensity of pain were measured by NRS 11-points scale and by Tender Points (TPs) evaluation. Pearson correlation analysis was used to calculate correlations between Pain Scale, Physical Synthetic Index of SF-36 and NRS/TRPs scores. Wilcoxon’s Test was used to compare baseline scores with subsequent ones.

RESULTS
From October 2012 to April 2013, 70 EBC pts entered the study. Exclusion criteria included severe osteoporosis or other co morbidities that contraindicate therapy with AIs and documented diagnosis of psychosis or cognitive deterioration. Median age was 64.8 years (43-88). The vast majority of the pts (584.3%) presented stage IA-IIA disease. Fifteen pts aged less than 55 years were menopausal, 13 have previously received chemotherapy as part of the planned adjuvant programme. Thirty-four pts (48.6%) had a baseline NRS>0 and 18 NRS>5; 26 pts presented at least 1 positive TP. Regarding the SCL 90-r analysis, all the pts have reported standardized scores within the normal ranges for somatization, anxiety and phobic anxiety. The analysis of SF-36 questionnaires indicated that pts showed a significant lower score in comparison to the reference population in the ISF Index (44.17 vs 48.7) and in the Physical Health Scale (47.5 vs 74.13). These differences could suggest a greater difficulty to do daily activities and a worse global index of self physical perception.
At baseline, there was a statistically significant correlation between Physical Pain Scale of SF-36 and NRS Pain score (p=0.001) and between Physical Synthetic Index of SF-36 and NRS Pain Score (p<0.001). No differences have been observed in the COPE NVI questionnaires between the studied population and the reference one.

CONCLUSION
Early BC pts who are candidate for endocrine adjuvant therapy with AIs often present an impairment in terms of physical activity (daily and working) and perception of physical pain. The analysis of these parameters along the 1st year of AI therapy and their correlation with MSK adverse events onset is ongoing.
**Title:** Sources of information and influence on decisions regarding contralateral prophylactic mastectomy: A prospective study

Swati A Kulkarni¹, Martha Van Haitsma¹, Kristen Wroblewski¹, Sarah Rabbit² and Kathy Yao². ¹University of Chicago, Chicago, IL and ²NorthShore University HealthSystem, Evanston, IL.

**Body:** Background: The number of women with a newly diagnosed ipsilateral breast cancer requesting contralateral prophylactic mastectomy (CPM) has increased significantly in recent years. Little is known about the sources of information women utilize as part of their decision making process regarding the choice of CPM.

**Methods:** A 55-item survey validated with breast cancer survivors was administered to 136 women with newly diagnosed breast cancer before they underwent surgical therapy at two institutions. Women were asked about the influence of medical personnel, individuals outside of medicine, websites and social media on their decision to keep or remove their healthy breast.

**Results:** The median age was 58 years (range 30-85); 69.2% were White, 26.7% were African American and 4.2% were Asian; Thirty-eight (28.6%) of patients had a first-degree relative with breast cancer. Seventy-five (58.6%) had a lumpectomy, 41 (32%) had a unilateral mastectomy and 12 (9.4%) had a CPM. About 80% of women felt the information they were given about breast cancer treatment was consistent. Fifty-five patients (41.7%) sought out extra information beyond what the doctor gave them. Sources of information that had strong or some influence were websites and books recommended by their doctor (44%), websites, followed books not recommended by their doctor (30.4%), and magazines (15.4%). Only 6.7% said that social media had some or strong influence on their decision making; 61.7% stated that they completely or mostly relied on their doctor’s advice to make treatment decisions and ranked their doctor’s spoken advice (74.3%) as the most important source of information in making their decision to keep or remove their healthy breast. However, Eighty-two women (62.6%) stated that information provided by staff did not include any information about removing the healthy breast. Forty-one (36%) stated that no one discussed contralateral breast cancer risk and 67 women (59%) stated that no one talked with them about the possibility that cancer could turn up somewhere else even if both breasts were removed. Of medical personnel, women stated that their surgeon (81%) had the most influence on their decision, followed by their oncologist (59.1%) and reconstructive surgeon (29.8%). When asked who influenced their decision outside of medicine, 58.7% of women stated that other breast cancer survivors influenced them. Reconstructive surgeons and the spouse/partner had more influence on those <50 years old compared to >50 years old (p=0.03). Oncologists (p=0.004), nurses and trained counselors (p=0.06-0.08), had more of an influence for African Americans compared to Whites.

**Conclusions:** Many women with newly diagnosed breast cancer do not receive information about CPM, contralateral breast cancer risk or how CPM affects recurrence from their physician. However, women still highly value their doctor’s advice and information provided by their doctor but one third of women state that websites identified on their own strongly influence their decision making for CPM. These findings highlight an opportunity for physicians to educate women about the utility of CPM as part of their surgical treatment.
Purpose/Objectives: RT is associated with acute treatment-related complications that can lead to poor quality of life (QOL) and fatigue. Exercise has been shown in other cancer treatment settings to improve negative outcomes. We conducted a prospective pilot study to explore the association between exercise, patient-reported outcomes (PROs), and radiation therapy (RT) toxicities.

Materials/Methods: Patients with surgically excised ductal carcinoma in situ or invasive breast carcinoma receiving curative radiation were enrolled. Each patient completed an exercise behavior/QOL survey at two time points: before the patient’s 5th fraction of radiation and during the last week of treatment. Limb girth measurements to evaluate lymphedema and assessment of shoulder range of motion (ROM) were completed on all patients at the same two time points. Skin toxicity was assessed weekly throughout radiotherapy. Exercise behavior was quantified with the Godin Leisure Time Exercise Questionnaire (metabolic equivalent [MET] hours per week). Patients with >7 METs/week were designated the exercise cohort (n=10) and those with <7 METs/week the non-exercise cohort (n=10). PROs were measured using standardized questionnaires. Continuous variables were compared using the Wilcoxon rank sum test and percentages were compared using Fisher’s exact test.

Results: Median patient age was 56 (range 28-70) years. Median MET in the exercise cohort was 12.9/week (range 7.5-35.0, n=10); 0.0/week in the non-exercise cohort (range 0-5.8, n=10). Women in the exercise cohort experienced significant improvement in depression scores over the course of treatment as compared to those who did not exercise (p=0.013). Those in the exercise cohort also reported less fatigue on the FACT-Fatigue subscale at treatment completion (exercise: 133.0; non-exercise: 121.0) (p=0.6). In addition, only 30% of exercisers suffered from grade 2 dermatitis compared to 70% of non-exercisers (p=0.2), despite a similar body mass index (26.4 exercise cohort versus 28.1 non-exercise). Exercisers also had greater ROM in the affected (91.7 vs. 85.2%, p=0.1) and contralateral shoulder (95 vs. 90%, p=0.048) at treatment completion. No differences in pain or sleep scores were noted and lymphedema was mild (<3cm) in the entire patient cohort.

Conclusion: The vast majority of current exercise oncology literature indicates that physical activity is an independent predictor of quality of life metrics in cancer patients. Our study notes a trend towards improved outcomes with increased exercise during radiation therapy, suggesting that accrual of additional patients to our pilot study is worthwhile. Ultimately, a concurrent exercise intervention may improve quality of life and reduce acute toxicity in patients undergoing breast radiation treatment.
Chemotherapy-induced fatigue and mitochondrial function in early stage breast cancer

Namrata I Peswani1, Kanchana Herath1, Brian P Dranka1, George M Lessmann1, Donna McAllister1, Raymond G Hoffmann1, Balaraman Kalyanaraman1 and Christopher R Chitambar1. 1Medical College of Wisconsin, Milwaukee, WI.

Introduction

Patients with early stage breast cancer receiving chemotherapy (CT) may experience persistent fatigue. The mechanisms for fatigue are not well understood. We hypothesized that CT-induced fatigue may involve perturbations in mitochondrial function. We therefore examined the development of fatigue in patients during adjuvant or neoadjuvant CT and measured mitochondrial function in their peripheral blood mononuclear cells (PBMCs).

Methods

Females with Stage I-III breast cancer patients, ages 35-75 years, were enrolled in an IRB-approved study. Patients with coexisting illnesses associated with chronic fatigue were excluded. Patients self-reported fatigue severity using the 14-question Fatigue Symptom Inventory (FSI) that rates fatigue intensity on a 10-point scale. Data [FSI and PBMCs] were collected prior to CT, mid-point in the course of CT, 2 - 3 weeks after completion of CT, and 3 and 6 months later. Mitochondrial function in PBMCs from patients was measured using a Seahorse Bioscience XF24 analyzer at corresponding times.

Results

Results on CT-induced fatigue are available for 67 patients. The overall fatigue score for each patient was measured as the sum of the scores for all 14 questions in the FSI. The average fatigue score for the 67 patients prior to chemotherapy was 20. Baseline fatigue scores for patients who had adjuvant CT were much greater than for patients receiving neoadjuvant CT (R2=1). Patient fatigue scores doubled after starting chemotherapy (average score 40, p < 0.001). Even though fatigue scores improved after treatment completion (average score 26 at 6 months), the scores did not return to baseline 6 months later (p=0.02). In our population, CT-induced fatigue did not correlate with patient age regardless of the CT regimen [r=0.3 and r= -.2, for doxorubicin plus cyclophosphamide (AC) vs docetaxel plus cyclophosphamide (TC) groups, respectively]) or a decrease in hemoglobin (r= -.3 and r=0.03, for AC and TC, respectively). None of these correlations could explain more than 9% of the change in treatment-induced fatigue (p=0.2).

Preliminary analysis of mitochondrial function in 12 patients shows that 9 patients had a decrease in mitochondrial reserve capacity with an increase in CT-induced fatigue following completion of 4 cycles of CT. 3 patients had a decrease in mitochondrial reserve capacity but reported no significant change in their fatigue scores. Mitochondrial function data on all patients have been collected and are presently being analyzed.

Conclusion

Our study shows that early stage breast cancer patients treated with adjuvant or neoadjuvant CT may experience fatigue that can persist for months after completion of treatment and cannot be explained by the CT regimen, age, or anemia. Our initial results suggest a link between CT-induced fatigue and changes in mitochondrial function in some patients. Mitochondrial function analysis and its correlation with fatigue for all 67 patients will be completed and reported at SABCS. Mitochondrial function may prove to be a biomarker for CT-induced fatigue in certain patients and may help identify patients for whom interventions that stimulate mitochondria biogenesis, including pharmacologic agents and exercise, may be beneficial in ameliorating this side-effect.
Title: Psycho-oncological intervention in breast cancer patients - A quantitative analysis of tumor associated fatigue treatment

Christian Eichler1,2, Pia Multhaupt2, Sibylle Multhaupt2, Friedrich Wolff1 and Mathias Warm2,4. 1Holweide Hospital, Cologne, Germany; 2Brustzentrum, Krankenhaus Holweide, Cologne, Germany and 3University of Witten/Herdecke, Witten, Germany.

Body: Abstract

Background: Although tumor associated fatigue (TAF) or cancer related fatigue (CRF) is not a new concept, no real headway has been made in the quantitative analysis of its successful treatment. Since 20 to 30% of all breast cancer patients suffer from anxiety and/or depression within the first year of their diagnosis, this issue needs to be addressed and a standard treatment protocol has to be developed. Multimodal approaches are currently being used including increased physical activity, pharmaceutical therapy, and psycho-oncological intervention. This study focused on developing a simple, reproducible protocol for the psycho-oncological support of tumor associated fatigue patients.

Methods: Between the year 2011 and 2012, 23 breast cancer patients fulfilled the diagnosis TAF requirements and were introduced into this study. All patients had received surgery and were currently being monitored in an adjuvant setting. Our method focused on a psycho-oncological support group using a predetermined, highly structured and reproducible treatment manual. Eight weekly, 90 minute sessions were conducted and patients were evaluated before and after this eight session block. Tumor fatigue specific questionnaires such as the multidimensional fatigue inventory (MFI) as well as the hospital anxiety and depression scale (HADS) were used in order to quantitatively evaluate patient TAF.

Results: Of the 23 patients enrolled in the study, only 7 patients fulfilled the TAF diagnostic criteria after the psycho-oncological group treatment. This represents a 70% reduction in diagnosable tumor associated fatigue. The HADS analysis showed a 33% reduction in patient anxiety as well as a 57% reduction in patient depression levels. The MFI scores showed a significant reduction in 4 of the 5 evaluate categories. With the exception of the "mental fatigue" MFI category all results were statistically significant.

Conclusion: This study showed that a highly structured, psycho-oncological group intervention will produce significant improvements in breast cancer patient tumor associated fatigue levels after only 8 sessions. This type of group therapy should be recommended as a standard of care for all TAF breast cancer patients.
Title: Examining diagnostic and therapeutic delays using patient reported outcomes

Odicie Fielder-Kimbrough¹, Mayra Serrano¹ and Kimlin T Ashing¹. ¹City of Hope, Duarte, CA.

Body: Background: Diagnostic and treatment delays in cancer patients lead to poorer outcomes. African American and Latina women with breast cancer (BC) are more likely to experience delays. This study explores delays in African American and Latina breast cancer survivors (BCS) and association with physician-patient communication variables.

Methods: A total of 320 BCS (88 African-American and 232 Latina-American) diagnosed with stage 0-3 BC were recruited from the California Cancer Registry and Hospitals. BCS completed a questionnaire assessing patient reported time to diagnosis and initiating treatment, and quality of care variables pertaining to physician-patient interaction.

Results: The mean number of days waited after finding a lump or abnormality and seeking diagnostic care to obtaining a cancer diagnosis was 57.9 days in this cohort. Bivariate analysis revealed that Latina BCS were significantly more likely to wait longer to obtain diagnosis. \( p = .033 \). Once diagnosed, African-American BCS were more likely to report greater days delay to initiating treatment \( p = .007 \). The mean number of days delayed for initiating treatment between African American and Latinas, however, was not significantly different, 35.3 v. 34.5 days \( p=.107 \). We investigated the surgeon-patient dyad in influencing delays. The better patients rated the relationship, the fewer days in delayed treatment reported \( p=0.038 \). Additionally, 11% of BCS \( n=34 \) did not believe that their doctors gave them enough information regarding their treatment. The regression analysis showed that the patients' evaluation of the adequacy of doctors' information was a significant predictor of days delayed treatment \( p<0.0001 \). If the patient believed that enough information was provided, then the treatment was initiated within 6 days compared to the 40 days average reported by patients who endorse inadequate information.

However, the majority of patients (87%) reported a "good" or "very good" relationship with her surgeon. Although the majority of patients reported a "good" or "very good" relationship with her Oncologist (65%), the relationship was rated as "poor" or "very poor" by 23% of patients. There was no statistical difference in rating of relationship with providers between the ethnic groups \( p=.865 \).

Conclusions: Our results suggest that poor patient-physician interactions are associated with BC diagnostic and therapeutic care delays in both African American and Latina BCS. As healthcare shifts towards improving variables of quality of care and patient satisfaction, attention to improving these interactions and further exploration will be warranted. Further, improving timely access to diagnostic and therapeutic care may reduce BC disparity and improve cancer equity.
Title: Socioeconomic factors and the use of complimentary and alternative therapies in patients with early stage breast cancer

Swetha Panati¹, Kamran Shahid¹, Kaylin S Watson¹, Lauren Adair¹, Sanjay Juneja¹, Kimberly Nguyen¹, Runhua Shi¹ and Gary V Burton¹. ¹LSU Health Sciences Centre- FWCC, Shreveport, LA.

Body: BACKGROUND: Treatments not considered a part of conventional cancer care are known as Complementary and Alternative Medicine (CAM) and are becoming increasingly popular. These CAM therapies, divided into Alternative Medical Systems (AMS), Mind-Body Interventions (MBI), Biological Based Therapies (BBT), Manipulative Therapies (MT) and Energy Therapies (ET), may or may not benefit patients (pts). Socioeconomic factors which may be associated with CAM use have not been well defined.

AIM: To further define socioeconomic factors associated with the use of specific CAMs in pts with early stage breast cancer.

METHODS: 513 early stage breast cancer pts were interviewed between 4/2012 and 6/2014 using an IRB approved survey. The pts were interviewed after completion of all adjuvant chemotherapy, radiation and at least 6 months of adjuvant hormone therapy. Data collected included pt demographics, age, race, employment, insurance, marital status, income, education, religion and residence. All pts had literacy testing using a validated reading test. Pts were questioned on the use of 36 specific CAMs within the 5 CAM divisions, why they chose to use CAMs, and their opinion relative to benefits. Chi square test was used to evaluate the data.

RESULTS: CAM use was common with 100% of pts using or participating in at least 1 CAM class. CAMS within the MBI division were used by most patients with statistically increased use seen in non-protestant religion and employed pts (p=0.02 and 0.04). BBT division use was associated with age (p=0.001) and marginal for being insured (p=0.06). AMS use appeared to be more common in unemployed pts but was not statistically significant (p= 0.07).

The most common specific CAMs used were prayer (95%), exercise (65%), deep breathing (39%), and music therapy (38%). Statistically significant socioeconomic associations with specific CAM subclass utilizations included: Advancing age was associated with herbal supplement use. White pts used PET, music and art therapy as compared to black pts who used dance therapy and progressive relaxation. Insured pts used herbal, yoga and pilates. Pts with high income participated in yoga while low income used deep breathing, progressive relaxation, music and dance therapy. Pts with higher reading level (education) used deep breathing, music, dance and pet therapy.

CONCLUSION: All pts, with early stage breast cancer, utilize CAMs and see their use as an important part of their cancer therapy. Specific CAM subgroups use was associated with advancing age, employment, income, race and reading levels. Although all pts use CAM therapy, the vast majority of CAMs pose no risk and could benefit the individual pt. Utilization of various herbal supplements, which could pose a risk, is seen in all socioeconomic groups, however, a statistically significant increased use was seen with advancing age and having insurance. This study, however, did not obtain information from the 2% of pts seen at our institution who abandoned all conventional therapy and were lost to follow-up.
Breast cancer in young women: Fertility preservation as a component of treatment planning and discussion

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Body: Background: As part of education and informed consent before cancer therapy, health care providers should address the possibility of infertility with patients (pts) treated during their reproductive years, and be prepared to discuss fertility preservation options and/or to refer all potential patients to Reproductive Specialists. Here we describe the recently established referral protocol of our institution with a fertility preservation centre. Both institutions belong to the Portuguese National Health System.

Methods: The referral protocol includes female pts, ≤ 39 years old, with a diagnosis of breast or gynecological cancer or sarcoma with indication to curative intention chemotherapy. The possibility of fertility preservation is primarily discussed at the cancer centre and only interested pts are referred to the reproductive clinic, where specialized counseling is provided. Fertility preservation procedures (FPPs) (oocyte, embryo and/or ovarian tissue cryopreservation) are conditional to a previous evaluation of the ovarian pool (OP). OP markers are: antral follicle count, follicle stimulating hormone (FSH) and anti-mullerian hormone (AMH). Only good prognosis cases are eligible for FPPs.

Results: After primary discussion with eligible breast cancer (BC) pts, 17 were referred to the reproductive clinic: median age at BC diagnosis was 34 yrs (29-38), median previous pregnancies number of 0 (0-2) and median number of children of 0 (0-2). Marital status: most (64%) pts had a companion but 36% did not. All pts except 1 were candidates for immediate chemotherapy, either in the adjuvant or neoadjuvant setting. In the adjuvant setting (9 pts), median time from surgery until starting systemic treatment was 61.5 (43-146) days (approx. 8.8 weeks); the case with a 146 day interval before chemotherapy was due to surgical complications and not to FPP. In the neoadjuvant setting (5 pts), median time between multidisciplinary decision and systemic treatment beginning was 17 (11-24) days (2.4 weeks). One patient was proposed to hormone therapy only after BC surgery and underwent FPP before systemic treatment. The timing for primary discussion of FPP was in multidisciplinary decision consultation. After counseling at the reproductive clinic, 2 pts declined FPP (%). For the other 13 pts oocyte cryopreservation was the FPP mostly used, although in one case embryo cryopreservation was performed. One case of ovarian hyperstimulation syndrome was observed but this FPP complication resolved without sequela and this event did not cause delay in chemotherapy treatment.

Conclusions: Our collaboration protocol allows for an efficacious referral of BC pts seeking fertility preservation counselling. Although BC patients may be focused initially on their cancer diagnosis, health care providers are encouraged to advise them regarding potential threats to fertility. Formal collaboration between cancer and reproductive centres, like the one described here, are crucial so as to allow for the widest array of options for fertility preservation and to prevent delay in cancer treatment. We intend to follow up these patients in order to realistically understand the impact of this practice in fertility and quality of life of cancer patients.
Deevakar Rogith¹, Rafeek A Yusuf¹, Shelley R Hovick², Bryan M Fellman³, Susan K Peterson³, Allison Burton-Chase⁴, Yisheng Li³, Elmer V Bernstam¹ and Funda Meric-Bernstam³. ¹University of Texas School of BioMedical Informatics, Houston, TX; ²Ohio State University, Columbus, OH; ³University of Texas MD Anderson Cancer Center, Houston, TX and ⁴Albany College of Pharmacy, Albany, NY.

INTRODUCTION: Breast cancer patients and providers are increasingly interested in personalized cancer therapy. Information-seeking behaviors and knowledge about personalized cancer therapy, cancer genetics, and molecular testing may influence patients’ participation in clinical trials and decision making regarding their care. We evaluated breast cancer patients’ knowledge and information seeking behaviors regarding personalized cancer therapy (PCT).

METHODS: The study population included newly registered female breast cancer patients at The University of Texas MD Anderson Cancer Center prior to their first clinical visit. Of 308 consecutive patients who were invited to participate, 100 (32%) completed a self-administered questionnaire assessing their knowledge and information seeking preferences regarding PCT. Knowledge regarding cancer genetics and PCT research was assessed using 16 true/false questions (Cronbach’s $\alpha=0.88$). A knowledge score was computed from the total number of correct responses.

RESULTS: Respondents were predominantly white (70%), older (median age 55 years; SD=12.9; range 26-84), educated (78% with college degree or higher) and higher incomes (54% >$50,000/year); 71% had been diagnosed with breast cancer for at most one year at time of participation. Knowledge regarding cancer genetics and PCT was moderate (M=8.68, SD=3.8). Although most participants (85%) could correctly identify the definition of PCT, many (59%) did not know that somatic mutations are not hereditary. Many (75%) knew that molecular testing can reveal risk for other hereditary cancers. Less than half (46.5%) knew about the availability of PCT in clinical trials. A minority (27%) indicated that they had sought information regarding PCT. They sought for information related to specific treatment options. Higher education (p<0.01) and income levels (p<0.05) were associated with higher knowledge scores and with seeking PCT information (p<0.01). Those who had previously undergone any genetic testing also were more likely to seek information about PCT (29.6% vs 9.9%, p<0.05). Other demographic and clinical variables like age, race, duration of illness, cancer stage did not correlate with the knowledge score or information seeking behavior.

CONCLUSION: Study participants could define PCT, but had limited knowledge of its availability and underlying treatment principles. This may be due, in part, to the fact that few participants had sought information about PCT. Understanding patients’ knowledge and prior information seeking regarding PCT may inform clinicians, who are likely to be patients’ initial source of information about PCT.
Body: Objective: Breast cancer is one of the most common cancers, and it accounts for 20% of malignant tumors of women. Psychological damage of the breast cancer patient is serious. In our institution, approximately 30% of the early breast cancer patients diagnosed as depressed mental status before operation. Breast cancer patients have to receive hormone therapy, chemotherapy, and radiation therapy for a long term after operation. Depressed mental status affects execution of the treatment, and the interruption of the treatment brings the risk of cancer recurrence. As a treatment for depressed mental status of cancer patients, medication and psychotherapy are common, but some previous reports showed exercise is effective. Exercises provided in these reports were various kinds and strength, and the standard prescription is not established. We investigate the effect of walking program as a mild aerobic exercise, whether it improves the depressed mental status and quality of life (QOL) of breast cancer patients.

Methods: 25 early breast cancer patients were recruited. Depressed mental status and QOL were assessed by Center for Epidemiologic Studies Depression Scale (CES-D) and Medical Outcomes Study 36-item Short Form Health Survey (SF-36). Physical activity was measured by accelerometer (Lifecorder PLUS, Suzuken), and estimated using metabolic equivalent foe task (MET). Participants mounted accelerometer after discharge, and baseline physical activity was recorded till 1 month after operation. After recording baseline physical activity, we instructed to perform walking or mild aerobic exercise. Scores of CES-D and SF-36, and physical activity data were measured again after two months.

Results: Physical activity after intervention was significantly increased as compared to baseline (8.5±5.5 vs 12.3±6.9 MET.h/week, p=0.02). At pre-operation phase, nine patients (36%) regarded as depressed mental status by CES-D, and depressed patients decreased to five at the time of 3 months after operation. A subscale of SF-36 (mental health) reduced significantly compared with pre-operation and 3 months after operation (p<0.01). Physical activity after intervention was correlated with the scores of CES-D and all subscales of SF-36.

Conclusion: This study demonstrated the possibility that walking program as a mild exercise improves mental status and QOL of perioperative breast cancer patients. It is necessary to examine in more detail for clinical application.
Title: Perception of women after undergoing microbiopsy following a positive breast cancer screening

Birgit Carly¹, Mireille Aimont¹, Nicolas Beauloye¹ and Fabienne Liebens¹. ¹CHU St Pierre, Brussels, Belgium.

Body: Background: Some authors have challenged the benefit of breast cancer mammographic screening due to high prevalence of overdiagnosis and overtreatment.

Purpose: To assess the anxiety and stress of women undergoing a microbiopsy following a false positive breast cancer screening and to evaluate whether this experience refrains them from continuing breast cancer screening.

Methods: 296 patients underwent breast microbiopsy for positive breast cancer screening B1, B2 and B3 lesions in our Breast Center between 2011 and 2013. Patients undergoing microbiopsy for B4 and B5 lesions were excluded from the study. The patients were thereafter interviewed by our two psychologists by phone within a time range of 6 to 18 months following the biopsy.

Results: 201 women answered the questionnaire (68% response). 5.5% had a breast lesion at risk and 75% had a benign lesion. Half of the women rated their anxiety as high (7-8 on a scale between 0 – 10) and 15% as very high (9-10/10) before the biopsy. While waiting for the result, 42% rated their anxiety as high (7-8/10) and nearly a third as very high (9-10/10). But 80% accepted to continue breast cancer screening and 90% would accept to undergo again a microbiopsy if necessary.

Conclusion: microbiopsy provides high stress in most patients undergoing it. Nevertheless, 90% of those who had a false positive results accept it and are willing to undergo a biopsy again if necessary.
Title: Exploratory post hoc analyses of patient-reported outcomes (PROs) in the IMELDA randomized phase III trial: Maintenance bevacizumab (BEV) ± capecitabine (CAP) after initial first-line BEV plus docetaxel (DOC) for HER2-negative metastatic breast cancer (mBC)

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Body: BACKGROUND The addition of CAP to maintenance BEV demonstrated statistically significant and clinically relevant improvements in progression-free survival (PFS [primary endpoint]; HR 0.38 [95% CI 0.27–0.55]; log-rank p<0.001) and overall survival (OS [secondary endpoint]; HR 0.43 [95% CI 0.26–0.69]; log-rank p<0.001) in patients (pts) without disease progression (PD) on initial first-line BEV–DOC for HER2-negative mBC in the IMELDA trial. This benefit was achieved despite the smaller than planned sample size due to premature recruitment discontinuation because of regulatory withdrawal of BEV–DOC.

METHODS Pts with HER2-negative measurable mBC, ECOG PS <2, and no prior chemotherapy for mBC were eligible. After 3–6 cycles of BEV–DOC, pts without PD were randomized to either BEV alone or BEV–CAP (BEV 15 mg/kg q3w; CAP 1000 mg/m^2 bid d1–14 q3w) until PD. PROs (secondary endpoint) were assessed using the EORTC QLQ-C30 completed at screening (before BEV–DOC), at randomization to CAP vs no CAP, then every 3 cycles until PD, and at (but not beyond) PD. Analyses of mean change from randomization were prespecified. A 28-day window around the scheduled timepoints from randomization was applied to maximize the number of questionnaires available for analysis. Exploratory post hoc analyses included mixed-model repeated measures (MMRM; modeling weighted treatment effect from randomization across all available timepoints) and responder analyses using the global health status/QoL subscale. Pts were categorized as having improved (≥10-point increase), stable (change of <10 points), or worsened (≥10-point decrease) scores from randomization [Osoba, 2005].

RESULTS Adherence with questionnaire completion was 65–85% for all assessment timepoints during the first year of maintenance therapy. MMRM analysis of the global health status/QoL subscale showed no difference between the treatment arms in change from randomization (least squares mean estimate 0.40 [95% CI −6.07 to 6.87]). Similar results were observed for other subscales, including the diarrhea symptom subscale.

<table>
<thead>
<tr>
<th>No. of pts (%)</th>
<th>BEV (N=94)</th>
<th>BEV–CAP (N=91)</th>
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<tbody>
<tr>
<td><strong>Week 9</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=51</td>
<td></td>
<td>N=59</td>
</tr>
<tr>
<td>Improved</td>
<td>15 (29.4)</td>
<td>17 (28.8)</td>
</tr>
<tr>
<td>Stable</td>
<td>26 (51.0)</td>
<td>34 (57.6)</td>
</tr>
<tr>
<td><strong>Week 18</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=29</td>
<td></td>
<td>N=57</td>
</tr>
<tr>
<td>Improved</td>
<td>11 (37.9)</td>
<td>12 (21.1)</td>
</tr>
<tr>
<td>Stable</td>
<td>12 (41.4)</td>
<td>30 (52.6)</td>
</tr>
<tr>
<td><strong>Week 27</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N=23</td>
<td></td>
<td>N=43</td>
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<tr>
<td></td>
<td>Week 36</td>
<td>Week 39</td>
</tr>
<tr>
<td>----------------</td>
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</tr>
<tr>
<td>Improved</td>
<td>4 (26.7)</td>
<td>14 (40.0)</td>
</tr>
<tr>
<td>Stable</td>
<td>9 (60.0)</td>
<td>17 (48.6)</td>
</tr>
</tbody>
</table>

N=15 N=35

**Week 36**

<table>
<thead>
<tr>
<th></th>
<th>N=35</th>
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<tbody>
<tr>
<td>Improved</td>
<td>14 (40.0)</td>
</tr>
<tr>
<td>Stable</td>
<td>17 (48.6)</td>
</tr>
</tbody>
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*No. of patients with completed questionnaires at both randomization and the respective week. Only weeks with ≥10 pts in both arms shown.*

**CONCLUSIONS** The IMELDA sample size was smaller than planned but protocol adherence with PRO completion was relatively high. Prespecified change from randomization and exploratory post hoc MMRM analyses of PROs suggest that the clinically meaningful PFS and OS benefit from adding CAP to BEV is achieved while maintaining QoL, with no difference between BEV and BEV–CAP treatments. Responder analyses over time showed improved or stable global health status/QoL scores in the majority of pts at each timepoint in both treatment arms.
Title: Stage-related risk categorization and influence of free margins on survival in triple negative early breast cancer - a population-based study of 2037 TNBC patients with adjuvant chemotherapy

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Body: Introduction: Triple negative breast cancer (TNBC) represents 10-20% of all breast cancer entities [1][2] and has a known aggressive behavior and poor outcome. Patients treated in the setting of randomized clinical trials often do not represent actual treatment characteristics in real-life scenarios. To determine the stage-related survival and effect of surgical performance in TNBC with current multimodal treatment, we set out to analyze data of a large population-based registry of primary breast cancers which covering >50% of all breast cancer cases in Germany.

Patients and methods: We analyzed data from a prospectively collected cancer registry of >200 certified breast units of the West-German Breast Center (WBC) in Germany from 2009-2011. From a cohort of 39570 primary breast cancer patients treated in this period, 12759 underwent adjuvant systemic therapy, out of which 2037 were TNBC cases with adjuvant chemotherapy. Inclusion criteria were triple negative breast cancers (Her2-new1+/2+ (Fish negative) and estrogen receptor (ER) and progesterone receptor (PR) <10%) and adjuvant chemotherapy, unilateral and non-metastasized breast cancer. Only those patients were included who have been followed-up within the first 3 years. Exclusion criteria were neoadjuvant chemotherapy, bilateral breast cancer and metastatic disease. The use of first, second and third generation chemotherapy was analyzed as well as the effect of clear/unclear resection margins and its impact on survival data.

Results: 2037 patients were eligible for this study. Overall survival rates were as follows: T1 a and T1b 100 %, T1c 90,7 %, T2 90,9 %, T3 68,1 % and T4 64,3 %. No statistical differences were detected in between stages T1 and T2, and also not in between T3 and T4. Combining T1/T2 and T3/T4 and performing group-wise comparisons, differences for combined stages were highly statistically significant (3.9 x E-09). Inflammatory TNBC was prognostically worst with a survival-rate of 33,3 % at 24-months. (p<0,001) Unclear resection-margins versus clear margins in TNBC exerted a negative impact on DFS (87 vs. 73 %; p=0,00002) and DDFS (p=0,0004). Age was an independent risk factor for survival with a cut-off at 35 years.(p=0,044) Third-generation chemotherapies (anthracycline+taxanes) were associated with a significant improved overall-survival at 24-months compared to first generation chemotherapies (non-anthracycline, non-taxane) (95 % vs. 87 %; p=0,0029)

Conclusion: Standard 3rd generation (anthracycline- and taxane-containing) chemotherapy and optimal surgical performance with clear margins is vital for patients with early, triple-negative breast cancer (TNBC). Within T1 and T2 stages, no stage-related deterioration of prognosis was detected, however these stages were markedly different from stages T3/T4, declining from 90-100% to 64-68 %.

This analysis of a large database of a population-based study demonstrates that tumor size, margins and guideline-adapted chemotherapy matter in triple-negative, early breast cancer.

[1] Schwentner et al. 2013
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-13-02
Average Grade: 0

Title: Nipple sparing mastectomy in patients with BRCA1 and BRCA2 mutations

Aidan T Manning¹, Andrea Pusic¹, Caitlin Wood¹, Anne Eaton¹, Michelle Stempel¹, Deborah Capko¹ and Virgilio Sacchini¹.  
¹Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Introduction: Nipple sparing mastectomy (NSM) is now performed with increasing frequency in both therapeutic and prophylactic breast surgery. The role of NSM in BRCA1 and BRCA2 mutation carriers has not been well described. The aim of this study was to review our experience with NSM in this high-risk population.

Methods: A review of the breast database was performed to identify all patients with documented BRCA mutations who underwent NSM at Memorial Sloan Kettering Cancer Center. Data extracted from the database included patient demographics, type of mutation, indication for surgery, type of reconstruction, and complications. For patients undergoing therapeutic mastectomy, data on disease stage, axillary procedures, and follow-up were also extracted.

Results: 177 NSMs (88 bilateral, 1 unilateral) were performed in 89 female patients with a documented BRCA mutation between September 2005 and December 2013. There were 56 patients with BRCA1 mutation, 26 with BRCA2 mutation, and 7 with genetic variants of uncertain significance. 26 patients had a therapeutic NSM for breast cancer (stage 0: n=6; stage 1: n=15; stage 2: n=5) and concurrent contralateral prophylactic mastectomy (CPM). The mean tumor size was 1.46cm (range, 0.1-3.5cm), and all were node negative following sentinel lymph node biopsy. 63 patients had NSM for prophylaxis. The mean age of patients undergoing therapeutic mastectomy was 41 years (range, 26-59) and prophylactic NSM was 39 years (25-59). There was an incidental diagnosis of ductal carcinoma in situ (DCIS) in 4 women undergoing CPM and 4 patients undergoing prophylactic NSM, including 1 patient diagnosed with bilateral DCIS, and an incidental diagnosis of atypia in 8 patients undergoing prophylactic NSM. In 26 patients undergoing therapeutic NSM, at median follow-up of 2.34 years (range, 0.45-6.06) there were no local or regional recurrences. One patient developed distant metastases and subsequently died from her disease, and 1 other patient died from metastatic ovarian cancer. In 63 patients undergoing prophylactic NSM, at median follow-up of 2.15 years (range, 0.11-8.14) there were no newly diagnosed breast cancers or deaths. Following NSM, 5 patients (5.6%) required subsequent excision of the nipple-areolar complex (3 cases for close or positive DCIS on final histology, 1 case for infection with necrosis, and 1 case for ongoing nipple discharge). All 89 patients had immediate breast reconstruction (tissue expander: n=80; permanent implant: n=8; autologous (DIEP) flap: n=1). Postoperative complications are shown in Table 1.

Postoperative complications following 177 nipple sparing mastectomies performed in 89 patients with BRCA mutations

<table>
<thead>
<tr>
<th></th>
<th>No. of Breasts; n (%)</th>
<th>No. of Patients; n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin desquamation</td>
<td>68 (38.4)</td>
<td>40 (44.9)</td>
</tr>
<tr>
<td>Necrosis requiring debridement</td>
<td>18 (10.2)</td>
<td>13 (14.6)</td>
</tr>
<tr>
<td>Infection</td>
<td>7 (4.0)</td>
<td>7 (7.9)</td>
</tr>
<tr>
<td>Hematoma</td>
<td>3 (1.69)</td>
<td>3 (3.4)</td>
</tr>
<tr>
<td>Complication requiring implant or tissue expander removal</td>
<td>6 (3.4)</td>
<td>6 (6.7)</td>
</tr>
</tbody>
</table>

Conclusion: NSM is an acceptable choice for patients with BRCA gene mutations undergoing therapeutic or prophylactic mastectomy with no evidence of compromise to oncological safety. This report shows an acceptable complication rate, and patients rarely required subsequent excision of the nipple-areolar complex.
Title: Variation in the use of mastectomy (MAST) in women with small node negative breast cancer (BC) treated at US academic institutions

Ines Vaz Luis¹,², Melissa E Hughes¹, Angel Cronin¹, Hope S Rugo³, Stephen B Edge⁴, Beverly Moy⁵, Richard Thériault⁶, Michael J Hassett¹, Eric P Winer¹ and Nancy U Lin¹. ¹Dana-Farber Cancer Institute, Boston, MA; ²Intituto de Medicina Molecular, Lisboa, NA, Portugal; ³University of California, San Francisco, CA; ⁴Baptist Cancer Center, Memphis, TN; ⁵Massachusetts General Hospital, Boston, MA and ⁶University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: More than two decades ago several trials have shown equivalent survival between breast conserving surgery (BCS) and MAST. Among a contemporary cohort of patients (pts) with Stage I BC who would be expected to be candidates for BCS; we examined the initial choice of surgery and factors associated with it.

Pts and methods: Prospective cohort study including pts with clinical Stage I BC treated at a National Comprehensive Cancer Network center that participated in the BC outcomes database from 2000-09. Descriptive analyses were performed examining the proportion of pts who initially underwent MAST vs BCS. Factors associated with initial surgery were analyzed using multivariable logistic regression.

Results: Of 10,249 pts with clinical Stage I BC, 2,361(23%) underwent MAST as the initial surgery and 7,888 (77%) BCS. Of those, 8% were ultimately converted to MAST. The median time from diagnosis to initial surgery was longer among the MAST group (4 vs. 6 weeks).

Patient, tumor, care and institutional factors were associated with higher rates of initial MAST: 30% of pts with <50 years of age had a MAST vs. 17% of those ≥70; 41% of pts with body mass index (BMI) < 18.5 kg/m2 (underweight) had a MAST vs. 20% of those with a BMI ≥30 kg/m2 (obese). There was significant institutional variation, with rates of initial MAST ranging from 14-30%.

Differences by tumor subtype were observed, 38% of pts with HER2+/HR- tumors had initial MAST vs. 22-28% among other subtypes. In the multivariate model, age, BMI, comorbidity, income, center, stage, tumor subtype, grade, histology and preoperative MRI were associated with the choice of initial surgery.

Multivariate logistic model to investigate factors associated with initial MAST

<table>
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<tr>
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<th>MAST vs BCS</th>
<th>OR</th>
<th>95%CI</th>
<th>P</th>
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<td>Age</td>
<td></td>
<td></td>
<td></td>
<td>&lt;0.01</td>
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<tr>
<td>&lt;50</td>
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<tr>
<td>4</td>
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<td>F</td>
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<td>G</td>
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<td><strong>Grade</strong></td>
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<td>1.1-1.4</td>
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<td><strong>Preoperative MRI</strong></td>
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</table>
Conclusions: Among a cohort of pts with small node negative BC, 23% elected to have MAST with significant variation associated with choice of treatment, while some of this variation is likely appropriate and clinically indicated, further studies to assess pt understanding of the tradeoffs between BCS and MAST is warranted. These findings need to be considered in light of the increasing number of pts who are choosing MAST/bilateral MAST.
Title: The role of preoperative MRI in negative margins after breast conserving surgery in patients with invasive breast cancer or purely DCIS

Elvira L Vos¹, Adri C Voogd², Cornelis Verhoef¹, Inge-Marie Obdeijn¹ and Linetta B Koppert¹. ¹Erasmus MC Cancer Institute, Rotterdam, Netherlands and ²Eindhoven Cancer Registry, Eindhoven, Netherlands.

Body: Introduction Clinical benefit of preoperative MRI in invasive breast cancer (IBC) patients and patients with purely ductal carcinoma-in-situ (DCIS) remains controversial. We aimed to study the role of preoperative MRI in negative margins after breast conserving surgery (BCS) and reexcision rate in a large population-based cohort.

Methods Retrospective analyses were performed in women diagnosed with IBC and purely DCIS and surgically treated between 2011-2013 extracted from the Eindhoven Cancer Registry. Patients were excluded in case of: neo-adjuvant systemic therapy, (clinical or pathological) tumor stadium T4, distant metastasis, and unknown resection margin status. According to preoperative MRI use, the study population was divided into a non-MRI and MRI group. All multivariable analyses were adjusted for baseline differences between non-MRI and MRI group with P<0.1. No information was available on the exact reason to perform MRI preoperatively in these patients.

Results A total of 3,116 patients were eligible of which 2,238 (71.8%) patients were treated by breast conserving surgery (BCS) and included for analyses. Preoperative MRI was performed in 592 (30.6%) IBC patients and 55 (18.3%) DCIS patients. In IBC patients, differences in non-MRI and MRI group were: median age (62 vs 58), histology (lobular type in 5.7% vs 24.5%), median tumor size (13 mm vs 15 mm), her2neu receptor status and specific tumor locations. Median (interquartile range) time between diagnosis and surgery in the non-MRI and MRI group were 21 (16-28) and 31 (22-40) days respectively (univariable and multivariable P<0.001). Negative margin was attained in 1,135 (84.4%) and 489 (82.6%) patients respectively (OR 0.88 95%CI 0.68-1.14 P=0.326). After adjustment for baseline differences and for factors associated with negative margin with P<0.1 (i.e. histology, tumor size, differentiation grade, progesterone receptor %, her2neu receptor status, regional lymph node stadium, and tumor location) MRI use was not associated with negative margin (OR 1.09 95%CI 0.81-1.45 P=0.587). Reexcision was performed in 96 (7.1%) and 62 (10.5%) patients (P=0.013). In case reexcision was needed, conversion to mastectomy occurred in 32 (2.4%) vs 20 (3.4%) patients (P=0.210). In patients with purely DCIS, only median age differed in non-MRI and MRI group (61 vs 57). Median (interquartile range) time between diagnosis and surgery in the non-MRI and MRI group were 22 (16-31) and 35 (23-49) days respectively (univariable P<0.001 and multivariable P=0.024). Negative margin was attained in 197 (80.1%) and 42 (76.4%) patients respectively (OR 0.80 95%CI 0.40-1.61 P=0.538). After adjustment for baseline differences and for factors associated with negative margin with P<0.1 (i.e. differentiation grade and tumor location) MRI use was not associated with negative margin (OR 1.51 95%CI 0.72-3.16 P=0.280). Reexcision was performed in 39 (15.9) and 10 (18.2) (P=0.672). In case reexcision was needed, conversion to mastectomy occurred in 12 (4.9%) vs 3 (5.5%) patients (P=0.859).

Conclusion In both IBC and DCIS patients, preoperative MRI delayed time between diagnosis and surgery, but was not associated with a higher percentage of negative margins after BCS.
Body: Introduction

Despite widespread adoption for the localization of impalpable breast lesions the wire guided localization (WGL) technique has a range of disadvantages including high re-operation rates. Radio-guided occult lesion localization (ROLL) has been proposed as an alternative technique, which may have superior clinical outcomes and be more flexible in its clinical application. A retrospective study of outcomes following ROLL therapeutic wide local excisions (WLE) and diagnostic excision biopsies (DEB) was performed. This documented 12 years experience of the ROLL technique at a single institution to provide a comprehensive analysis of the largest UK based series of ROLL excisions and sentinel node occult lesion localisations (SNOLL).

Methods

942 patients were identified who had been referred with non-palpable breast lesions from 2000-2012. 576 patients underwent WLE following a biopsy proven diagnosis of breast cancer. 366 patients underwent DEB. Prospective data collection was performed using bespoke proformas by the operating clinician. These data were supplemented with a retrospective analysis of prospectively collected data held on patient electronic records including evidence of radiological excision on specimen radiographs and histopathological characteristics including excision margin status. Sub-group analysis was performed to examine the outcomes from "same day" and "next day" protocols for ROLL/SNOLL procedures and to examine the effect of residual radioactivity levels in the excision bed on margin status.

Results

99.0% of ROLL WLE returned histological diagnoses of invasive cancer or DCIS and 98.4% of radiological abnormalities were identified on post-excision specimen radiographs. 97.5% of radiological abnormalities were identified on post-excision radiographs following DEB. Surgical localisation was rated as easy in 92.3% of excisions and 29 adverse events were recorded as a result of the localisation procedure (3.1%). Complete histological excision was recorded in 77.8% of the WLE sub-group based on defined margin criteria with a median specimen weight of 50g. 31.7% of DEB were pathologically upgraded to a diagnosis of breast cancer. No significant difference was seen in complete excisions rates between "next day" (76.4%, n=250) and "same day" (78.8%, n=326) ROLL protocols (p= NS). No significant difference was seen when levels of residual excision bed radioactivity and complete excision rates were compared (p=NS). Sentinel Lymph Node (SLN) localisation was successful in 97.6% of cases (n=205) with an SLN positivity rate of 12.7%. No significant difference was identified between SLN localisation rates for "next day" vs. "same day" SNOLL protocols (p=NS). The presence of microcalcification as the radiological abnormality (p=0.0005), underestimation of lesion size on pre-operative imaging (p=0.0005) and symptomatic referral (p=0.001) were factors that predisposed to incomplete excision margin status.

Conclusions

ROLL/SNOLL can be safely and effectively used to accurately localize impalpable breast lesions in agreement with current evidence. In addition to highly accurate localization, ROLL also has technical and logistical advantages that may make it more acceptable than WGL for clinicians and patients.
**Title:** Flat epithelial atypia on core biopsy and upgrade to cancer: A systematic review and meta-analysis

Anatoliy V Rudin¹, Hoskin L Tanya¹, Ann M Farrell¹ and Amy C Degnim¹. 'Mayo Clinic.

**Body:** BACKGROUND: Flat epithelial atypia (FEA) is a recently described breast lesion that may coexist with cancer or atypical ductal hyperplasia (ADH). Currently, there is no consensus on whether to surgically excise FEA diagnosed by core needle biopsy. Our aim was to perform a systematic review and meta-analysis of pertinent studies to determine the frequency of upgrade to cancer or ADH at surgical excision of "pure" FEA (no other high risk lesion on core biopsy).

**METHODS:** A retrospective search was performed using MEDLINE, Embase, Scopus and Web of Science databases from 1/2003- 4/2014 to capture studies on core biopsy diagnosed FEA followed by surgical excision. Search terms were: FEA, flat epithelial atypia, DIN1A, columnar cell, breast diseases, core needle biopsy. Inclusion criteria were: 1) manuscript with original data on FEA diagnosed on core needle biopsy, 2) data included outcome of cancer at surgical excision, 3) English Language.

**RESULTS:** Of 224 articles, 30 met inclusion criteria.

<table>
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<th>Study</th>
<th>Total Pure FEA cases</th>
<th>%</th>
<th>N excised</th>
<th>N upgraded to cancer</th>
<th>% upgraded</th>
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<tr>
<td>Flegg, 2010</td>
<td>NA</td>
<td>NA</td>
<td>5</td>
<td>2</td>
<td>40</td>
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<td>Sohn, 2011</td>
<td>36</td>
<td>66.7</td>
<td>24</td>
<td>2</td>
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<tr>
<td>Lavoue, 2011</td>
<td>60</td>
<td>100</td>
<td>60</td>
<td>8</td>
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<td>Rakha, 2011</td>
<td>24</td>
<td>100</td>
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<td>Soloranzo, 2011</td>
<td>33</td>
<td>84.8</td>
<td>28</td>
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<td>Verschuur-Maes, 2011</td>
<td>69</td>
<td>34.8</td>
<td>24</td>
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<td>Peres, 2012</td>
<td>128</td>
<td>79.7</td>
<td>102</td>
<td>10</td>
<td>10</td>
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<td>Uzoaru, 2012</td>
<td>145</td>
<td>65.6</td>
<td>95</td>
<td>3</td>
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<tr>
<td>Bianchi, 2012</td>
<td>190</td>
<td>100</td>
<td>190</td>
<td>18</td>
<td>9</td>
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<tr>
<td>Biggar, 2012</td>
<td>51</td>
<td>100</td>
<td>51</td>
<td>3</td>
<td>6</td>
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<td>Yamaguchi, 2012</td>
<td>17</td>
<td>47.1</td>
<td>8</td>
<td>1</td>
<td>13</td>
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<td>Polom, 2012</td>
<td>20</td>
<td>100</td>
<td>20</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Khoumais, 2013</td>
<td>104</td>
<td>90.4</td>
<td>94</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>
Across 1420 patients with pure FEA in 30 studies, observed proportions with upgrade to cancer varied from 0-40% with a pooled estimate of 11% (95% CI: 8-15%) using a random effects model. Test for heterogeneity was statistically significant (p < 0.0001, I² statistic=58%). After excluding 7 studies with <50% (or unreported %) of all FEA cases excised, heterogeneity decreased (p=0.07, I²=32%) and the pooled estimate of cancer upgrade was 9% (95% CI: 7-12%). For upgrade to ADH, 693 subjects with pure FEA were analyzed from 21 studies. The percent upgraded to ADH ranged from 0-60% with significant heterogeneity (p < 0.0001, I² = 64%). Excluding 4 studies with <50% of FEA cases excised did not improve heterogeneity (p < 0.0001 and I² = 66%). The random effects model pooled estimate of upgrade to ADH was 16% (95% CI: 11-23%) for the 21 studies and 17% (95% CI: 12-25%) for the subset of 17 studies.

CONCLUSION: The pooled upgrade rate of FEA to cancer was 9-11% and upgrade to ADH was 16-17%. For patients with FEA on core needle biopsy, surgical excision should be strongly considered.
Title: Prophylactic mastectomy in young women with breast cancer

Cristiane Metran Nascente¹, Frances Wright¹, Christel Helwig¹, Nim Li¹, Rodica Mandel¹, Alex Kiss² and Ellen Warner¹.
¹Sunnybrook Health Sciences Centre, Toronto, ON, Canada and ²ICES, Toronto, ON, Canada.

Body: Background: In recent years women with unilateral breast cancer (BC) have been increasingly requesting contralateral or bilateral prophylactic mastectomy (PM) despite only requiring unilateral mastectomy (M) or lumpectomy (L) respectively. This trend has been observed even in women at low risk for contralateral BC, particularly among younger women.

Objective: Determine the rates of ‘unexplained’ PM (UPM) and factors associated with this choice, among a prospective cohort of newly diagnosed young women with BC at our centre.

Methods: We reviewed the records of all patients diagnosed with BC at age ≤ 40 years from Feb ‘08 to Sept ‘13 at Sunnybrook who consented to have their clinical data entered in a prospective research database. Psychosocial data - HADS [Hospital Anxiety and Depression Scale] and IES [Impact of Event Scale] - were prospectively collected from all consenting women at baseline and at completion of active treatment. Among the 149 patients treated with curative intent, 43 received unilateral M, 59 L and 47 contralateral or bilateral PM. These groups were compared with respect to demographics, risk factors, use of pre-operative MRI, pathology, treatment and surgeon gender. Differences in variables between the groups were analyzed using analysis of variance for continuous variables and chi-square test for categorical variables.

Results: The mean age of all patients was 35 years old (21-40 range). Among the 47 patients who had PM, the first surgery was L for 15 (32%), unilateral M for 27 (57%) and bilateral M for 5 (11%). Twenty-four (51%) fulfilled high risk criteria (19 BRCA mutation, 4 strong family history, 1 chest radiation) and 2 (4%) had suspicious findings in the contralateral breast. The other twenty-one patients (45%) underwent UPM for "peace of mind" alone. Thirty-three (33%) of UPM patients had T3-4 tumors. There were no statistically significant differences between the UPM group and the combined M plus L groups, except for more multicentric/ multifocal disease in the UPM groups (43% vs 9% p≤0.0001).

Discussion: Fourteen percent of young women at our centre, a 1/3 with a relatively poor prognosis had UPM. Data correlating surgical choice with baseline and follow-up psychological variables will be presented. There appears to be a need for an educational/psychological intervention at the time of BC diagnosis for non-high-risk women contemplating preventive breast surgery to ensure that such a choice is truly informed.
Title: Initial experience with an ambulatory extended recovery program for patients undergoing mastectomy

Aidan T Manning¹, Danielle Cassella¹, Stacy Ugras¹, Beverly Tseng-Reyes¹ and Lisa Sclafani¹. ¹Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Introduction: An ambulatory surgical program has been introduced at Memorial Sloan Kettering Cancer Center for patients undergoing select procedures that require a single overnight stay. The aim of this study was to review the initial experience with this program for patients undergoing mastectomy and to determine the rate and cause of unanticipated hospital admission.

Methods: All patients undergoing mastectomy with or without implant based reconstruction from March 2013 to February 2014 inclusive were entered into the Ambulatory Extended Recovery (AXR) program and data were recorded in a prospectively maintained AXR database. Data on patient demographics, type of procedure performed, and whether the patient remained on the AXR program were extracted. Electronic Medical Records were reviewed for all patients who required hospital admission in order to determine the reasons for this.

Results: 926 consecutive patients (905 female, 21 male) requiring mastectomy with or without implant based reconstruction were entered into the AXR program during this one-year period (mean age was 51 years, range 21-90). The procedures performed were as follows: bilateral mastectomy with reconstruction (n=433, 46.8%); bilateral mastectomy without reconstruction (n=48, 5.2%); unilateral mastectomy with reconstruction (n=255, 27.5%); and unilateral mastectomy without reconstruction (n=190,20.5%), with or without axillary procedures. Reconstructive procedures deemed suitable for the AXR program included tissue expander or permanent implant insertion only. 861 of 926 patients (93%) remained on the AXR program and were discharged following overnight stay. 62 patients (6.7%) (61 female, 1 male) did not complete the AXR program and required hospital admission (mean age, 52 years; range, 22-81). 3 additional patients (0.3%) required hospital admission on occasions that the AXR unit was at maximum capacity. Reasons for admission are shown in Table 1. Of the 26 patients with postoperative hematoma, 17 were brought back to the Operating Room for definitive management and 9 patients were treated conservatively. Following admission, most patients (52 of 62, 83.9%) were fit for discharge after 1 day. Of the remaining 10 patients, 9 were discharged after 2 days and 1 after 5 days.

Reason for hospital admission for 62 of 926 patients scheduled for breast surgery on the Ambulatory Extended Recovery (AXR) program

<table>
<thead>
<tr>
<th>Reason for Admission</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematoma</td>
<td>26</td>
</tr>
<tr>
<td>Pain control</td>
<td>17</td>
</tr>
<tr>
<td>Nausea</td>
<td>5</td>
</tr>
<tr>
<td>Urinary Retention</td>
<td>4</td>
</tr>
<tr>
<td>Cardiac Issues</td>
<td>5</td>
</tr>
<tr>
<td>Other (respiratory issue, poor mobility, social admission, fall on ward, pyrexia)</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
</tr>
</tbody>
</table>

Conclusion: Unilateral and bilateral mastectomy, with or without implant based reconstruction, is safely performed in the setting of an AXR program. Only a small minority of patients will subsequently require hospital admission, most commonly for management of postoperative hematoma or inadequate pain control.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P2-13-09  
**Average Grade:** 6.80

**Title:** Implication of breast-conserving therapy in the subtype era of breast cancer

Sanghwa Kim¹, Hyung Seok Park¹, Jee Ye Kim¹, Jegyu Ryu¹, Seho Park¹ and Seung Il Kim¹. ¹Yonsei University College of Medicine, Seoul, Korea.

**Body:**

**Background**

Molecular subtypes of breast cancer are one of the key factors to predict clinical outcomes in women with breast cancer. Some specific subtypes like triple-negative breast cancer (TNBC) show more aggressive behaviors and worse clinical outcomes than luminal A subtype, thus there are conflicting data regarding optimum local surgical strategy for TNBC. We evaluate the clinical outcomes of women who underwent breast-conserving therapy (BCT) compared to those underwent mastectomy regarding to breast cancer subtypes using a large single center cohort.

**Methods**

A total of 5353 women who underwent BCT or mastectomy due to primary breast cancer from 1990 to 2010 were retrospectively reviewed. Cases with initial distant metastases or those with neoadjuvant chemotherapy were excluded. Clinicopathological characteristics, overall survival (OS), and recurrence-free survival (RFS) were analyzed using the Chi-square test, Kaplan-Meier survival analysis, and log-rank test. Cox proportional hazard models were used for multivariate analyses. In order to explore the role of BCT in TNBC, we performed sub-group analysis using patients with TNBC in the cohort.

**Results**

BCT was performed in 1866 cases and mastectomy in 3487 cases. The mastectomy group had higher T stage (T2-3: 53.5% vs. 26.5%, p<0.001), higher N stage (N2-3 17.1% vs. 5.1%, p<0.001), and more HER2 over-expression (11.6% vs. 8.3% p<0.001) than the BCT group had. The BCT group consisted of more Luminal A and TNBC subtypes than the mastectomy group. (Luminal A 56.2% vs 41.6%, TNBC 19.9% vs 12.7%, all p<0.001)

The 5-year RFS rates of the BCT group in luminal A, B, and TNBC were better than those in the mastectomy group (Luminal A 93.7% vs 89.4%, p<0.001, Luminal B 94.9% vs. 86.3%, p=0.01, and TNBC 92.9% vs 79.8%, p<0.001). However, the 5-year RFS of HER2-enriched subtype was not significantly different according to the operation type (p=0.85). The BCT group in luminal A and TNBC showed better OS than the mastectomy group (Luminal A, p=0.002, TNBC, p<0.001), however, the difference of OS between the BCT and mastectomy groups in luminal B and HER2-enriched subtypes was not significant (p>0.05).

In multivariate analyses, T and N stage, breast cancer subtypes, histologic grade, and the status of adjuvant chemotherapy were independent prognostic factors for RFS and OS (all p<0.05). However, The statistical significance of RFS and OS of local therapy, BCT vs. Mastectomy, according to breast cancer subtypes in the univariate analyses disappeared in multivariate analyses (HR for RFS=0.90, 95%CI=0.70-1.16, HR for OS=0.83, 95%CI=0.614-1.122). In the sub-group analysis, BCT showed comparable outcomes compared to mastectomy in patients with TNBC in multivariate analyses. (HR for RFS=0.89, 95%CI=0.38-2.06, HR for OS=1.007, 95%CI=0.987-1.028)

**Conclusions**

Clinical outcomes were not affected by surgical approaches regarding to breast cancer subtypes. BCT is an acceptable surgical approach regardless of breast cancer subtype, and even in selective patients with TNBC.
Title: Assessment of diagnostic and therapeutic value of ductoscopy biopsy in single intraductal papillary lesion compared with open surgery

Zheli Xu¹, Wanying Xing¹, Qiang Li², Deli Xing³ and Yiqi Gu¹. ¹Breast Surgery of the 3rd Clinical Medical College of Norman Bethune Health Science Center of Jilin University, Changchun, Jilin, China; ²China-Japan Union Hospital of Jilin University, Changchun, Jilin, China and ³China-Japan Union Hospital of Jilin University, Changchun, Jilin, China.

Body: Purpose
Papillary lesions (PL) account for 1–2% of all breast neoplasms and fiberoptic ductoscopy is a practical and direct method compared with open biopsy in diagnosis and treatment of intraductal papillary lesions. This study aimed to assess efficacy of ductoscopy in the diagnosis and management of single intraductal papillary lesion.

Methods
A total of 232 patients at China-Japan Union Hospital of Jilin University who were diagnosed with single intraductal papillary lesion by fiberoptic ductoscopy were enrolled from March 2011 to November 2013. All patients underwent ductoscopic papillomectomy before open surgery. The final pathologic diagnoses were made by using specimen from both ductoscopic papillomectomy and surgeries. Any intraductal papillomatous lesion or surface abnormalities were considered as positive findings during ductoscopy.

Results
Histopathologic investigations of surgically excised or ductoscopically removed lesions revealed that 217 out of 232 cases were positive. In 187 cases pathological changes were only found in ductoscopic papillomectomy specimen, while the number of cases which was only found in open biopsy was 24. In 6 cases ductoscopic papillomectomy and surgeries specimen both showed positive.

Conclusions
The accuracy of ductoscopy biopsy in diagnosis was 88.9% (193/217) and specificity was 100%. In 187 out of 217 cases (86.2%) ductoscopic papillomectomy alone could completely remove the lesion. Ductoscopy is an effective tool for diagnosis and treatment of single intraductal papillary lesion.
Title: Multimodality perioperative analgesia with paravertebral nerve block and gabapentin reduces narcotic use and hospital length of stay in mastectomy patients

Zandra H Cheng¹, Vlad Frenk¹, Jennifer D Bishop¹, Theresa Bowling¹ and Helen A Pass¹. ¹Stamford Hospital, Stamford, CT.

Body: Postoperative pain control is the major determinant in hospital length of stay (LOS) in patients undergoing mastectomy. Using a multimodality approach for peri-operative analgesia (PA) with paravertebral nerve block (PVB)(regional anesthesia) as well as including pre- and postoperative oral gabapentin, we significantly reduced both LOS and narcotic usage (NU) when compared to PVB alone or conventional postoperative management (CPM) with on demand postoperative pain medications. A single institution, retrospective chart review of patients undergoing mastectomy from 2009 to 2014 was performed (n = 129; 84 bilateral, 45 unilateral) with a subset analysis performed on patients undergoing tissue expander (TE) reconstruction (n=86) or bilateral mastectomies (n=84). Patients were grouped by PA type (CPM, PVB by catheter infusion, and PVB with gabapentin (PVB+G)). Data were analyzed via using Student t-test and significance was defined as p<0.05. As seen in the table below, LOS and NU decreased with increasing multimodal PA approach. LOS was significantly decreased by PVB+G compared to CPM and PVB for all mastectomies, bilateral mastectomies, and mastectomies with tissue expander (TE) reconstruction. NU was significantly decreased by PVB+G compared to CPM and PVB for all mastectomies and bilateral mastectomies, and trended toward decreasing NU in TE reconstruction.

<table>
<thead>
<tr>
<th>All (n = 129)</th>
<th>(1) CPM (n=51)</th>
<th>(2) PVB (n=35)</th>
<th>(3) PVB+G (n=53)</th>
<th>p 1 vs 3; p 2 vs 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOS (days)</td>
<td>2.3 (0.84)</td>
<td>2.1 (0.71)</td>
<td>1.61 (0.54)</td>
<td>&lt;0.0001; &lt;0.0005</td>
</tr>
<tr>
<td>NU (mg)</td>
<td>73 (42.9)</td>
<td>52 (32.3)</td>
<td>39 (25.3)</td>
<td>&lt;0.001; 0.04</td>
</tr>
</tbody>
</table>

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<thead>
<tr>
<th>TE reconstruction (n=86)</th>
<th>(1) CPM (n=25)</th>
<th>(2) PVB (n=21)</th>
<th>(3) PVB+G (n=40)</th>
<th>p 1 vs 3; p 2 vs 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOS (days)</td>
<td>2.2 (0.50)</td>
<td>2.3 (0.63)</td>
<td>1.61 (0.44)</td>
<td>&lt;0.001; &lt;0.001</td>
</tr>
<tr>
<td>NU (mg)</td>
<td>86 (32.6)</td>
<td>55 (34.4)</td>
<td>43 (36.3)</td>
<td>&lt;0.001; 0.10</td>
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</table>

<table>
<thead>
<tr>
<th>Bilateral (n=84)</th>
<th>(1) CPM (n=20)</th>
<th>(2) PVB (n=24)</th>
<th>(3) PVB+G (n=40)</th>
<th>p 1 vs 3; p 2 vs 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOS (days)</td>
<td>2.4 (0.7)</td>
<td>2.2 (0.72)</td>
<td>1.65 (0.52)</td>
<td>&lt;0.009; &lt;0.001</td>
</tr>
<tr>
<td>NU (mg)</td>
<td>84 (37.4)</td>
<td>58 (32.4)</td>
<td>38 (24.5)</td>
<td>&lt;0.001; 0.0066</td>
</tr>
</tbody>
</table>

NU = narcotic use normalized to morphine sulfate

We have found employing a multimodality approach to PA with the addition of the GABA analogue, gabapentin, to regional anesthesia via PVB catheter infusion significantly improves the postoperative course of patients undergoing mastectomy procedures by decreasing LOS and NU.
Title: Does the implementation of an enhanced recovery programme impact on post-operative outcomes in populations with significant comorbidity and social deprivation?

Ee Von Woon¹, Adrian Wong¹, Juliette Murray¹ and Alison Lannigan¹. ¹Wishaw General Hospital, Wishaw, Lanarkshire, United Kingdom.

Body: Background:
Enhanced Recovery Programmes (ERP) are a well established evidence-based model of care which are intended to reduce the impact of surgery and safely reduce the length of inpatient stay for surgical patients. In Lanarkshire, Scotland, the ERP was introduced in Wishaw General Hospital in 2012 but has yet to be extended to neighbouring Monklands Hospital. We audited the impact of the ERP by comparing post-operative outcomes between these district general hospitals in the same health board servicing adjacent catchment areas which are both in areas of significant social deprivation.

Materials and Methods:
All patients who underwent breast surgery from August 2012 to August 2013 inclusive were identified from a prospectively collected electronic database. Parameters analysed included ASA grades, length of postoperative stay, rate of post-operative complications and re-admissions. The relative social deprivation of patients was calculated by cross referencing their postcodes with the Scottish Index of Multiple Deprivation (SIMD) 2012.

Results:
294 and 152 patients underwent 336 and 161 breast operations in Wishaw and Monklands respectively. The mean age of these patients was 57 in Wishaw and 54 in Monklands. 30% of patients in Wishaw and 19% of patients in Monklands had ASA grade 3 (range = 1 to 3, p=0.08). In both hospitals, the most common diagnosis was breast cancer (Wishaw: n=257, 76%; Monklands: n=110, 68%; p=0.17) and the most common procedure performed was wide local excision (Wishaw: n=235, 70%; Monklands: n=114, 71%; p=0.73). The mean postoperative stay was 0.9 days in Wishaw compared to 2.0 days in Monklands (p<0.001). Postoperative complications were higher in Monklands (n=37, 23%) compared to Wishaw (n=52, 15%, p=0.04). There was no significant difference in the ASA grades of patients who developed complications, rates of readmissions, A&E visits or reoperation (p>0.05). Although the Wishaw patient cohort is living in significantly more deprived areas (mean SIMD rank=2543) compared to the Monklands patient cohort (mean=2915, p=0.02), there was no significant difference in deprivation status between patients with complications in these two catchment areas (p=0.65).

Conclusion:
The ERP is a safe and effective protocol for breast surgery patients with low complication rates and its implementation halved the inpatient admission time in our cohort. The savings derived from this would outweigh the running costs of the ERP.
Title: Predictors of bilateral mastectomy in breast cancer patients

Yun Fu1,2, Zhigang Zhuang2, Michelle Dewing1,4, Apple Sophia3 and Chang R Helena1. 1Revlon/UCLA Breast Center, , David Geffen School of Medicine at UCLA, Los Angeles, CA; 2Shanghai First Maternity and Infant Hospital, Tongji University School of Medicine, Shanghai, China; 3David Geffen School of Medicine at UCLA, Los Angeles, CA and 4University of Colorado Health Breast Surgery, Colorado Springs, CO.

Body: Background: Breast cancer surgery has experienced an evolutilional change from radical mastectomy to conservative techniques due to the migration of stage of disease at diagnosis and adjuvant use of multimodality treatment. While breast conservation is preferred by most, the trend of bilateral mastectomy has been on the rise in the United States. The aim of this study is to determine factors that may affect patients’ choice of bilateral mastectomy.

Methods: This is a retrospective study of 376 patients diagnosed with primary invasive breast cancer who were treated by bilateral or unilateral mastectomy (BM or UM) at the Revlon/UCLA Breast Center between Jan. 2002 and Dec. 2010. Patients in the bilateral mastectomy (BM) group were further divided into groups of bilateral mastectomy for bilateral breast cancer and for unilateral breast cancer and contralateral prophylactic mastectomy.

Results: When compared with the UM group, the following factors were found to be associated with the BM: younger age (p<0.001), pre-menopause (p<0.001), having a family history of breast cancer (p<0.001) or ovarian cancer (p=0.017), BRCA 1 and 2 mutations (p<0.001, p=0.011, respectively), more breast biopsies (p=0.007), history of breast augmentation (p=0.014), more MRI study within 6 months before the surgery (p=0.029), more likely to have reconstruction surgery (p<0.001) and sentinel lymph node biopsy (SLNB) (p<0.001). Multivariate analysis indicated that patients with smaller tumor size (p<0.001, OR 0.087), negative nodes (p<0.001), sentinel lymph node biopsy as nodal surgery (p<0.001, OR 0.259), BRCA mutation (p=0.020, OR 6.537) and positive family history (p=0.001, OR 2.732) were more likely to choose bilateral mastectomy with reconstruction using tissue expanders or implants (p<0.001, OR 4.546).

Conclusion: Bilateral mastectomy is associated with lower TN stage, requiring only SNLB, presence of BRCA mutation and/or high risk family history. Tissue expanders or implants based reconstructions were more frequently chosen by patients with bilateral mastectomy.
Title: Does therapeutic mammoplasty reduce mastectomy rates?

Jennifer Pollard¹, Pang Wong¹, James Mansell², Juliette Murray¹, Alison Lannigan¹, Julie Doughty², Laszlo Romics³, Sheila Stallard² and Christopher Wilson². ¹NHS Lanarkshire, Wishaw; ²Western Infirmary, Glasgow and ³Victoria Royal Infirmary, Glasgow.

Body: Introduction: Therapeutic mammoplasty (TM) is increasing in popularity as a method for enhancing breast conserving surgery. Studies have shown it’s oncologically safe, whilst improving cosmetic outcome1,2,3. Therapeutic mammoplasty has been gaining popularity in West of Scotland over the past five years. Initially it was thought this may reduce requirement for mastectomy and immediate reconstruction. We have recorded type of surgery patients would have required had they not been suitable for TM, looking at changing demographic of surgical workload.

Methods: Prospective data collected about patients undergoing TM in West of Scotland since 2011 in Victoria Infirmary, WesternInfirmary and in NHS Lanarkshire. We reviewed clinical indications for TM, surgical alternative, Body Mass Index (BMI) and smoker status.

Results: Seventy-nine patients were identified. In 67 cases, alternative surgical option of mastectomy or standard conservation was recorded. Mean BMI was 29. 41% of patients had contralateral surgery for symmetry at the same time. In 28 cases (35%) TM avoided need for mastectomy. In 39 cases (49%) it was felt that cosmetic result would be improved by TM compared with standard conservation. During the study period, rates of mastectomy with immediate reconstruction as a proportion of total number of treated cancers have remained similar.

Conclusions: Whilst introduction of therapeutic mammoplasty in our region has improved options offered to patients and likely cosmetic outcomes, it has not had a major impact in reducing mastectomy rates or demand for immediate reconstruction. It has probably increased surgical workload of plastic surgeons as these cases are often performed as joint procedures.

References:


Title: Intraoperative ultrasound guided wire bracketing of nonpalpable breast lesions for excision

David T Rock¹, Aimee L Stewart¹ and Samith Sandadi¹. ¹Regional Breast Care, 21st Century Oncology, Fort Myers, FL.

Body:

Background. Mammographic wire localization is the primary means of localizing nonpalpable breast lesions for excision. Unfortunately, that procedure is associated with patient discomfort and vasovagal responses, scheduling difficulties, and problems with inaccurate wire placement or wire displacement. In addition, the wires often enter far from the target and take an indirect course resulting in high positive margin rates. We reviewed our experience with intraoperative ultrasound guided bracketing of nonpalpable lesions to determine if the accuracy of the excision and patient factors could be improved.

Methods. Patients that underwent intraoperative ultrasound guided wire bracketing were identified by electronic chart review. After the patient was anesthetized, high frequency linear array ultrasound was used to identify the target lesion. Under direct ultrasound visualization, the lesions were bracketed with 4 hook-wires placed perpendicular to the chest wall at the radial and antiradial margins of the lesion. Excision of the lesion was then performed with circumferential dissection around all 4 localization wires.

Results. Intraoperative localization was planned on 119 patients. The lesion was identified by preoperative ultrasound in 110 of those patients (92%). The remaining patients required mammographic localization. In 43 patients (85%) the lesion was identified for bracketing, in the remaining 17 patients the biopsy clip was seen by ultrasound and bracketed. Only 3 patients had positive margins (no tumor touching ink) requiring re-excision (2.7%).

Conclusions. The results of our experience show that intraoperative ultrasound guided wire bracketing is a reliable alternative to mammographic localization and is associated with a lower positive margin rate than reported for mammographic wire localization with a single wire. In addition, patient comfort is improved, cost is reduced, and there are no scheduling issues. The technique is especially useful for oncoplastic excisions where the incision is remote from the target lesion.
Title: Impact on survival of primary tumor resection in women with de novo metastatic breast cancer. The GEICAM Alamo I-III breast cancer registry (1990-2001)

Sara López-Tarruella1, María José Escudero17, Miguel Martín1, Carlos Jara2, Ángel Guerrero3, Ana Lluch4, Ana Santaballa5, Purificación Martínez del Prado6, Juan Lao7, Emilio Alba8, Antonio Fernández9, Raquel Andrés10, Antonio Liombart11, Norberto Batista12, Ignacio Porras13, José Manuel López-Vega14, Encarnia Adrover15, Lourdes Calvo16 and Eva Carrasco17.

1IIS Gregorio Marañón, Madrid, Spain; 2F H de Alcorcón, Alcorcón, Spain; 3IVO, Valencia, Spain; 4H C Valencia, Valencia, Spain; 5H U La Fe, Valencia, Spain; 6H U Basurto, Bilbao, Spain; 7H G U Miguel Servet, Zaragoza, Spain; 8C H Virgen de la Victoria, Málaga, Spain; 9CHUA, Albacete, Spain; 10H C U Lozano Blesa, Zaragoza, Spain; 11H U Arnau de Vilanova, Lleida, Spain; 12H U de Canarias, Sta Cruz de Tenerife, Spain; 13C H Reina Sofía, Córdoba, Spain; 14H U Marqués de Valdecilla, Santander, Spain; 15C O Juan Canalejo, A Coruña, Spain; 16ISCIII, Madrid, Spain and 17GEICAM, San Sebastián de los Reyes, Madrid, Spain.

Body: Introduction: Retrospective data from institutional series and population-based databases have suggested a potential benefit of the primary tumor (PT) surgery in de novo metastatic breast cancer (MBC) patients (pts). Recently reported prospective data from 2 randomized trials and a multicenter registry questioned the real role of the local approach in the modern individualized systemic treatment era. Methods: The ALAMO (A) is a retrospective analysis of pts diagnosed with BC between 1990 and 2001 across 56 GEICAM hospitals in Spain. Patterns of BC presentation (tumor and host characteristics), treatment and survival were recorded in 3 cohorts, AI (1990-93, 4529 pts, closed by 2000), AII (1994-97, 10453 pts, closed by 2003) and AIII (1998-2001, 10675 pts, closed by 2007). MBC pts at first diagnosis excluding those without complete information about their PT surgery were included. Descriptive, Kaplan-Meier and Cox regression analyses were carried out. Results: 5.5% (N=1415) of the ALAMO database pts were initially diagnosed with MBC, 1331 fulfilled the present analysis criteria (327 from AI, 619 from AII and 385 from AIII). Median age was 63.1 years (range: 21.6-96.0), 51.8% had single-organ metastasis, and their distribution according to the predominant site of disease was skin/soft tissue (16.2%), bone (33.7%), and visceral (48.4%). Surgery of the PT was done in 44.5% (N=592) of pts (512 with radical procedures, 46 with palliative procedures and 34 unknown); besides, 427 pts underwent axillary dissection. Initial local treatment was the choice for 380 pts (358 surgery and 22 radiotherapy), 722 received initial systemic therapy (480 chemotherapy, 214 endocrine treatment and 28 both), 29 received best supportive care and for 200 pts the treatment sequence could not be established. Pts in the surgery (S) group were younger (19.5% vs 11.8% were <44 years-old in the S vs non-S group respectively), with oligometastatic disease (61.9% vs 43.9% with single-organ involvement in the S vs non-S group respectively) and with different sites of disease (40.2% vs 54.3% with visceral and 39% vs 29.8% with bone metastasis in the S vs non-S group respectively). With a median follow-up of 1.9 years, the 5-yr overall survival (OS) was 25.4% in the entire de novo MBC population, with a median OS of 3.3 yrs in the S-group vs 1.9 yrs in the non-S-group (HR 1.69, p<0.0001). Subgroup analyses showed a benefit of PT surgery in OS regardless the number of metastasis and site of disease, but didn't show this benefit analyzing pts according to BC subtypes. The multi-adjusted HR for surgery was 1.38 (p=0.037). The multivariate Cox regression analysis model included the site of disease (p=0.028), the histopathologic grade (p=0.019) and the hormone receptor status (p=0.007). Discussion: The Alamo data line up with previously reported population-based registries, which highlight the better survival outcome of de novo MBC pts undergoing PT surgery. However, the consideration of the biological heterogeneity of BC has changed the landscape of systemic treatment. Only well designed randomized controlled trials will have the power to discriminate between a consistent bias and a real biologic effect of the PT surgery.
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-13-18
Average Grade: 5.67

Title: Oncologic outcome of 2,217 patients with breast cancer after negative sentinel lymph node biopsy without axillary lymph node dissection

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Body: Background
Sentinel lymph node biopsy (SLNB) is a standard method for nodal staging and enables to omit axillary lymph node dissection after negative sentinel lymph node (SLN). False-negative rate of SLNB has been reported as 5-7%. To dissolve the concerns regarding the axillary recurrence due to false-negative cases after negative SLNB, we investigated oncologic outcomes of a large number of breast cancer patients with negative SLN after SLNB.

Methods
A total of 2,475 patients with clinically node-negative breast cancer underwent SLNB at Severance hospitals between December 1998 and December 2013. ⁹⁹m Tc radiocolloid or blue dye was used in SLNB with periareolar intradermal injection technique. Intraoperative frozen examination of SLN was performed. If frozen test for SLN showed negative result, further axillary node dissection was omitted. Survival rates were estimated by the Kaplan-Meier method. Multivariate survival analysis was performed using the Cox-regression hazard model.

Results
Among 2,475 patients, 2,217 patients had no metastatic focus on SLN. A mean of 2.4 sentinel lymph nodes was dissected in all of the patients. At a median follow-up of 36 months, only 13 patients (0.6%) had axillary recurrence, and a median time to the recurrence was 20 months (range 7-45 months). During the follow-up period, 22 local, 16 regional, and 44 distant recurrences were observed. In multivariate analysis for axillary recurrence, tumor size and refusal of systemic therapy were demonstrated to be independent risk factors for axillary recurrence (hazard ratio (HR) 1.77, 95% confidence interval (CI), 1.08-2.89, \( P=0.024 \); HR 0.15, 95% CI, 0.03-0.75, \( P=0.021 \)). Disease-free survival and axillary recurrence-free survival rates at 3 years were 96.5% (95% CI, 0.960-0.970) and 99.2% (95% CI, 0.990-0.994), respectively.

Conclusions
The axillary recurrence rate was very low in patients with negative SLN. Our findings supported that SLNB is a reliable procedure and its oncologic safety is not affected by the chance of axillary recurrence after negative SLNB.
Intraductal Papillomas and upgrade rates on excision

<table>
<thead>
<tr>
<th>Upgrade rate(%) in the routine excision studies</th>
<th>Upgrade rates(%) in the observation group</th>
</tr>
</thead>
<tbody>
<tr>
<td>19</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>8.9</td>
</tr>
<tr>
<td>39</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>1.6</td>
</tr>
<tr>
<td>23</td>
<td>0</td>
</tr>
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<td>3.1</td>
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<td>11.5</td>
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<td>Numb of studies</td>
<td>7</td>
</tr>
<tr>
<td>Average rate</td>
<td>17.23</td>
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<td></td>
<td>1.79</td>
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</table>

Clinical presentation included: Palpable mass, nipple discharge, itching, mammographic mass asymmetry or calcifications. This study found 69.3% of papillary lesions presented as mammographic findings including nodules asymmetry (28%), calcifications (24%), masses (16.7%). The remaining 29.3% of the papillary lesions presented as palpable masses (14.7%), discharge (4%), itching (2.67%), and pain/tenderness (1.3%).

Conclusion: IDPs present as a radiologic finding in 2/3 of the cases and a clinical complaint in the remaining patients. A review of the upgrade rates in the literature shows a range of 0-39%. Although larger cores from vacuum assisted biopsies may lower the upgrade rates, a good number of studies show rates that do not justify observation only. As concerning is the association of papillary lesions with atypia and malignancy in 32.3% of our patients indicating a possible causal relation. Our data and review of the literature indicate that short of a prospective randomized trial to settle this question, IDPs should be routinely excised.
Title: Randomized clinical trial comparing 2-octylcyanoacrylate versus intradermic suture with nylon: Similar cosmetic results with different safety profile

Ruffo Freitas-Junior¹, Thiago S Becker¹, Rosemar MS Rahal¹ and Regis R Paulinelli¹. ¹Federal University of Goias (UFG), Goiania, Goias, Brazil.

Body: Introduction: There are multiple options available for closing surgical incisions. This study compared the cosmetic results between the use of 2-octylcyanoacrylate and nylon sutures in elective breast surgery. Objectives: To compare the efficacy and cosmetic outcome between the adhesive and conventional suturing with nylon on the cutaneous synthesis mammary surgeries. Methods: we performed a prospective, randomized, controlled trial with 79 patients, 37 in group 2-octylcyanoacrilate and 42 in the group with nylon suture. We evaluated the surgical aspect of the scar in 40 and 180 days, the occurrence of complications (such as dehiscence, hematoma, infection and allergic reactions), the size of the wound and breast lesions, surgical time and skin closure time, hemoglobin and preoperative WBC, age, height and weight of patients. Statistical analysis was performed by t test and chi-square. Results and discussion: the study was stopped before the end of the recruitment of patients for the presence of a greater number of dehiscence in the adhesive group (OR: 11.42, 95% CI 1.36 - 96.02, p=0.007). There were no significant differences between the groups regarding other complications, not in relation to surgical time and the aesthetic appearance of scars after 40 and 180 days. The average size of the wound was greater in the adhesive group than in the suture, being respectively 32.97 (+ 10.54) mm and 27.64 (+ 9.56) mm, no correlation of size with the largest number of dehiscence. Conclusion: The results showed that the cosmetic scar appearance of 2-octylcyanoacrilate is equivalent to those obtained with the intradermal nylon suture, but the risk of dehiscence is higher.
2014 San Antonio Breast Cancer Symposium

Title: Multiple radioactive seed localization in breast conserving surgery for multifocal or extensive DCIS

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Body: Background: Radioactive seed localization (RSL) has been introduced as an alternative to wire localization for guiding surgical excision of nonpalpable breast lesions. The goal was to determine whether the use of multiple seeds makes conservative management of the breast in case of extensive or multifocal ductal carcinoma in situ (DCIS) feasible.

Methods: A retrospective chart review was performed which included women with confirmed pure DCIS undergoing multiple seed RSL followed by breast conserving surgery (BCS). Outcome measures included positive margin and re-operation rates, specimen weight and the need for oncoplastic reconstruction after resection of the primary lesional area.

Results: Between January 2008 and September 2013, 28 patients with DCIS were localized with multiple (≥ 2) 125I seeds. Multiple seeds were implanted because of multifocal DCIS in 12 patients (42.9%) or because of extensive DCIS in 16 patients (57.1%). Seventeen patients (60.7%) underwent simultaneous oncoplastic reconstruction.

The mean specimen weight was 81.5 grams (median 64, range 12- 340 grams), and varied from 42.4 grams (median 35, range 12 - 101 grams) in the patients without oncoplastic reconstruction to 103.5 grams (median 84.5, range 46 - 340 grams) in the patients with oncoplastic reconstruction. The positive margin rate was 25% (7/28). Of these, 3 were focally positive and 4 were more than focally positive and required a re-excision.

Conclusions: By using multiple 125I seeds to localize extensive or multifocal DCIS, breast conserving surgery becomes an effective and safe option in this group of patients with acceptable outcome measures.
Title: Treatment of benign breast tumor with ultrasound-guided radiofrequency ablation

Zheli Xu1, Denghua Sun1, Guang Sun1, Yanxi Liu1 and Xinyu Feng1. 1Breast Surgery of the 3rd Clinical Medical College of Norman Bethune Health Science Center of Jilin University, Changchun, Jilin, China.

Body: Purpose To evaluate the safety and efficacy of radiofrequency ablation (RFA) as a therapy for benign breast mass in terms of ablation lesion size and temperature.

Methods This study had institutional review board approval, and written informed consent was obtained. A total of 230 patients (122 solid cases and 108 cystic cases) with core-needle biopsy-proved in benign breast masses were enrolled. Under US-guidance, the RF energy was delivered through a 15-mm 13-gauge monopolar tip needle electrode by using the temperature-controlled mode. 80°, 85°, 90°, 95° were maintained for 10 minutes. The tumor and surrounding breast tissue were ablated with a saline-cooled RF electrode followed by immediate surgical resection. Resected specimens were examined by hematoxylin and eosin (H&E) staining and nicotinamide adenine dinucleotide (NADH) diaphorase staining to assess tumor viability.

Results All ablation procedures were performed successfully. For the 122 treated patients with solid tumor (≤ 2cm in diameter at 90° & 95°, ≤ 1.5cm in diameter at 85°, ≤ 1.0cm in diameter at 80°) nicotinamide adenine dinucleotide in its reduced form–diaphorase staining showed no evidence of viable cells. For the 108 treated patients with cystic tumor (≤ 1.0cm in diameter at 90° & 95°, ≤ 0.5cm in diameter at 80° & 85°) there was no evidence of viable malignant cells.

Conclusion RFA is a safe and promising minimally invasive treatment for solid tumor with diameter less than 2.0cm, while for cystic tumor with diameter less than 1.0cm at 90°. Because the thermal solidification range is larger than the damage area in cystic tumor, RFA is more suitable for solid tumor.

Key words breast tumor; ultrasound-guided; radiofrequency ablation.
Title: The oncologic safety of nipple-areolar complex skin sparing mastectomy compared with skin sparing mastectomy: 8 years follow up results

Kyung Jun Yeu¹, Jeong Yeong Park¹, Jung Eun Choi¹, Su Hwan Kang¹, Young Kyung Bae² and Soo Jung Lee¹. ¹Yeungnam University College of Medicine, Daegu, Korea and ²Yeungnam University College of Medicine, Daegu, Korea.

Body: Background: Skin sparing mastectomy (SSM) has been conducted in breast cancer patient because of both oncologic safety and cosmetic satisfaction. Preservation of nipple-areolar complex (NAC) is helpful to keep more natural breast shape, but, can cause anxiety about local recurrence. This study reviewed long term follow-up result of SSM and nipple-areolar skin sparing mastectomy (NASSM), retrospectively.

Patients and methods: This study included 272 primary breast cancer patients who received SSM (94 patients) or NASSM (178 patients) except bilateral breast cancer from September 1996 to December 2008. Frozen section was conducted for analysis of NAC resection margin status. In case of positive resection margin, NAC was sacrificed. Local recurrence and overall survival of SSM and NASSM group was analyzed.

Results: The mean follow-up was 94.9 months. 81 NAC resection margins (29.8%) were invaded by tumor cells. The positive resection margin of NAC was associated with presence of ductal carcinoma in situ (p=0.005), especially extensive intraductal component (p=0.005) and invasive carcinoma with multiplicity (p=0.048). The patients in NASSM group tended to have more worse disease free survival than those in SSM group (75.3% vs 86.2%, P=0.087). But, in analysis of only local recurrence including NAC, there were 25 cases (14.0%, 7 in skin flap and 18 in NAC) of local recurrence in NASSM group and 8 (8.5%) in SSM group. Local recurrence free survival of the NASSM group was 86.0% and that of the SSM group was 91.5% (P=0.278). Distant recurrence after surgical treatment for local relapse occurred in only one SSM case. There was no significant difference for the overall survival between NASSM and SSM group (97.8% vs 96.8%, p=0.556).

Conclusion: In this study, result of long term follow up showed that patients in NASSM group tend to have more local recurrence than patients in SSM group, even if there is no statistically significance. However, surgically well-controlled local recurrence of skin flap and/or NAC did not affect on overall survival. NASSM is alternative method for SSM with oncological safety and better cosmetic outcome.
Title: DCIS in low resource settings: The black swan of breast health care?

Miriam Mutebi¹, Lydia Cairncross¹, Eugenio Panieri¹ and Hannah Simonds¹. ¹Groote Schuur Hospital, University of Cape Town, Western Cape, South Africa.

Body: In situ carcinomas of the breast may constitute between 25-30% of all screen detected tumors in countries that practice routine screening. Of the in situ tumors, at least 80% are ductal carcinomas in situ (DCIS). In the United States, DCIS accounts for at least 25% of all newly diagnosed breast cancers. Presentations may vary in non screened populations.

Aim: To determine the clinical presentation and surgical management of patients presenting with isolated ductal carcinoma in situ at a single tertiary centre in the Western Cape, South Africa over a 5 year period. (Jan 2008-Dec 2012)

Secondary: To review the diagnostic techniques most commonly used.

Results:

42 patients with isolated DCIS were identified (41 females and 1 male) with an average age of 58 years. DCIS comprised less than 1% (42/3768) of all breast malignancies managed in this period. Most patients presented with a breast lump (23/42). 6 patients presented with nipple ulceration and 5 patients with a nipple discharge. 8 patients had their lesions picked on mammography. The diagnosis was made on core biopsy in 14 patients while 8 patients required excision of the palpable lump to make the diagnosis. 6 patients had a punch biopsy, 4 patients underwent a micro-ductectomy and 8 patients required a stereotactic biopsy. 2 patients had a diagnostic ROLL performed.

In terms of primary surgical management, 23 patients underwent a primary mastectomy, 6 patients had a WLE and 6 patients had a ROLL with therapeutic intent. 3 patients declined surgery and 1 was transferred to a different unit. 3 patients were poor surgical candidates and were placed on tamoxifen only. In terms of axillary management, 9 patients had a concurrent axillary clearance and 9 patients had a sentinel lymph node biopsy.

Of the 12 patients who had initial BCT (either ROLL or WLE), half required mastectomy for close or involved margins and 2 patients required re-excision of margins. 2 patients had an immediate reconstruction and 2 underwent a delayed reconstruction. All the axillary node clearances and sentinel lymph nodes were negative for metastatic disease.

Considerations: The spectrum of presentation of breast malignancies differs markedly in resource limited and unscreened populations. The commonest clinical presentation for DCIS was a breast lump and our incidence of isolated DCIS (1%) was much lower than that reported in international series. A number of patients with failed core biopsies required excision biopsies for diagnosis. ROLLS and stereotactic biopsies were also used to make a diagnosis, but the skills and equipment for this are frequently lacking in most regional centers. The inappropriately high axillary clearance rate for our patients could be explained by diagnostic concerns over concurrent invasive disease and the previously limited availability of sentinel lymph node biopsy facilities.

Conclusion: Though rare, the management of DCIS in this set up serves to highlight the challenges of diagnosing and managing breast malignancies in LMICs. Practical interventions like increasing health worker training in core biopsy methods, and in the use of supportive aids like ultrasound, alongside increasing human capacity in cancer diagnostics, could help to improve the management scope of cancers in LMICs.
2014 San Antonio Breast Cancer Symposium

**Publication Number:** P2-13-25
**Average Grade:** 7.17

**Title:** Cytology and histopathology evaluation of sub-nipple tissue during intraoperative and postoperative time to predict neoplastic involvement of the nipple in patients with breast carcinoma

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**Body:** Introduction: The analyses of sub nipple tissue (SNT) have been used by some surgeons to preserve or not the nipple in nipple sparing mastectomy for breast carcinoma. Therefore, the intraoperative study of SNT becomes an important tool. However, it is uncertain if the SNT evaluation can safely predict the nipple involvement for carcinoma. The aim of this study was to evaluate the accuracy, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) of intraoperative imprint cytology, intraoperative frozen section and postoperative paraffin histopathology of SNT to predict involvement of the nipple in women with breast carcinoma.

Methods: It was realized a prospective study with 38 consecutives breast carcinoma women (stage 0, I, II and III) underwent to mastectomy. It was excluded inflammatory carcinoma and clinical evident nipple involvement. After mastectomy, the nipple areolar complex were dissected in an approximate thickness of 5mm simulating nipple-sparing flap dissection. Then, the SNT were dissected and submitted to imprint citology and frozen section during intraoperative time. Subsequently, it were submitted to routine paraffin histopathology analysis. The nipple was examined separately by paraffin histopathology (considered the gold standard). We considered any atypical cells like positive findings in all exams (cytology, frozen and paraffin).

Results: The mean of patient’s age was 59 years, the mean of tumor size was 34 mm in clinical exam and 31mm in pathological exam. The clinical and mammographic means of distance from tumor to nipple were 23 and 34 mm, respectively. The imprint cytology frozen section and paraffin histopathology of SNT showed: sensitivity 42.9%, 42.9% and 57.1%; specificity 80.6%, 96.8% and 100%; accuracy 73.7%, 86.8% and 92.1%; PPV 33.3%, 75% and 100%; NPV 86.2%, 88.2% and 91.2%, respectively. When we associated both intraoperative exams (imprint cytology and frozen section), the specificity (80.6% x 100%, p=0.01) and accuracy (76.3% x 92.1%, p=0.02) were worse than postoperative exam. The false negative of postoperative analyses was 8.8%.

Conclusion: These preliminaries outcomes showed a moderate sensitivity and good specificity of three exams, the low false negative rate of postoperative paraffin exam. Our dates suggest that SNT evaluation is a good method to predict nipple involvement and possibly the postoperative evaluation (histopathology) is better than intraoperative evaluation (imprint cytology and frozen section).
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-13-26
Average Grade: 0


Mahvish Muzaffar1, Swapnil D Kachare1, Timothy L Fitzgerald1, Jan H Wong1, Kathryn Verbanac1 and Nasreen A Vohra1. 1East Carolina University, Brody School of Medicine, Greenville, NC.

Body: INTRODUCTION: The value of primary tumor resection in patients with metastatic breast cancer is a topic of ongoing debate. We aimed to analyze the trend and impact of primary tumor resection on survival over the last two decades using a national database. We hypothesized that there would be a decreasing utilization of primary tumor resection over time with an increase in disease specific survival.

METHODS: All patients with stage IV breast cancer at diagnosis between the years 1988 and 2011 were identified in the SEER database. Univariate and multivariate descriptive and survival analyses were performed.

RESULTS: A total of 41,601 patients with stage IV breast cancer were included in the study, 98.9% (41,162) were females and 1.1% were males. Forty percent underwent surgery. Table 1 summarizes the other significant differences in demographic and tumor-related characteristics of patients who did and did not receive PTR. Over the 23- year study period there was a statistically significant temporal trend of decreased primary tumor resection (62% of patients underwent PTR in 1988, 42.4% in 2000 and 27.7% in 2011). On univariate analysis, patients who underwent PTR had a greater median disease-specific survival (DSS), (34 vs. 18 months, p<0.0001). Younger age (p<0.0001), non-African American race (p<0.0001), lower T and N-stage (p<0.0001), positive hormone receptor status (p<0.0001), lower grade (p<0.0001), mucinous histology (p<0.0001), radiation therapy (p<0.0001), and surgery performed in the latter years (p<0.0001) were also associated with improved DSS. On multivariate analysis increasing age (p<0.0001), AA race (p=0.0001), higher T and N stage (p<0.0001), negative hormone receptor status (p<0.0001), higher grade (p<0.0001), no history of radiation therapy (p=0.002), and surgery in earlier years were associated with increased mortality (p<0.0001). Not undergoing PTR was independently associated with increased mortality, (p<0.0001).

CONCLUSIONS: Results from this retrospective study suggest a survival advantage in patients with stage IV breast cancer who undergo primary tumor resection. However, there has been a marked reduction in the number of patients undergoing surgery, most likely reflecting more focused patient selection. Ongoing randomized controlled trials will help address the impact of primary tumor surgery on survival of patients diagnosed with metastatic disease.

Table 1: Comparison of patient and tumor characteristics in the surgery vs no surgery group (all p<0.0001)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Surgery</th>
<th>No surgery</th>
</tr>
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<tbody>
<tr>
<td>Female (%)</td>
<td>16,328 (98.7)</td>
<td>24,750 (99.1)</td>
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<tr>
<td>White Race (%)</td>
<td>13,198 (79.7)</td>
<td>19,328 (77.4)</td>
</tr>
<tr>
<td>T1 stage (%)</td>
<td>3,067 (18.5)</td>
<td>2,096 (8.4)</td>
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<tr>
<td>N1 stage (%)</td>
<td>4,173 (25.2)</td>
<td>5,834 (23.4)</td>
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<tr>
<td>ER Positive (%)</td>
<td>9,642 (58.3)</td>
<td>12,300 (49.3)</td>
</tr>
<tr>
<td>PR Positive (%)</td>
<td>7,532 (45.5)</td>
<td>9,346 (37.4)</td>
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<tr>
<td>Her2 Positive (%)</td>
<td>414 (4.5)</td>
<td>745 (3.0)</td>
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<tr>
<td>Grade II (%)</td>
<td>4,844 (29.3)</td>
<td>5,400 (21.6)</td>
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<tr>
<td>Infiltrating ductal cancer (%)</td>
<td>11,539 (69.7)</td>
<td>12,461 (50.0)</td>
</tr>
<tr>
<td>Radiation Therapy (%)</td>
<td>6,338 (38.3)</td>
<td>6,937 (27.8)</td>
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</tbody>
</table>
Title: Nipple sparing mastectomy: Risks of wound complication in the setting of neo-adjuvant or adjuvant chemotherapy and/or radiation therapy

Selyne Samuel\textsuperscript{1}, Rebecca Viscusi\textsuperscript{1}, Amy Waer\textsuperscript{1}, Victor Gonzalez\textsuperscript{1}, Pavani Chalasani\textsuperscript{1}, Craig Hurst\textsuperscript{1}, Ethan Larson\textsuperscript{1}, Robert Livingston\textsuperscript{1} and Michele Ley\textsuperscript{1}. \textsuperscript{1}University of Arizona, Tucson, AZ.

Body: Background: Surgical care of breast cancer has evolved significantly over the past 40 years. Nipple sparing mastectomy (NSM) and skin sparing mastectomy (SSM) have become an increasingly used surgical management for women with malignant breast disease. To date, there are limited recommendations about the role of NSM in patients receiving aggressive adjuvant therapy. The purpose of this investigation is to determine whether NSM in the setting of neo-adjuvant or adjuvant chemotherapy and/or radiation therapy increased the risks for wound complications.

Methods: A retrospective chart review of nipple sparing mastectomies at a single institution was performed from 2007 to 2014. Multiple data points including neo-adjuvant or adjuvant chemotherapy and/or radiation therapy, obesity, smoking history, and type of reconstructive surgery were examined in detail.

Results: We counted the procedures by breasts affected and identified 76 NSM in the time period which met criteria. Of the 76 NSMs, 27 breasts received neo-adjuvant(20) or adjuvant chemotherapy and/or radiation(7) therapy. 21 breasts in the NSM group developed wound complications including skin flap necrosis (5), total nipple necrosis and loss (11) hematoma (2) infection(2) and seroma(1). The complications were seen in 11 in the non adjuvant treated setting (14%) and 9 (33%) in the adjuvant therapy setting.

Conclusions: Nipple sparing mastectomy are emerging as safe and adequate options for the management of malignant breast disease. Our results show there is a significant risk of wound complication associated with neo-adjuvant and adjuvant chemotherapy and radiation therapy in the setting of nipple sparing mastectomy. We are pursuing the development of new surgical techniques and guidelines to reduce these risks in these high risk patients.
Title: Flap fixation reduces seroma in patients undergoing mastectomy: A significant implication for clinical practice

James Van Bastelaar1,2, Arianne Beckers1, Maarten Snoeij1 and Yvonne Vissers1,2. 1Atrium Medical Center, Heerlen, Limburg, Netherlands and 2Orbis Medical Center, Sittard, Limburg, Netherlands.

Body: Background
Seroma formation is a common complication following mastectomy for invasive breast cancer. Seroma formation can cause problems in wound healing and infection, thus leading to seroma aspirations and repeated visits to the out-patient clinic. The key to reducing seroma formations seems to partly lie in the obliteration of dead space. However, the techniques used to achieve this goal are subjects of much controversy and debate. Mastectomy flap fixation (FF) is achieved by reducing dead space volume using interrupted subcutaneous sutures. We hypothesized that obliteration of the dead space following mastectomy would significantly reduce seroma formation, resulting in fewer outpatient visits and seroma aspirations. This study, aiming to reduce seroma formation after mastectomy, is a retrospective study that compared conventional mastectomy with mastectomy and flap fixation.

Methods
All patients undergoing mastectomy due to invasive breast cancer were eligible for inclusion. From May 2012 – March 2013 all patients undergoing mastectomy in 2 hospitals were treated using flap fixation. The skin flaps were sutured on to the pectoral muscle using polyfilament absorbable sutures. The data was retrospectively analyzed and compared to a historical control group (HC) that was not treated using flap fixation (May 2011 – March 2012). In the HC group, only the skin was sutured. Closed suction drainage was applied to all patients in both groups.

Results
180 patients were included; 92 in the FF group and 88 in the HC group. There were no significant differences in patient demographics. 36% of patients developed seroma in the group that underwent flap fixation; 59% of patients developed seroma in the historical control group (P=0.002). Seroma aspiration was performed in 15% of patients in the flap fixation group as opposed to 43% of patients in the control group (P<0.001). Multivariate analysis showed that the effect of flap fixation varied with the extent of surgery: whereas flap fixation reduced seroma formation in patients undergoing simple mastectomy or mastectomy with sentinel node recovery, FF did not reduce seroma formation in patients undergoing mastectomy with axillary lymph node dissection (i.e. modified radical mastectomy; P=0.04 for interaction).

Effects of flap fixation on seroma formation stratified by operation type

<table>
<thead>
<tr>
<th></th>
<th>Historical Control</th>
<th>Flap fixation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mastectomy</td>
<td>8/14 (57%)</td>
<td>1/6 (17%)</td>
<td>0.16</td>
</tr>
<tr>
<td>Mastectomy and SN</td>
<td>26/42 (62%)</td>
<td>13/52 (25%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Modified Radical Mastectomy</td>
<td>18/32 (56%)</td>
<td>19/34 (56%)</td>
<td>0.98</td>
</tr>
</tbody>
</table>

In contrast, flap fixation was associated with fewer seroma aspirations in all types of surgery (P=0.80 for interaction).

Effects of flap fixation on seroma aspiration stratified by operation type

<table>
<thead>
<tr>
<th></th>
<th>Historical Control</th>
<th>Flap fixation</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mastectomy</td>
<td>6/14 (43%)</td>
<td>1/6 (17%)</td>
<td>0.35</td>
</tr>
<tr>
<td>Mastectomy and SN</td>
<td>19/42 (45%)</td>
<td>7/52 (14%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Modified Radical Mastectomy</td>
<td>13/32 (41%)</td>
<td>6/34 (18%)</td>
<td>0.04</td>
</tr>
</tbody>
</table>
Conclusion
Patients undergoing mastectomy flap fixation displayed a significant reduction in seroma formation and fewer patients were subjected to seroma aspiration. Patients undergoing flap fixation required fewer seroma aspirations. Flap fixation is an effective surgical technique in reducing dead space and therefore seroma formation in patients undergoing mastectomy for IBC.
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-13-29
Average Grade: 8.00

Title: External dosimetry and in vivo measurements improve surgical comprehension of intraoperative radiotherapy using Intrabeam™

Pierre-François Dupré¹, Dounia Bouzid², Petra Miglierini¹, Olivier Pradier¹, Dimitris Visvikis², Julien Bert² and Nicolas Boussion².
¹Cancer Institut CHU, Brest, France and ²LaTIM INSERM UMR 1101 CHU Brest, Brest, France.

Body:

Background

IORT is an option for patients with limited access to radiotherapy or patients wishing to avoid the prolonged course of External Beam radiotherapy (EBRT). The recent 5-years results of the non inferiority trial TARGIT-A suggest that with a short follow-up the 5-years risk for local recurrence is greater for the IORT arm. Several options can be suggested to improve those results.

Objectives

We choose to enhance surgical comprehension of IORT with Intrabeam™ by performing in vivo measurements comparing to external dosimetry taking into account tissues heterogeneities using a preoperative CT scan.

Material and Methods

Nine patients enrolled in french randomised trial RIOP-InCA underwent Intrabeam™ procedure for breast cancer treatment. Each patient had a preoperative CT scan to simulate the treatment on a Monte Carlo platform using an x-ray source model; in order to calculate the dose actually delivered by the Intrabeam™ system which takes into account the tissues heterogeneities. In vivo measurements using thermoluminescent dosimeters are also performed to evaluate the dose to the skin. Comparisons are done between simulated and measured data. Relative depth dose curves are also compared.

Results

The median age was 68.3 years [58-87]. The mean maximal diameter of lumpectomy was 71.3 mm [50-125]. The median size was 10.8 mm [5-19] and the median margin status was 12.8 mm [2-32]. 2 patients had micro metastatic involvement of sentinel lymph node without axillary clearance. SBR score was 1 for 5 patients and 2 for 2 patients. All histological subtype were ductal, one patient presented associated DCIS. 6 patients presented luminal A phenotype, 2 luminal B and 1 luminal B with HER-2 neu over-expression. 2 patients received supplemental EBRT (HER-2 neu over expression and SLN micro metastatic involvement). 2 patients had adjuvant sequential chemotherapy and all had adjuvant ani-aromatase hormono-therapy.

In vivo measurements on the skin using TLDs gives a mean dose of 1.3 Gy ± 1.1 Gy [0.1-4.9 Gy] in comparison with external dosimetry which had a mean dose of 1.5 Gy ± 0.8 Gy [0.3-6.2 Gy] at the same positions. No patient received a dose superior to the prescription of 6 Gy. Relative depth dose curves give a mean deviation of +36.7% ± 24.8% and +50.6% ± 16.8% in the tangential and perpendicular axes respectively from the manufacturer. Using the shielding allow a reduction of the dose by 10 concerning the ribs.

<table>
<thead>
<tr>
<th>Results</th>
<th>Relative deviation</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean deviation dose to the skin</td>
<td>In vivo/external</td>
<td>13.3%</td>
</tr>
<tr>
<td>Mean deviation dose</td>
<td>Shielding/ no shielding</td>
<td>-60.2%</td>
</tr>
<tr>
<td>Depht Dose curve</td>
<td>Tangential axe</td>
<td>36.7%</td>
</tr>
<tr>
<td></td>
<td>Perpendicular axe</td>
<td>50.6%</td>
</tr>
</tbody>
</table>

Conclusions

Results about the dose as a function of depth show clearly that we cannot consider breast as equivalent to water at this energy and may taking in account breast density. It seems to be important to spread the skin correctly from the incision, to recover carefully the applicator and use the shielding in order to avoid secondary effects as skin necrosis and ribs failures.

Our X-ray source model allows to have realistic dose distribution which hepls in better surgical comprehension of IORT particularly in the set up of the applicator.
Immediate implant-based breast reconstruction following total skin-sparing mastectomy: Defining the risk of preoperative and postoperative radiation therapy on surgical outcomes

Anne Warren Peled¹, Frederick Wang¹, Robert D Foster¹, Rachel Lentz¹, Michael Alvarado¹, Cheryl A Ewing¹, Laura J Esserman¹, Barbara Fowble¹ and Hani Sbitany¹. ¹University of California, San Francisco, CA.

Body: Background
Radiation therapy is an increasingly common adjuvant treatment in breast cancer therapy. As total skin-sparing mastectomy (TSSM) and immediate reconstruction becomes more widely performed, further defining the risks of various treatment regimens on surgical and reconstructive outcomes after TSSM is important. In this study, we assess the effects of premastectomy and postmastectomy radiation therapy on outcomes following TSSM and immediate prosthetic reconstruction.

Methods
All patients undergoing TSSM and immediate tissue expander/implant reconstruction at our institution between 2006 and 2012 were identified from a prospectively maintained database. Cohort 1 included patients undergoing TSSM and reconstruction without any radiation. Cohort 2 included patients with a prior history of radiation before TSSM and reconstruction. Cohort 3 included patients undergoing radiation after TSSM and reconstruction. Complication rates were compared between cohorts.

Results
A total of 580 patients were identified undergoing 903 breast reconstructions following TSSM. Cohort 1 included 727 breasts, cohort 2 included 63 breasts, and cohort 3 included 113 breasts. Compared to patients without radiation, patients with prior radiation were more likely to develop severe infection (7.3% vs. 20.6%, p = 0.001), incisional breakdown (3.1% vs. 9.5%, p = 0.01), and expander/implant loss (5.1% vs. 20.6%, p < 0.0001). Similarly, patients with postmastectomy radiation had higher rates of severe infection (22.1%, p < 0.0001) and expander/implant loss (17.7%, p < 0.0001) compared to patients without radiation, though equivalent rates of incisional breakdown (6.1%, p = 0.57). All three cohorts showed similar low rates of partial or complete nipple-areolar complex necrosis (1.2% vs. 3.2% vs. 0%, respectively).

Conclusions
Both preoperative and postoperative radiation following TSSM and immediate prosthetic reconstruction result in higher, but acceptable, complication risks. Risks of infection and expander/implant loss in irradiated patients who have undergone TSSM are similar to those reported after skin-sparing mastectomy. Further, the low rates of complications related to nipple-areolar complex skin preservation in irradiated patients are equivalent to those seen in non-irradiated patients, supporting the safety of performing TSSM in patients who require radiation therapy.
Title: Complications of breast reconstruction in a provincial population of breast cancer survivors

Elaine S Wai¹,³, Ling Hong Lu², Cheryl Alexander¹, Mary L Lesperance², Mary L McBride¹,³, Scott Tyldesley¹,³ and Chris Taylor³. ¹BC Cancer Agency â–“ Vancouver Island Centre, Victoria, BC, Canada; ²University of Victoria, Victoria, BC, Canada and ³University of British Columbia, Vancouver, BC, Canada.

Body: Background: Breast cancer is the most prevalent cancer in women in Canada. There are varying reports of the use of breast reconstruction in various populations and very few prospective trials or population reports of complications related to breast reconstruction in breast cancer patients. The goal of this study is to describe the use of breast reconstruction in breast cancer survivors in British Columbia, the risk of complications after breast reconstruction, and factors associated with a higher risk of complications.

Methods: Electronic Records from the BC Cancer Agency were used to identify women diagnosed with invasive breast cancer between 2001-2008 in British Columbia. Administrative hospital records used with permission of the British Columbia Ministry of Health, obtained through PopulationData BC, were used to obtain details about all surgeries, hospital admissions and associated complications for the cohort. Analyses of reconstruction for those treated with breast-conserving surgery (BCS) versus mastectomy were done separately. For those who underwent mastectomy following BCS, complications after BCS before mastectomy were attributed to the BCS, and those that occurred after mastectomy were attributed to the mastectomy. These analyses included a description of the type of reconstruction procedures used in this era, and the frequency and types of complications after the reconstruction procedures. Logistic regression was used to identify risk factors for complications post reconstruction.

Results: The cohort consisted of 18,642 women; 4868 had mastectomy alone, 8633 had BCS alone, 2894 had both, and 2247 had neither surgery. Of those undergoing BCS (11527), 746 had reconstructive procedures. Most of these were separate procedures; 55% were ≤50 years of age. The most common complication was "miscellaneous complication of internal prosthetic devices, implants or grafts". One hundred eighty (14%) of those with reconstruction after BCS had complications within 30 days of the reconstruction procedure, 134 (18%) had complications within one year of the procedure.

Of those undergoing mastectomy (7762), 7497 had their mastectomies within 1 year of diagnosis. Ninety-six had bilateral mastectomies. 1766 underwent reconstructive procedures; 74% were immediate procedures, 57% were ≤ 50 years of age. The most common complication was "miscellaneous complication of internal prosthetic devices, implants or grafts". One hundred sixty-seven (9%) of those with reconstruction after (or with) mastectomy had complications within 30 days of the reconstruction procedure, 227 (13%) had complications within one year of the procedure. Regression analysis showed that no radiotherapy (OR=0.65) and premenopausal status (OR=0.70) were significantly associated with a lower risk of complication post reconstruction.

Conclusion: Complications related to reconstructive procedures for breast cancer happened in the minority of breast cancer survivors. They were more common in those with BCS, and were more likely for those who underwent adjuvant radiotherapy or were postmenopausal.
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-14-03
Average Grade: 5.60

Title: Mastectomy without drains: Flap adhesion and closure of dead space using TissuGlu® vs standard wound closure (SWC) - a parallel cohort comparison

Stefan Paepke1. 1Klinikum rechts der Isar der Technischen Universität München, Frauenklinik, Munich, Bavaria, Germany.

Body: Background: Mastectomy(ME) remains associated with high rates of minor complications and occasional revision surgery for wound healing/seroma. Closed suction drain use in an effort to reduce seroma related complications is the current standard procedure but drain efficacy has been contested in multiple reports. Mechanical closure of dead space with suturing techniques has been shown to be effective but this is often not practical in these patients and increases operating time. Fibrin sealants may reduce early drainage volume but have not been shown to allow elimination of drains. Recent reports have shown that closure of dead space can be achieved through the use of a new resorbable surgical adhesive (TissuGlu®, Cohera Medical, Pittsburgh) allowing for the elimination of drain use in that procedure. Our work with this adhesive began in 2013, with use initially limited to revisions and patients at high risk of postoperative complications; later extended use to ME in the general patient population without drain placement. Here we present the results of our prospective observation of postoperative outcomes in this early series.

Materials & Methods: A parallel cohort comparison was performed for non-inferiority of TG/No Drains vs. SWC with respect to postoperative fluid management and seroma related complications. All primary ME +/- SLNB and/or ALND were prospectively documented for operative procedure variables and outcomes, including male patients and bilateral cases. 56 MEs patients (66 MEs) from Feb 2013 through Feb 2014 were documented and analyzed. Total drained volume, aspirations, minor wound healing complications and need for revision surgery were compared.

Results: Patient demographics and procedure variables were comparable in the two groups.

<table>
<thead>
<tr>
<th>Pt demographics &amp; procedure variables</th>
<th>SWC</th>
<th>TG/ND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>35</td>
<td>21</td>
</tr>
<tr>
<td>Mean Age</td>
<td>61.5</td>
<td>62.7</td>
</tr>
<tr>
<td>Mean BMI</td>
<td>25.7</td>
<td>25.7</td>
</tr>
</tbody>
</table>

Mean total drained + aspirated fluid per ME was 78 ml in the TG/No Drain group vs. 512 ml in the SWC group, an 85% reduction (p=0.00). Four MEs required aspiration in the test group (15%) vs. 10 (25%) in the SWC group (ns). There were 3 revisions in the SWC group and none in the test group (ns).

Outcomes

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>SWC</th>
<th>TG/ND</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (mastectomies)</td>
<td>40</td>
<td>26</td>
<td></td>
</tr>
<tr>
<td>Mean Days with drains</td>
<td>6.4</td>
<td>0</td>
<td>-100%</td>
</tr>
<tr>
<td>Mean Total Drained Volume/mast. (ml)</td>
<td>463</td>
<td>0</td>
<td>-100%</td>
</tr>
<tr>
<td>Mean Total Drained + Aspirated (ml)/mast</td>
<td>512</td>
<td>78</td>
<td>0.000 -85% Mann-Whitney</td>
</tr>
<tr>
<td>Number (%) of mast. aspirated</td>
<td>10</td>
<td>25%</td>
<td>4  15% 0.539 -38% Fisher's Exact due to Small # of incidence</td>
</tr>
<tr>
<td>Number (%) of mast. w minor complications</td>
<td>13</td>
<td>33%</td>
<td>7  27% 0.625 -17% two proportion</td>
</tr>
<tr>
<td>Number (%) of mast. Requiring revision</td>
<td>3</td>
<td>8%</td>
<td>-   0% 0.273 -100% Fisher's Exact Due to Small # of incidence</td>
</tr>
</tbody>
</table>
**Discussion/Conclusions**: Our experience suggests a clear advantage in the TG/no-drain approach in mastectomy. In addition to the improved patient comfort and earlier discharge with TG, patients with drains had significantly higher total fluid collection and still required more aspirations on average than the test group. Non-inferiority for minor complications was not only achieved, but there seems to be a trend toward reduced complications in the no drain cohort. A prospective, randomized study is planned to verify our initial impressions.
Title: Is the combination of fat grafts and platelet rich plasma effective and safe? An experimental study in rats

Ruffo Freitas-Junior¹, Alexandre R Blumenschein¹, Marise AR Moreira¹, Maria-Auxiliadora PC Cysneiros¹ and Roseana N Pereira¹. ¹Federal University of Goias (UFG), Goiania, Goias, Brazil.

Body: Autologous fat grafts and lipofilling can be used for breast reconstruction after breast conserving therapy. Fat is considered an ideal filler because of its low cost, ease of harvest, abundance in the human body and low immunogenic and allergic reaction due to its autologous nature. Platelet rich plasma (PRP) is a plasma fraction, with platelet count above baseline, generally obtained via centrifugation of blood. PRP theoretically promotes tissue regeneration due to fact that it concentrates a greater amount of growth factors essential in the process of tissue regeneration and neovascularization. This paper aims to examine if the association of fat grafts and PRP improves graft viability in female rats, through an experimental, randomized and blinded study, which involved 47 animals. These animals underwent fat graft harvest from their inguinal fat deposits and fat grafting subcutaneously to their cranial region. In 22 animals the fat graft was mixed with PRP and in 25 the fat was grafted by itself. After a 100 day period, the animals were sacrificed and the fat grafts were analyzed using scores from 0 (absent) to 4 (abundant), in optical microscopy by two independent and blinded pathologists, by means of the following variables: fat graft cell viability, fat necrosis, tissue inflammation and fibrosis. Regarding fat graft cell viability, the PRP group scored moderate/abundant in 63% of the cases and the fat graft only group scored absent/slight in 72% of the cases (p<0.05). The PRP group presented lower fat necrosis scores and lower tissue inflammation scores when compared to the fat graft only group (p<0.05). The presence of tissue fibrosis was rarely observed in both groups. Tumors (dermoid cysts) within the fat grafts were observed in 3 animals in which the grafts were mixed with PRP. It is concluded that PRP improves the viability and integration of fat grafts in rats, but more studies are needed to fully understand the exact mechanisms that lead to this improvement and assess the safety of the method for use in humans.
Title: Prediction of local recurrence following mastectomy

Sarah Al-Himdani, Simon Timbrell, Kian Tan, Julie Morris and Nigel Bundred. 'University Hospital of South Manchester NHS Foundation Trust, Manchester, United Kingdom.

Body: Background: Rates of local recurrence following mastectomy vary considerably in the literature, ranging from 1.7% to 11.5%. Previous studies have attempted to identify risk factors for recurrence and whether these relate to aggressive disease or to inadequate local treatment. We aimed to identify local recurrence rates at our institution to determine risk factors associated with local recurrence.

Methods and materials: A retrospective study of all women undergoing mastectomies for unilateral T1-T3 breast cancer between 2000-2005 was undertaken. Kaplan-Meier curves were created and the log-rank test calculated to assess for differences between groups. The incidence of local, regional and systemic recurrence was identified at 5 and 8 years post-mastectomy. Incomplete excision/margin involvement was defined as margins 1mm or less. Demographic, biological and treatment-related factors associated were used to identify predictors of local recurrence.

Results: In total, 581 women were studied (mean age: 59; range: 22-96); 462 simple mastectomies (SM) and 106 skin-sparing mastectomies (SSM) which included 199 (34%) screen-detected and 382 (66%) symptomatic cancers. Mean follow up was 6.7 years (range: 5-12.4) and longest was 12.4 years. SSM patients had a lower mean age (p=<0.001), with a smaller tumour size (p=0.022), fewer positive lymph nodes (p=<0.001) and less co-existing ductal carcinoma in situ (DCIS) (p=<0.001) than SM patients. Recurrence rates for SM were 5.6% and 6.9% and for SSM, recurrence rates were 5.7% and 9.0% at 5 and 8 years respectively. Recurrence in node negative patients was 2.2% at 5 years. Predictors of local recurrence were node positivity (HR 8.03; 95% CI 3.2-20) and margin involvement/close margins (HR 3.34; 1.64-6.8).

Conclusion: No significant difference between recurrence rates in SSM and SSM groups was identified. The presence of over four positive lymph nodes and incomplete excision margins indicates an increased risk of recurrence and in these patients, immediate SSM reconstruction should be considered prudently.
Title: A systematic review of the clinical evidence regarding PIP breast implants

Umar Wazir¹, Abdul Kasem¹ and Kefah Mokbel¹. ¹London Breast Institute, Princess Grace Hospital, London, United Kingdom.

Body: Background: Mammary implants marketed by Poly Implant Prothèse (PIP) were found to contain industrial grade silicone and this caused heightened anxiety and extensive publicity regarding their safety in humans. These implants were used in a large number of patients worldwide for augmentation or breast reconstruction.

Methods: Articles were identified by searches of Medline, PubMed, Embase and Google Scholar databases up to March 2014 using the terms: "PIP", "Poly Implant Prothèse","breast implants" and "augmentation mammoplasty" or "silicone" In addition the websites of regulating bodies in Europe, USA and Australia were searched for reports related to PIP mammary implants.

Results: PIP mammary implants are more likely to rupture than other implants and can cause adverse effects in the short to the medium term related to the symptoms of rupture such as pain, lumps in the breast and axilla and anxiety. Based on peer-reviewed published studies we have calculated an overall rupture rate of 14.5% (383/2635) for PIP implants.

Conclusions: There is no evidence that PIP implant rupture causes long-term adverse health effects in humans so far. The long-term adverse effects usually arise from inappropriate extensive surgery, such as axillary lymph node dissection or extensive resection of breast tissue due to silicone leakage.
Body: Purpose: Immediate implant-based breast reconstruction followed by postmastectomy radiation therapy (PMRT) is controversial because of the risk of compromised treatment plans, particularly left-sided plans including internal mammary nodes (IMNs) as target. Using direct-to-implant reconstruction with anatomical implants may improve plans due to their unique shape over the chest wall. This single-institution study evaluated the effects of immediate breast reconstruction with anatomical implants on the quality of PMRT delivered by 3-dimensional conformal radiation therapy (3D-CRT).

Methods and Materials: Patients undergoing immediate direct-to-implant reconstruction with anatomic implant, performed by a single surgeon, were treated between 2008 and 2013. For each patient, 2 plans were created and calculated, including or excluding IMNs. No electron fields were used. The primary endpoint was the dose distribution among reconstructed breast (RB), heart, lungs and IMNs and between right and left breasts. Six patients were treated with the Varian RPM system for left sided BC due to anterior heart position.

Results: Of 29 consecutive patients, 11 received right-sided and 18 received left-sided PMRT. For plans excluding IMNs, mean Dmean was 49.09 Gy (98.2% of the prescribed dose) for right and 48.51 Gy (97.0%) for left RBs. For plans including IMNs, mean Dmean was 49.15 Gy (98.3%) for right and 48.46 Gy (96.9%) for left RBs. Mean RB D95 ranged from 42.05 Gy (left side with IMNs) to 45.15 Gy (right side with IMNs). Mean IMN Dmean was almost identical for left- and right-sided treatment (47.89 Gy and 47.27 Gy, respectively; \( P=.340 \)). Heart doses were very low, with mean Dmean values of 1.25 Gy (range, 0.83-1.46) and 1.56 Gy (range, 1.23-2.10) for left-sided plans excluding and including IMNs, respectively (\( P<.001 \)). Mean lung V20 values ranged from 13.80% for left-sided treatment excluding IMNs to 19.47% for right-sided treatment including IMNs.

Conclusion: PMRT can be delivered effectively and safely by 3D-CRT after direct-to-implant breast reconstruction with anatomical implants. The reconstructed breast and the IM chain coverage were excellent and the heart dose was very low, (probably due to the use of the RPM system). Lungs V20 was comparable with other publication. Our study support the hypothesis that immediate breast reconstruction per se is not an impediment to the delivery of high-quality PMRT by modern 3D-CRT technology. Based on our findings, reconstructive surgeons should consider the use of anatomical implants for immediate breast reconstruction in patients requiring subsequent PMRT.
Title: Variation in UK reconstructive practice in the face of post-mastectomy radiotherapy

James R Harvey, Nigel J Bundred, Cliona C Kirwan, Ashu Gandhi and Paula J Duxbury. 1Manchester Academic Health Sciences Centre, University of Manchester, Manchester, United Kingdom and 2Nightingale Centre, UHSM, Manchester, United Kingdom.

Body: Approximately 30-40% of women are not offered immediate breast reconstruction because the possibility of post-mastectomy radiotherapy (PMRT) is unknown at the time of mastectomy. Breast reconstruction may be delayed until final pathology is available and need for radiotherapy established. Surgical literature is replete with studies of varying quality, reporting complication rates for a range of reconstructive procedures, highlighting the need for surgical trials of reconstructive techniques in women at risk of PMRT. Decisions for these patients are complex, involving multiple clinicians including surgeons and oncologists. To inform a surgical trial design, we aimed to determine current UK surgical practice and gain an understanding of the drivers behind decision-making.

Methods: A questionnaire, validated in a pilot population, was posted to Consultant members of the Association of Breast Surgery (UK). We collected data on current practice in conducting Delayed, Immediate and Delayed-immediate reconstructive surgery. We collated data on type and volume of procedure performed and factors affecting decision-making including delay to adjuvant treatment, risk of complications, perception of patients' quality of life (QoL) and aesthetic satisfaction.

Results: Of 355 surgeons, 130(37%) responded. Of these, 77% felt the current evidence base was not adequate to guide surgical decisions and 80% felt a need for further trials to guide best treatment. Despite a lack of scientific evidence demonstrating a difference in cosmesis or QoL between Immediate and Delayed reconstruction, 85% felt there is not equivalent cosmesis and 71% felt there is not equivalent QoL between the two groups. There is considerable heterogeneity in reconstructive approach to patients at risk of PMRT (Table 1). Delayed reconstruction remains the most popular option, being regularly used by 94% of surgeons despite only 34% of surgeons believing the majority of patients are satisfied with the approach. Significantly fewer surgeons perform Immediate implant based reconstruction (with or without ADM) than Delayed (p<0.01). Implant reconstruction is performed by 71% of surgeons in patients at risk of PMRT, but only 44% of surgeons felt patients were happy with the final results.

The three most important drivers in making a reconstructive choice were 1. Effect of PMRT on the cosmetic result 2. Minimising risk of complications and avoiding delay to adjuvant treatment 3. Pre-operative uncertainty over the need for PMRT.

Table 1

<table>
<thead>
<tr>
<th>Method</th>
<th>Never</th>
<th>Sometimes</th>
<th>Often</th>
<th>Always</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delayed</td>
<td>5</td>
<td>50</td>
<td>51</td>
<td>19</td>
</tr>
<tr>
<td>Immediate implant</td>
<td>58</td>
<td>41</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>Immediate ADM+Implant</td>
<td>45</td>
<td>49</td>
<td>14</td>
<td>5</td>
</tr>
<tr>
<td>2-stage (delayed-immediate) Expander to permanent reconstruction</td>
<td>28</td>
<td>55</td>
<td>33</td>
<td>5</td>
</tr>
<tr>
<td>Immediate Autologous</td>
<td>29</td>
<td>59</td>
<td>22</td>
<td>7</td>
</tr>
</tbody>
</table>

Conclusions: Surgeons employ a variety of approaches to reconstruction in the face of PMRT, the most common approach being delayed reconstruction. Decision-making is based upon individual surgeon’s perception of risks including likely delay to adjuvant therapy and effect of PMRT on the reconstruction. Drivers appeared to be more surgeon-centred rather than patient-based. There is awareness of a lack of evidence to support decision-making and the need for high quality studies. Randomised clinical trials are needed to provide an evidence base for outcomes.
Title: Technical results and complication rates after nipple-sparing mastectomy and direct-to-implant breast reconstruction using porcine acellular dermal matrix for implant coverage

Roland Reitsamer¹ and Florentia Peintinger¹. ¹University Hosptial Salzburg, Breast Center Salzburg, Paracelsus Medical University, Salzburg, Austria.

Body: Objective:
To evaluate the feasibility and complication rates of nipple-sparing mastectomy (NSM) and direct-to-implant breast reconstruction using a porcine acellular dermal matrix (ADM) for implant coverage.

Methods:
NSM and direct-to-implant breast reconstruction using a porcine ADM for implant coverage was performed in a series of 91 breasts in 63 patients. Technical results, complications, and cosmetic results were collected from patient records.

Results:
Short-term complications within one month comprised minor complications as minimal nipple necrosis in 13.2%, and infection in 1.1%. Major complications, as hemorrhage with surgical evacuation in 4.4%, and implant loss due to skin breakdown in 4.4%, totaled in 8.8%. Within three months after primary surgery 3 further severe complications with reoperation occurred. In 87.9% no second surgical intervention was necessary and cosmetic results were good or excellent. After a mean follow-up of 12 months (range 5 - 35 months) no further complications could be observed. Patient satisfaction was high for 92.3% of the patients and subjective cosmetic result was excellent in 90.1% and satisfactory in 2.2%.

Conclusion:
Direct-to-implant breast reconstruction using a porcine ADM for implant coverage after NSM is an innovative approach resulting in a high patient satisfaction. The technique is challenging but feasible and complication rates are acceptably low.
Title: Antibiotic prophylaxis in prosthesis-based mammoplasty: A systematic review

Naisi Huang¹,², Mengying Liu¹,² and Jiong Wu¹,². ¹Shanghai Cancer Center, Fudan University, Shanghai, China and ²Shanghai Medical College, Fudan University, Shanghai, China.

Body: Background Implants are used in over two-thirds of breast reconstructions and all aesthetic augmentations. Although considered as an aseptic surgery, infection rate after prosthesis-based breast surgery is reported to be 2% to 2.5% of patients and represents the leading cause of morbidity after reconstructive and aesthetic surgery. Therefore, prophylactic antibiotics is supported by several studies to prevent surgical site infection (SSI) and capsular contracture (CC). However, there is no high quality evidence on antibiotic prophylaxis in prosthesis-based mammoplasty.

Methods An electronic search was conducted in Medline, Embase, and Cochrane. Studies of prosthesis-based breast surgery with control group and antibiotic prophylaxis were included. Two authors independently screened, assessed and then extracted information from the included studies. Mantel-Haenszel method was used to conduct meta-analysis. All analysis was performed by STATA 12.0. Studies that were not qualified to be included into meta-analysis were summarized and described.

Results 15 studies were included into analysis. Average Jaded score for RCT studies was 2.5 (1-4). Average Newcastle score for non RCT studies was 7.55 (4-9). 3 studies compared systematic antibiotic prophylaxis with no antibiotic use, while no significant difference was observed. Compared with antibiotic prophylaxis within 24 hours, extended systematic antibiotic prophylaxis more than 24 hours postoperatively could significantly reduce infection rate (RR=0.638, CI 0.453-0.898). The average SSI rate was 4.6% in extended antibiotic group versus 11.1% in control group. 15.38 patients needed to be treated to prevent 1 case of SSI. However, in subgroup analysis, extended antibiotic prophylaxis could significantly decrease SSI rate in implant reconstruction surgery (RR=0.508, CI 0.349-0.739), but not in aesthetic breast surgery (RR=1.458, CI 0.602-3.528). Topical antibiotic irrigation could reduce CC rate (RR=0.472, CI 0.316-0.707), while might not be able to reduce infection rate. The average CC rate was 4.86% in topical antibiotic prophylaxis group, versus 6.81% in control group. 51.28 patients needed to be treated to prevent 1 case of Baker grade III or IV CC. Cephalosporins were the most commonly preferred antibiotic regimen in included studies. Nevertheless, there was no consensus of antibiotic prophylaxis combination or timing.

Conclusions Extended systematic antibiotic prophylaxis will significantly reduce SSI rate, especially in implant breast reconstruction. Topical antibiotic irrigation would decrease CC rate, while might not be able to reduce infection rate. Cephalosporins are generally recommended as antibiotic prophylactic regimen which cover the most commonly identified implant–associated bacteria. Risk factors such as chest irradiation and diabetes should be take into consideration when prescribing antibiotic prophylaxis. More better-designed RCTs are awaited to demonstrate the proper antibiotic regimen in prosthesis-based breast surgery to reduce complications.
Title: Integrated immunologic assessment of tumor infiltrating lymphocytes (TILs) and peripheral blood to assess synergy of cryoablation (cryo) plus ipilimumab (ipi) in early stage breast cancer (ESBC) patients (pts)

Body: Background: In pts with ESBC, cryo combined with cytotoxic T-lymphocyte antigen 4 blockade was well tolerated and did not delay standard-of-care mastectomy. As observed in mice, cryo+ipi may liberate tumor-associated antigens, synergistically activate tumor-reactive T-cells, and confer long-term anti-tumor immunity. Because singular biomarkers of response to immunotherapy have not been well defined, we conducted an integrated immunologic assessment to explore potential predictors of immune activation and response.

Methods: Serial blood and pre-/post-treatment tumor tissue were collected from 18 pts treated with cryo (6 pts), single-dose ipi at 10mg/kg (6 pts), or cryo + ipi (6 pts). A Meso Scale Discovery platform was used to measure plasma cytokine interferon gamma (IFNγ). Multiparameter flow cytometry was used to evaluate peripheral and intratumoral T-cell and myeloid cell quantity, T-cell phenotype (effector versus regulatory), proliferation state (Ki67), and activation state (inducible costimulator [ICOS] expression). Finally, a DNA deep sequencing platform was used to conduct T-cell repertoire analysis of peripheral T-cells and TILs.

Results: Sustained >2-fold elevations (1 month post-treatment) in plasma IFNγ were observed in the majority (4/6) of pts receiving cryo/ipi (median 6-fold increase), but in the minority of pts receiving cryo (0/6, median 0-fold) or ipi (2/6, median 0-fold). Similarly, sustained >2 fold elevations in ICOS expression in peripheral CD3+CD4+ T-cells, a known pharmacodynamic marker of ipi, were observed in the majority (5/6) of pts receiving cryo/ipi (median 4-fold increase), but in the minority of pts receiving cryo (0/6, median 0-fold) or ipi (2/6 ipi; median 1-fold). No trends were observed in peripheral myeloid derived suppressor cells. Analysis of TILs by flow cytometry identified increased numbers of proliferating CD8+ T-cells (CD8+Ki67+) in ipi and cryo/ipi groups relative to cryo alone; furthermore, the ratio of proliferating (CD8+Ki67+) to regulatory (CD4+CD25+FoxP3+) cells was enhanced in the cryo/ipi group. Finally, analysis of T-cell repertoire in TILs demonstrated that cryo/ipi generated an influx of novel T-cell clones, with select clones surging dramatically in predominance and circulating within the periphery.

Conclusions: Utilizing an integrated assessment, we identified evidence of immunologic synergy with combination cryo/ipi versus either therapy alone. Of the tested parameters, peripheral CD4+ ICOS expression, plasma IFNγ, Ki67-gated TIL effector/regulatory ratios, and clonal repertoire analysis were identified as promising biomarkers of immune activation. These findings will inform a prospective assessment of potential immunologic biomarkers of immune response and clinical benefit in a phase 2 study of cryo-immunotherapy in ESBC.
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Average Grade: 6.83

Title: HER2 discordant results in local vs. central testing in the phase 3 nelipepimut-S trial and implementation of Leica Bond Oracle HER2 Immunohistochemistry (IHC) System for low and intermediate levels (1+, 2+) of HER2 protein expression as a companion diagnostic

Michelle Melisko¹, Elizabeth A Mittendorf², Sufia Safina³, Michael Schenker⁴, Murray A Brunt⁵, Maria Litwiniuk⁶, John Mackey⁷, Katarina Petrakova⁸, Svitlana Alieva⁹, Lacey Chance¹⁰, Gavin S Choy¹⁰, Mark Ahn¹⁰, Adamm Hamm¹¹, Sonia Kumar¹² and Hope S Rugo¹. ¹University of California, San Francisco, CA; ²U.T. MD Anderson Cancer Center, Houston, TX; ³Republican Clinical Oncology Center, Kazan, Republic of Tatarstan, Russian Federation; ⁴Sf Nectarie Oncology Center, Craiova, Romania; ⁵University Hospital of North Staffordshire, Stoke on Trent, United Kingdom; ⁶Greater Poland Cancer Center, Poznan, Poland; ⁷Cross Cancer Institute, Edmonton, AB, Canada; ⁸Masaryk Memorial Cancer Institute, Cancer Care Clinic, Brno, Czech Republic; ⁹Donetsk Regional Antitumor Center, Donetsk, Ukraine; ¹⁰Galena Biopharma, Inc, Portland, OR; ¹¹Aptiv Solutions, Durham, NC and ¹²Leica Biosystems, Danvers, MA.

Body: Background: The distinction between HER2-positive (IHC 3+ or 2+ with FISH ratio >/= 2) and not overexpressing HER2 has been the focus of many diagnostic tests over the past years in association with development of HER2-targeted therapies. The paucity of therapies developed for the low to intermediate HER2 protein expression populations has resulted in limited attention to their diagnostic precision and accuracy. The development of NeuVax™ (nelipepimut-S; Galena Biopharma, Inc.) in the defined population requires a HER2 IHC 1+/2+ diagnostic that precisely and accurately ensures identification of targeted patients. We describe discordance rates between local and central testing performed to identify tumors with HER2 IHC 1+ or 2+ expression that supports the development of a method to validate HER2 1+ and 2+ (FISH < 2.2) patients who receive nelipepimut-S adjuvant therapy.

Methods: The Prevention of Recurrence in Early Stage, Node-Positive Breast Cancer with Low to Intermediate HER2 Expression with NeuVax™ Treatment (PRESENT) study, is a multicenter, multinational, prospective, randomized, double-blind, controlled Phase 3 study assessing efficacy and safety of the peptide vaccine nelipepimut-S, in HLA A2 or A3 positive patients with early stage, node positive breast cancer expressing low and intermediate levels (IHC 1+/2+) of HER2 protein. PRESENT 2-step screening includes HLA testing and central lab confirmation of HER2 1+ or 2+ expression using the DAKO HercepTest.

Results: As of 2 June 2014, 1454 patients underwent central IHC testing for HER2 and had a quantifiable result of 0, 1+, 2+, or 3+ for both local and central test. Per local testing, 61% (HER2 1+, n=612; HER2 2+, n=275) were eligible and 39% (HER2 0, n=468; HER2 3+, n=99) were ineligible. Of those eligible by local testing, 67.5% (n=599) were confirmed as eligible per central testing for a discordance rate of 32.5% (n=288). Of the 288 discordant samples tested centrally, 73.6% (n=212) and 26.4% (n=76) were reported as HER2 0 and 3+, respectively. 8.7% (76/877) of patients found to be HER2 1+ or 2+ by local testing were determined to be HER2+ (IHC3+) by central testing.

Conclusions: Current tests for HER2 expression are defined by their ability to determine 3+ positivity, yet significant discordance still occurs with nearly 9% false negative rate in this trial. Similarly, marked discordance exists between local and central laboratory test results for HER2 by IHC at 1+/2+ levels of expression. The relatively high discordance rate observed may be due, in part, to the lack of a validated IHC assay for low-to-intermediate expression of HER2 (0, 1+, and 2+). In order to improve accuracy of testing and to develop a companion diagnostic for nelipepimut-S, the Leica Bond Oracle HER2 IHC System has been validated to determine samples across the IHC spectrum (0, 1+, 2+ and 3+) and is now incorporated into HER2 screening for the PRESENT trial as a companion diagnostic to increase accuracy, precision, and specificity in discerning HER2 1+ and 2+ patients.
Title: A Phase Ib Study of an adjuvant GM-CSF-Secreting Breast Cancer Vaccine

Karen S Anderson¹, Beth Overmoyer², Christine Canning², Jennifer Savoie², Garrick Wallstrom¹, Eric P Winer² and Glenn Dranoff². ¹Biodesign Institute, Tempe, AZ and ²Dana-Farber Cancer Institute, Boston, MA.

Body: Background: Vaccination with tumor cells engineered to secrete granulocyte-macrophage colony stimulating factor (GM-CSF) generates potent, specific, and long-lasting anti-tumor immunity in multiple tumor models. Here, we present analysis on the safety, feasibility, and biologic activity of an autologous GM-CSF-secreting breast cancer vaccine in the high-risk adjuvant setting.

Methods: Following IRB approval and patient consent, 18 patients with stage II-III breast cancer underwent tumor procurement for vaccine development at the time of breast surgery. Patients were required to have at least 4 cm of primary tumor, or have received neoadjuvant chemotherapy with at least 2 cm of residual tumor. 11 patients had insufficient tumor following neoadjuvant chemotherapy. 7 patients had sufficient tumor harvested to produce and subsequently receive vaccine therapy. Breast cancer cells were transduced with a replication defective adenoviral vector encoding GM-CSF and irradiated. Vaccinations were started 4-12 weeks after completion of all chemotherapy, immunotherapy, and radiation therapy. Vaccines were delivered subcutaneously (sc) and intradermally (id) weekly for three weeks, then every other week for a total of 6 doses. Immune monitoring included skin biopsies of vaccine sites, measurement of leukocyte populations, and proteomic-based assessment of antibody responses.

Results: Tumor cell yields ranged from 0.07-31.6 x 10⁶ cells. Dose levels were based on cellular yield, ranging from 10⁵- 4 x10⁶ cells/dose for the 7 patients with sufficient cell numbers. Vaccinated patients were 32-65 years of age, and all received 6 vaccines total. Three patients developed relapse within one year of the start of vaccinations, one of whom died at 14 months. The remaining four patients remained disease-free 23-34 months from start of vaccine. Toxicities related to treatment were mild and included Grade I/II local injection-site reactions, as well as grade I/II fatigue, fever, upper respiratory symptoms, cough, and joint pain. One episode of grade 3 fatigue was observed. Increases in antibody responses (p < .05) were observed for 17 antigens in at least 4 out of 5 patients evaluated.

Conclusion: For larger tumors, breast cancer cells can be harvested from a subset of patients in sufficient number for autologous vaccine production at the time of breast surgery. Autologous vaccination can induce immune responses with limited toxicity. The proteomic-based identification of antigen-specific immune responses following vaccination will be presented.
Title: A phase 1/2 study of Ad.p53 DC vaccine with indoximod immunotherapy in metastatic breast cancer

Hatem H Soliman1, Susan E Minton1, Roohi Ismail-Khan1, Hyo S Han1, Nicholas N Vahanian2, Charles J Link2, Gene Kennedy2, Howard Streicher3, Daniel Sullivan1 and Scott J Antonia1. 1Moffitt Cancer Center, Tampa, FL; 2NewLink Genetics Inc, Ames, IA and 3National Cancer Institute, Bethesda, MA.

Body: Background: Indoleamine 2,3 dioxygenase (IDO) is a tryptophan-catabolizing enzyme that causes immunosuppression in the tumor microenvironment. Indoximod is an IDO pathway inhibitor. Preclinical data suggests indoximod enhances the activity of dendritic cell (DC) vaccines. Ad.p53 is an adenovirus used to generate autologous dendritic cell (DC) vaccines against p53 epitopes. We initiated a phase 1/2a trial of indoximod + Ad.p53DC to explore the safety and efficacy of the combination along with response to subsequent chemo. The phase 1 safety data were previously presented and the treatment was well tolerated with no DLTs. (Soliman, ASCO 2013) This abstract includes new phase 2a safety/efficacy data and updated outcomes on all phase 1/2 metastatic breast cancer patients who received Adp53DC+indoximod and any subsequent response to salvage chemo.

Methods: The phase 2a study combined indoximod 1600mg PO BID with up to 6 Ad.p53 DC vaccinations q2wks. The trial used a single arm, Simon two stage design (n=12 in 1st stage, 25 in 2nd stage) with objective response as the primary endpoint. One response out of 12 was required for progression into second stage. The study had 90% power to detect 20% response rate with a p=.09. Patients with measurable, metastatic breast cancer, <3 lines of chemo in metastatic setting, p53 IHC >5%, ECOG 0-2, no autoimmune disease were eligible. Study treatment continued until disease progression or unacceptably toxicity.

Results: Twelve phase 2 patients were accrued, 9 (7 TNBC, 2 ER+/HER2-) received ≥1 dose of Ad.p53DC+indoximod (3 did not due to rapid disease progression during vaccine preparation). Six patients had ≥1 prior line of chemo. Seven (58%) subjects experienced any grade AE, there were no treatment related AEs ≥G3. All treatment attributable AEs were G1-2, <10% frequency, and included anemia, nausea, lymphopenia, photophobia, and headache. All discontinuations were due to disease progression. Best response to immunotherapy in phase 2=1 SD, 8 PD. Phase 2 median TTP = 6.85 wks (3.8-18.1), OS = 18.1 wks (3.8-52) with 3 patients alive as of June 2014. Five patients who received ≥2 cycles of chemotherapy after immunotherapy demonstrated 1 PR, 3 PD, 1 pending scan. For the entire phase 1/2 breast cohort (21 in phase 1, 9 in phase 2) the median TTP = 9.85 weeks (3.8-22.1), OS=38.7 (3.8-122.1) weeks, with 1 patient from the phase 1 cohort alive as of June 2014. Ten out of 21 evaluable patients (1 CR, 7 PR, 2 SD, 11 PD) had clinical benefit from chemotherapy after immunotherapy (9 patients had rapid decline and did not get one full cycle of therapy). All but one of the responders had seen prior chemotherapy in the metastatic setting (including a CR after 4 prior lines of chemo). Median OS in the chemo responders was 69.4 wks (30.1-122.1).

Conclusions: Indoximod+Ad.p53DC was well tolerated. Across phase 1/2 the best response to immunotherapy alone was SD in 4 pts while 10 of 21 (47%) (including 1 CR) responded to subsequent chemotherapy in this largely pretreated cohort. There may be a chemosensitization effect of indoximod+Ad.p53DC. Future trials should combine this treatment with chemotherapy in appropriately selected patients.
**Title:** Discovery of fully human monoclonal antibodies as therapeutic candidates for the treatment of HER2-negative breast cancer

Christy Boozer¹, Paul Algate¹, Aurelio Bonavia¹, Mark Branum¹, Po-Ying Chan-Hui¹, Alison Fitch¹, Brad Greenfield¹, Claire Sutherland¹ and Kristine Swiderek¹. ¹Theraclone Sciences, Seattle, WA.

**Body:** Unlike HER2-positive breast cancer, there are limited treatment options for patients diagnosed with triple negative and endocrine resistant HER2-negative breast cancer, and as such HER2-negative breast cancer represents a significant unmet medical need. The goal of this work is to use Theraclone’s proprietary I-STAR platform to mine the memory B cell immune repertoire of breast cancer patients for the discovery of therapeutically relevant monoclonal antibodies (mAbs) and targets that may be exploited as candidates for treatment of HER2-negative breast cancer.

Matched serum and PBMC samples were collected from breast cancer patients at multiple clinical sites. The selected patient populations included, but were not limited to, patients who were treatment naïve, those who had received immunotherapy or adjuvant chemotherapy, and patients who had an exceptional clinical response to treatment and/or disease. Serum antibody binding to a diverse panel of 5 well characterized breast cancer-derived cell lines of luminal and basal sub-types was determined. Utilizing this approach, patients with a robust serological profile across multiple breast cancer cell lines were identified and prioritized for memory B cell repertoire analysis via the I-STAR platform. Using a high throughput and miniaturized, multiplex flow cytometry assay, the secreted IgG antibodies from over 85,000 individually enriched and expanded B cell clones were screened for binding to the tumor cell line panel and a large number of positive B cell clones were identified. The screening hits can be binned into several unique binding profiles, many of which were confirmed to be shared across multiple patient samples. Deep sequence analysis of the variable regions of the antibodies produced by the B cell clones demonstrated that several of the screening hits were derived from clonally related B cells; the majority of the screening hits represented antibodies derived from unique B cell clones. A representative set of these antibodies was expressed recombinantly for further in vitro characterization.
Title: Bi-specific antibodies targeting signaling pathway crosstalk are a new breast cancer immunotherapeutic strategy

Yanliang Zhang, Edwige Gros, Sarabjit Chagar, Jian Cao, Heyue Zhou, Kouros Motamed, Gunnar F Kaufmann and Yanwen Fu. 1Sorrento Therapeutics, Inc, San Diego, CA.

Body: All of the currently approved therapeutic anti-cancer antibodies are monospecific and therefore only capable of interfering with the biological function of a single molecular target. However, breast cancers mostly involve crosstalk of often synergistic signal transduction pathways, and thus, isolated blockade of a single signal transduction pathway is frequently met by escape mechanisms, such as upregulation of redundant pathways, rendering the monospecific immunotherapy less effective.

Using both chemical and molecular biology techniques, Sorrento has developed new approaches to generate IgG-like bi-specific antibodies (BsAbs) targeting either two compensating signal transduction pathways, such as HER family members, or a breast cancer specific antigen and an immuno-regulatory molecule such as PDL1 or PD1. The chemical biology method, which involves specific hetero-dimerization of two half antibody molecules using bio-orthogonal chemistry, was used to generate an anti-c-Met and anti-PDL1 chemical bi-specific antibody (CBA). Lastly, employing a molecular biology approach, an anti-EGFR and ErbB3 scFv-Fc bi-specific antibody was produced. Progress will be presented on in vitro characterization and cell-based functional assays of these BsAbs.
Title: c-MET is a potential therapeutic target for antibody-drug conjugates in breast cancer

Yanwen Fu1, Edwige Gros1, Alice Lee1, Kimberly Johnson1, Hong Zhang1, Silpa Yalamanchili1, Kouros Motamed1, Gary Chen1, Bryan Jones1, David Miao1 and Gunnar F Kaufmann1. 1Sorrento Therapeutics, Inc, San Diego, CA.

Body: The transmembrane receptor tyrosine kinase c-MET plays a key role in malignant transformation of epithelial cells by activating signal transduction pathways essential for cellular proliferation, survival, migration and invasion. Over expression of c-MET, with or without gene amplification, has been reported in primary breast cancers and correlates with poor prognosis. Inhibition of c-MET signaling, via tyrosine kinase inhibitors (TKIs) or antagonistic antibodies, is usually not sufficient for sustained treatment efficacy, thus, we believe that antibody drug conjugates (ADCs) offer the promise and potential of delivering more potent anti-tumor activity. We have generated ADCs containing a proprietary human anti-c-MET antibody (STI-D0606) with either a tubulin inhibitor or a DNA damaging agent. STI-D0606, a fully human antibody (IgG1) selected from Sorrento's G-MAB antibody library, was conjugated with a cytotoxin via site-specific bioconjugation. The conjugates retained binding affinity and showed potent cell killing in a variety of c-MET-positive cell lines. Progress will also be reported on overall efficacy of c-MET ADCs in a preclinical xenograft model.
**Title:** Overall survival (OS) in the IMELDA randomized phase III trial of maintenance bevacizumab (BEV) with or without capecitabine (CAP) for HER2-negative metastatic breast cancer (mBC)

Joseph Gligorov¹, Jose Bines², Emilio Alba³, Giorgio Mustacchi⁴, Saverio Cinieri⁵, Vineet Gupta⁶, Jean-Yves Pierga⁷, Hakan Bozcu⁸, Rabab Gaafar⁹, Sudeep Gupta¹⁰, Guillermo Lopez Vivanco¹¹, Xiaojia Wang¹², Romulo Costa¹³, Kadri Altundag¹⁴, Ewa Chmielowska¹⁵, Sabine de Ducla¹⁶, Ulrich Freudsprung¹⁶, Paulo Cortes¹⁷ and Dinesh Doval¹⁸.

¹APHP Tenon, IUC-UPMC, Paris, France; ²Instituto Nacional de Cancer, Rio de Janeiro, Brazil; ³Hospital University Clinic Virgen de la Victoria, Málaga, Spain; ⁴Medical Oncology, University of Trieste, Trieste, Italy; ⁵Medical Oncology Department and Breast Unit, Brindisi, Italy; ⁶Bangalore Institute of Oncology, Bangalore, India; ⁷Institut Curie, Université Paris Descartes, Paris, France; ⁸Akdeniz University Medical Faculty, Antalya, Turkey; ⁹National Cancer Institute, Cairo University, Cairo, Egypt; ¹⁰Tata Memorial Hospital/Center, Mumbai, India; ¹¹Hospital Universitario Cruces, Vizcaya, Spain; ¹²Zhejiang Cancer Hospital, Hangzhou City, China; ¹³Instituto do Câncer do Estado de São Paulo, São Paulo, Brazil; ¹⁴Hacettepe University Cancer Institute, Ankara, Turkey; ¹⁵Centrum Onkologii Prof. F Lukaszcyzyka, Bydgoszcz, Poland; ¹⁶F. Hoffmann-La Roche Ltd, Basel, Switzerland; ¹⁷University Hospital of Santa Maria, Lisbon, Portugal and ¹⁸Rajiv Gandhi Cancer Institute & Research Center, Delhi, India.

**Body:** BACKGROUND The open-label randomized phase III IMELDA trial demonstrated that adding CAP to maintenance BEV until disease progression (PD) after initial BEV–docetaxel (DOC) provides statistically significant and clinically meaningful improvements in both progression-free survival (PFS [primary endpoint]; hazard ratio [HR] 0.38 [95% CI 0.27–0.55]; log-rank p<0.001) and OS. We present OS in subgroups representing stratification factors and clinically important populations.

METHODS Patients (pts) with HER2-negative measurable mBC, ECOG PS <2, and no prior chemotherapy for mBC were eligible. After 3–6 cycles of BEV–DOC, pts without PD were randomized to BEV alone or BEV–CAP (BEV 15 mg/kg q3w; CAP 1000 mg/m² bid d1–14 q3w) until PD. Stratification factors were estrogen receptor (ER) status, visceral metastases, response status, and lactate dehydrogenase (LDH) concentration. OS from randomization was a secondary endpoint. The planned sample size of 360 enrolled pts (290 randomized) was calculated assuming a PFS HR of 0.70 (median PFS 5.8 → 8.3 months) with 80% power at 2-sided α=0.05 after 244 PFS events. Recruitment was stopped prematurely after regulatory withdrawal of the BEV–DOC combination but pts who had already been enrolled and randomized were followed as originally planned.

RESULTS Between Jun 2009 and Mar 2011, 284 pts were enrolled and treated. Of these, 99 were not eligible for randomization (most commonly due to PD [41%] or AEs/toxicity [31%]) and 185 (65%) were randomized. At the time of the primary PFS analysis, representing study closure, median follow-up (from randomization) was 31.6 months. Median OS from randomization was 23.7 months in the BEV arm and 39.0 months in the BEV–CAP arm (events in 36% of pts). The HR for OS in the two randomized arms showed consistency between subgroups, favoring the BEV–CAP arm in all subgroups analyzed.

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>No. of events/No. of pts (%)</th>
<th>Unstratified HR (95% CI)</th>
<th>1-y OS rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BEV</td>
<td>BEV–CAP</td>
<td>BEV</td>
</tr>
<tr>
<td>All</td>
<td>53/94 (56)</td>
<td>33/91 (36)</td>
<td>0.43 (0.26-0.69)⁹</td>
</tr>
<tr>
<td>&lt;65 y</td>
<td>46/81 (57)</td>
<td>27/77 (35)</td>
<td>0.51 (0.32-0.82)</td>
</tr>
<tr>
<td>≥65 y</td>
<td>7/13 (54)</td>
<td>6/14 (43)</td>
<td>0.50 (0.16-1.60)</td>
</tr>
<tr>
<td>Triple negative</td>
<td>16/21 (76)</td>
<td>10/25 (40)</td>
<td>0.44 (0.19-0.99)</td>
</tr>
<tr>
<td>Hormone receptor positive</td>
<td>37/73 (51)</td>
<td>23/66 (35)</td>
<td>0.53 (0.31-0.89)</td>
</tr>
<tr>
<td>ER positive⁹</td>
<td>36/69 (52)</td>
<td>23/64 (364)</td>
<td>0.53 (0.32-0.90)</td>
</tr>
<tr>
<td>ER negative⁹</td>
<td>17/25 (68)</td>
<td>10/27 (37)</td>
<td>0.44 (0.20-0.99)</td>
</tr>
<tr>
<td>&lt;3 metastatic organ sites</td>
<td>17/40 (43)</td>
<td>17/48 (35)</td>
<td>0.75 (0.38-1.49)</td>
</tr>
<tr>
<td>≥3 metastatic organ sites</td>
<td>36/54 (67)</td>
<td>16/43 (37)</td>
<td>0.39 (0.22-0.71)</td>
</tr>
<tr>
<td>Visceral metastases⁹</td>
<td>38/65 (58)</td>
<td>23/62 (37)</td>
<td>0.43 (0.26-0.73)</td>
</tr>
<tr>
<td>Status</td>
<td>No visceral metastases</td>
<td>Complete or partial response</td>
<td>Stable disease</td>
</tr>
<tr>
<td>-------------------------------</td>
<td>------------------------</td>
<td>-----------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Event-free survival rate (%)</td>
<td>15/29 (52)</td>
<td>36/68 (53)</td>
<td>14/22 (64)</td>
</tr>
<tr>
<td>OS survival rate (%)</td>
<td>10/29 (34)</td>
<td>24/68 (35)</td>
<td>6/20 (30)</td>
</tr>
<tr>
<td>Hazard Ratio</td>
<td>0.76 (0.34-1.70)</td>
<td>0.61 (0.37-1.03)</td>
<td>0.22 (0.08-0.63)</td>
</tr>
<tr>
<td>Stratified analysis</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

aStratified analysis. bStratification factor.

CONCLUSIONS. Combining maintenance BEV with CAP until PD after initial BEV–DOC for mBC provides a statistically significant and clinically meaningful improvement in OS (secondary endpoint), seen consistently irrespective of baseline characteristics.
**Title:** A phase II study of bevacizumab in combination with modified FOLFOX6 in heavily pretreated patients with HER2-negative metastatic breast cancer

Ting Li¹ ², Biyun Wang¹ ², Xichun Hu¹ ², Zhonghua Wang¹ ², Jian Zhang¹ ², Si Sun¹ ², Jun Cao¹ ², Fangfang Lv¹ ², Leiping Wang¹ ², Sheng Zhang¹ ², Chen Ni¹ ², Zhenhua Wu¹ ² and Jie Xie¹ ². ¹Fudan University Shanghai Cancer Center, Shanghai, China and ²Shanghai Medical College, Fudan University, Shanghai, China.

**Body: Background**
Bevacizumab combined with modified FOLFOX6 (mFOLFOX6) is a standard regimen for colorectal cancer with good tolerability. Our previously published study by Sun et al showed a moderate efficacy and excellent safety of mFOLFOX6 for metastatic breast cancer (MBC). This study was to determine the efficacy and safety of adding bevacizumab to mFOLFOX6 in heavily pretreated patients with human epidermal growth factor receptor 2 (HER2)-negative MBC.

**Patients and Methods**
In this open label, single-arm phase II study, bevacizumab, 5 mg/kg every two weeks or 7.5 mg/kg every three weeks, in combination with mFOLFOX6, which were oxaliplatin 85mg/m², leucovorin 400mg/m² and 5-Fu 400mg/m² intravenously on day 1 following 5-Fu 2400 mg/m² continuous intravenously 46 hours every 2 weeks, was administered to patients who failed at least 2 prior chemotherapy regimens in metastatic setting. The prior exposure to taxane, anthracycline, vinorelbine, capecitabine and gemcitabine was 97.9%, 93.8%, 64.6%, 64.6% and 81.3%, respectively. The primary objective was progression free survival (PFS), and secondary objectives included objective response rate (ORR), overall survival (OS) and safety.

**Results**
48 patients were enrolled with a median of 49.5 years old (range, 34 to 73 years old) and a median of 3 prior chemotherapy regimens (range, 2 to 6). A median of 4.0 cycles (range, 0.5 to 8.0 cycles) were delivered with 45 patients undergoing treatment discontinuation, including 7 (14.6%) due to completion of 6 cycles, 1 (2.1%) due to completion of 8 cycles, 18 (37.5%) due to disease progression, 5 (10.4%) due to adverse events, 4 (8.3%) due to withdrawal of informed consent, 2 (4.2%) due to physician’s decision, 2 (4.2%) due to economic reasons and 6 (12.5%) with unknown causes. The median PFS was 6.0 months (95% confidence interval [CI], 3.6 to 8.5 months), ORR was 48.8% and median OS was 10.2 months (95% CI, 8.5 to 11.9 months). Most adverse events were grade 1 or 2 and grade 3 or 4 toxicities occurring in more than one patient were neutropenia (75.0%), leukopenia (50.0%), thrombocytopenia (20.8%) and anemia (6.3%).

**Conclusion**
Adding bevacizumab to mFOLFOX6 has significant anti-tumor activity and excellent safety in heavily pretreated HER2-negative MBC patients, which warrants conduct of further confirmative trials.

**Adverse events compared with our historical mFOLFOX6 trial**

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Sun et al</th>
<th>Li et al</th>
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<tr>
<td>Grade 3-4, n (%)</td>
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*<0.05
Chemotherapy as the third line or beyond treatment for metastatic breast cancer

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<tr>
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<th>Regimens</th>
<th>Median Line, range</th>
<th>Number of Patients</th>
<th>ORR (%)</th>
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<tr>
<td>Li et al</td>
<td>Bevacizumab+mFOLFOX6</td>
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<td>Vinorelbine</td>
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NA=Not Available, TTP=Time To Progression
Title: Characteristics and outcome of breast cancer patients enrolled in cancer therapy evaluation program (CTEP) sponsored phase I clinical trials

Filipa Lynce¹, Larry Rubinstein² and Pamela Harris². ¹Washington Cancer Institute, MedStar Washington Hospital Center, Washington, DC and ²Cancer Therapeutic Evaluation Program, National Cancer Institute, Rockville, MD.

Body: Background: Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer-related death among women. Given the availability of approved therapies and large clinical trials, historically few breast cancer patients are referred for consideration of a phase I trial. Although efficacy is not the endpoint of phase I studies, we were interested in determining whether clinical benefit rates differed in patients with breast cancer from patients with other cancers enrolled in phase I solid tumor trials. In addition a better knowledge of the characteristics and outcomes of patients with breast cancer enrolled in phase I clinical trials may contribute to better clinical trial design and patient selection.

Methods: We performed a retrospective analysis of all Cancer Therapy Evaluation Program (CTEP) sponsored phase I trials for patients with solid tumors, from 1992 to 2012. CTEP phase I database which is maintained by Theradex Inc was queried. Patients treated on phase I/II trials or including hematological malignancies were excluded. We conducted an analysis of demographic variables, variables related to disease characteristics and outcomes of patients with breast cancer and compared them to patients with other oncological diagnoses enrolled in the same trials.

Results: A total of 8119 patients who participated in 225 trials were identified. Of these 1367 (16.8%) patients had breast cancer. Compared with patients with other oncological diagnoses, breast cancer patients were older (51.9 vs. 45.4 years), less likely to be white (73% vs. 81.6%, p<0.001) and more likely to have received a high number of previous lines of therapy (34.2% of patients with breast cancer had received 6 or more prior regimens vs. 11.9% of patients with other cancers, p<0.001). Performance status (PS) was similar between the two groups of patients with the majority of patients presenting with PS of 1 (57.3% in the breast cancer group and 60.9% in the non-breast cancer group). The clinical benefit rate was higher for patients with breast cancer (56.1% vs. 42%, p<0.001). Breast cancer patients remained on study longer than non-breast cancer patients (mean 136.3 vs. 95.8 days, p<0.001). The toxicity related deaths were less than 1% in both groups. Discontinuation of treatment due to toxicity was observed in 6.6% of patients with breast cancer and 5.6% of patients with other cancers.

Conclusions: An analysis of the CTEP-sponsored phase I trials revealed that breast cancer patients, enrolled in these trials, were older and more heavily pre-treated than patients with other types of cancer. However they seem to have better outcomes and similar toxicities when compared to the other patients. These data suggest that patients with breast cancer who have likely exhausted treatment with approved agents should be considered for phase I trials.
Title: Potential benefits of hypnosis sedation on different modalities of breast cancer treatment

Martine Berliere¹, Sarah Lamerant¹, Philippe Pietet², Aurore Lafosse¹, Laurence Delle Vigne¹, Fabienne Roelants¹, Christine Watremez¹, Marie-Agnes Docquier¹, Lafita Fellah¹, Isabelle Leconte¹ and François Duhoux¹. ¹Cliniques Universitaires St Luc (UCL), Brussels, Belgium and ²Grand Hopital de Charleroi (GHdC), Charleroi, Belgium.

Body: Background: In oncology, hypnosis has been used for pain relief in metastatic patients but rarely for induction of anaesthesia.

Material and methods: Between January 2010 and February 2014, 220 patients from our breast clinic (Cancer Center - Cliniques universitaires Saint-Luc - Universite catholique de Louvain) were included in an observational, non randomized study approved by our local ethics committee. 110 consecutive patients underwent breast surgery (lumpectomy or mastectomy +/- axillary lymph node dissection or sentinel lymph node biopsy) while on general anaesthesia (group I) and 110 consecutive patients underwent the same surgical procedures while on hypnosis sedation (group II). The stages and the tumor characteristics were well balanced between the two groups. After surgery, 28 patients received chemotherapy in group I and 27 patients in group II. Radiotherapy was administered to 96 patients of group I and 95 patients of group II. Currently, 83 patients of group I and 82 patients of group II are receiving endocrine therapy. Different parameters were studied for each treatment modality.

Results: Duration of hospitalization was statistically significantly reduced in group II vs. group I (3.3 days vs. 4.4 days) (CI 95% range: -1.48 -0.72, p=0.000000578) for all surgical procedures. The same results were observed for mastectomies alone (3.1 vs. 5.3 days) (CI 95 % range: -3.19 -1.31, p=0.0012) and for lumpectomies (3.1 vs. 4.3 days) (CI 95 % range: -1.024 -0.364, p=0.00065). The number of post-mastectomy lymph punctures was reduced in group II (1 to 3 (median value n=1.6) vs. group I (2 to 5 (median value n=3.1, p=0.01), as was the quantity of lymph removed (103 ml versus 462.7 ml) (p=0.0297) in the group of mastectomies.

Concerning chemotherapy, the incidence of asthenia was statistically decreased (p=0.015) in group II. There was a statistically non significant trend towards a decrease in the incidence of nausea/vomiting and muscle pain in group II (respectively p=0.1 and p=0.2).

The frequency of severe radiodermatitis (p=0.01) and post-radiotherapy asthenia (p=0.01) were significantly reduced in group II. Finally, compliance to endocrine therapy was improved in group II (p=0.05), while incidence of hot flashes (p=0.00029), joint or muscle pain (p=0.000139) and asthenia (p=0.00002) were statistically significantly decreased in group II.

Discussion: Hypnosis sedation exerts beneficial effects on nearly all modalities of breast cancer treatment. The absence of a significant benefit for chemotherapy-induced nausea/vomiting and muscle pain observed is probably due to the small number of patients receiving chemotherapy in our study.

Conclusion: Benefits of hypnosis sedation on breast cancer treatment are very encouraging and further promote the concept of integrative oncology.
Body: Objective: Treatment of patients with a history of breast cancer who are diagnosed as newly found solid pulmonary nodule is still controversial. Our study is to find out whether the pulmonary nodule represents a primary cancer, metastasis, or benign lesion secondary to breast cancer can be differentially prognostic.

Methods: A total of 165 consecutive patients who underwent surgery or biopsy for pulmonary nodules between 2007 and 2013 after curative operation for breast cancer were reviewed. The postoperative survival rate was analyzed and the difference in survival rate was assessed between groups of pulmonary nodules representing a primary cancer, metastasis, and benign lesion.

Results: Among these 165 patients, median age was 59 and mean follow-up was 51 months (range from 5 to 398). The pathologic diagnoses of pulmonary nodules were pulmonary metastases of breast cancer in 71 patients (43%), primary lung cancer in 59 (35.8%), and other diagnoses in 35 (21.2%) (hyperplasia in 11; pneumonia in 9; granuloma in 6; sclerosing hemangioma and pulmonary fibrosis in 4 each). In those who were diagnosed as malignant pulmonary tumor, about one half (56.4%) were secondary malignant and the rest were metastatic. There was no statistically difference in median age or disease free interval between operation date of breast surgery and pulmonary surgery or biopsy among these groups. However, those who were diagnosed as primary malignant or benign lesion had significantly higher incidence of isolated pulmonary nodule shown on their CT scan results (P=0.036). The incidence of positive lymph node involvement and negative ER/PR status was significantly higher in metastatic breast cancer patients (63.8% and 70.6%, respectively). Among patients with metastatic pulmonary nodules, rates of discordance between primary breast tumor and metastasis foci were 20.3%, 23.7% and 14.7% for ER, PR and HER2, respectively, which changed a lot the regimen used for treatment of metastatic breast cancer. Both the 5-year and 10-year disease-free survival from the initial mastectomy were significantly shorter in metastatic breast cancer patients (72.9%, 35.3%) when compared with patients with primary pulmonary cancer (82.9%, 53.8%) or pulmonary benign lesions (91.6%, 67.8%). Prognostic factors in metastatic breast cancer patients were a disease-free interval of >60 months with 10-year survival of 48.3% (P<0.05), solitary lung metastasis is associated with a survival rate of 78.9% after 5 years and of 48.7% after 5 and 10 years, and this is statistically significant compared to multiple metastases.

Conclusions: We think that pulmonary lumpectomy is the best option in selected cases of solitary or multiple pulmonary nodules from breast cancer, which can be useful for differential diagnosis, predicting prognosis and deciding the drug treatment strategy in some cases.
Title: Isolated loco-regional recurrence in the breast: Re-quadrantectomy and systemic treatment

Victoria Costanzo1, Veronica Fabiano1, Mercedes Maino1, Federico Colo1, Reinaldo Chacon1, Adrian Nervo1, Jorge Nadal1, Martin Loza1, Jose Loza1, Daniel Mysler1 and Mora Amat1. 1Instituto Alexander Fleming, Buenos Aires, Argentina.

Body: Background: Local or regional recurrence in breast cancer is associated with poor prognosis. Mastectomy is the classic indication in these situations. However, second attempts of breast conservation in previously radiated patients with small, histologically favorable local relapse have been reported. Also, prospective data (The CALOR Trial) suggest that adjuvant chemotherapy should be recommended in patients with isolated loco-regional recurrence, especially if the recurrence is HR negative. The purpose of our study was to determine the frequency of use of re-quadrantectomy and systemic treatment in isolated loco-regional recurrence (ILR) in breast cancer.

Methods: Retrospective review of the database of our institution. Inclusion criteria: ILR in surgical bed, breast or lymph nodes with complete resection (mastectomy, re-quadrantectomy or axillary resection) with or without systemic treatment and radiotherapy. Findings: 4695 patients were analyzed from april 2000 to april 2014. 78 patients had a ILR, 66 were analyzed. Median age 49.5 years (27-86). 54.5% of the patients were postmenopausal and 16.6% had bilateral involvement at diagnosis. Characteristics of the primary tumor: Mean tumor size 2 cm, 66.6% were HR positive, 6% HER2 positive, 13.6% TN and 14% unknown. Initial treatment: breast conserving surgery 62 p (sentinel node 25, axillary node dissection 36); mastectomy 4 patients. 42 patients underwent adjuvant chemotherapy, 57 patients radiotherapy and 62 patients hormonotherapy. Time to recurrence: 94.3 months. Characteristics of the ILR: Mean tumor size 1.8 cm. 65% were HR positive, 12% triple negative, 6% HER positive and 17% unknown. Treatment at relapse was as follows: 27 re-quadrantectomy, 34 mastectomy, 1 lymph node dissection; all these patients had previous quadrantectomy. The other patients underwent to mastectomy (1) and complete resection of the lesion (3). 29 patients received chemotherapy (43.9%), : 70% anthracyclines and taxanes, 6% trastuzumab, 30% other and 65% received hormonotherapy. Local progression free survival 30.7 months; distance progression free survival 100 months. Local relapse was evidenced in 30% of patients who had a re-quadrantectomy procedure.

Conclusions: Second attempt of breast conservation in previously radiated patients with small, histologically favorable ILR and long recurrence free interval disease is feasible as described. The target population is of good prognosis as evidenced the long free disease interval at recurrence (94.3 m), the mean size of tumor at recurrence (1.8 cm) and the high proportion of HR positive disease. The percentage of local recurrence after re quadrantectomy is as described in some reports, 30% (20-35%). The indication of chemotherapy was mostly in HR negative or HER 2 positive tumors, but the low number of patients does not permit a valid comparaison.
2014 San Antonio Breast Cancer Symposium

Publication Number: P2-18-06
Average Grade: 0

Title: Low dose capecitabine is effective and relatively nontoxic in breast cancer treatment

John Carpenter¹. ¹University of Alabama, Birmingham, AL.

Body: Capecitabine has been widely used in treatment of both early and advanced breast cancer in the United States since 1998. The dose limiting toxicity is diarrhea, which is dose limiting and can be severe. Lower doses are widely used without any apparent compromise in efficacy and are consistently less toxic (see Naughton, Clinical Breast Cancer 2010). Here a continuous dose regimen of capecitabine 1000 mg. daily was used in 24 patients with breast cancer, both early and advanced, from March 2012-September 2013. Four received the drug as adjuvant treatment, 8 as preoperative therapy, and 12 for advanced disease. Duration of treatment ranged from 0.5 to 15 months. Toxicity was modest: 1 had moderate nausea, none experienced vomiting, 2 mild anemia (one with marrow replacement by tumor), 2 mild palmar irritation, and 1 grade 1 thrombopenia. No diarrhea was seen. One patient died of hepatic necrosis and portal vein thrombosis; the relationship to treatment was uncertain. 12/20 with measurable disease experienced a response to treatment (1 complete and 11 partial responses by RECIST criteria), 1 had stable metastatic disease for 9 months, and 7 had progression of disease. The median duration of response was 5 months (range 2-15 months). Use of capecitabine in this dose and schedule for treatment of breast cancer appears comparably effective to higher conventional doses and dramatically less toxic.
Title: Can synthetic 2D mammography be used to select patients in whom there is no need to review the digital breast tomosynthesis from which they were constructed? A review of 1871 consecutive mammogram sets of patients presenting symptomatically or for follow up

Simon DH Holt¹, Ali Moalla¹, Helen R Williams¹, Khaldoun MY Nadi¹, Anita M Huws¹, Amrita Gurung¹, Daniel Thomas¹ and Yousef M Sharaiha¹. ¹Peony Breast Care Unit, Prince Philip Hospital, Llanelli, Carmarthenshire, United Kingdom.

Body: Introduction: A synthetic 2D mammogram can be created by combining the individual optimally enhanced 1mm slices of a digital breast tomosynthesis (DBT). Theoretically this could help overcome the problems associated with standard 2D mammograms (superimposition of structures hiding small cancers) and the significantly increased reading time and decreased conspicuity of microcalcifications associated with DBT. It may avoid the need for the double x-ray exposure required for combination 2D and DBT currently being suggested to maximise the specificity and sensitivity of mammography. The hypothesis we wished to test is, “if the synthetic 2D is normal, is there any advantage to looking at the DBTs as well?”

Method: We have reviewed 1000 consecutive cases presenting symptomatically or to our follow up clinic all of which underwent DBT on a Hologic Dimensions machine. From the 3D data sets synthetic 2D mammograms were constructed (Hologic C-view technology). One breast radiologist with 12 years experience of interpreting mammograms and more recently 4 years experience in interpreting DBTs was asked to first review the 2D synthetic mammograms (each breast CC and MLO) and report them before then reviewing the DBTs and issuing a final report. The mammograms were reported M1 to M5 using the standard BIRADs criteria. The BIRADs scores for each breast were recorded prospectively and entered into a database.

Results: 1000 consecutive patients were studied between October 2013 and March 2014. The average age of the women was 58.1 years (range 29 to 92). Of these some were under follow up after mastectomy so in total there were 1871 individual mammogram sets reported. Table 1 summarises the correlation between C-view and DBT reporting. The correlation between the two modalities is very close, but importantly there was only one patient in whom the C-view was reported normal or benign (M1 or M2) but the DBT reported a possible abnormality (M3). However, 31 cases reported as suspicious or malignant M3, 4 or 5 by C-view were subsequently downgraded to benign after review of the DBT.

<table>
<thead>
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<th>BIRADS Classification - Synthetic 2D v DBT</th>
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<tr>
<td>Synthetic 2D</td>
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<tr>
<td>M1</td>
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<td>DBT</td>
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<td>M4</td>
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<td>M5</td>
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Table 1

Conclusion: In a symptomatic and follow up clinic, our study suggests that much radiologist’s time and x-ray exposure to the patient could be saved by using synthetic 2D mammograms derived from the DBT data rather than using separate 2D studies. Only if the C-view is reported M3, 4 or 5 is it necessary to review the DBT but for all these patients the DBT is already available without further x-ray exposure or recall.
Clinical, anatomical and histological characteristics of breast lesions visualized by photoacoustic mammography; first clinical study in CK project

Elham Fakhrejahani, Masae Torii, Yasufumi Asao, Iku Yamaga, Toshiyuki Kitai, Masako Kataoka, Shotaro Kanao, Masahiro Takada, Tsuyoshi Shiina and Masakazu Toi. Kyoto University, Kyoto, Japan; Kishiwada City Hospital, Kishiwada, Osaka, Japan and Canon Inc, Tokyo, Japan.

Background: Photoacoustic mammography (PAM) is an optical imaging technique potentially capable of imaging breast vasculature as well as measuring hemoglobin oxygen saturation (SO2) in focal breast lesions. We presented the initial observation on first 20 cases using the first generation PAM (PAM-01) prototype made by Canon Inc. (Tokyo, Japan) (spatial resolution: 2mm) in SABCS 2013. Here we report the clinico-pathological characteristics of all cases recruited in the first clinical study in CK project (Kyoto University/Canon joint research project) between Aug 2010 and March 2012.

Methods: 57 patients were recruited in IRB approved study at Kyoto University Hospital, Japan. Forty-two breast harboring lesions and when possible contralateral breasts were evaluated by PAM-01. Axial maximum intensity projection (MIP) were obtained and signals from consecutive MIPs confirmed to be associated with the tumor location in MRI by an expert breast radiologist were considered to be the region of interest (ROI). Histological sections from the widest area of the lesions were evaluated post-excisional by immunohistochemistry using anti-CD31 as endothelial marker and anti-carbonic anhydrase IX (CA IX) as hypoxia marker. Histological slides were scanned and divided into 1680 squares (0.84x0.84 mm²) (Hamamatsu Inc. Japan) for image analysis. Total vascular perimeter (TVP)-in mm- was calculated for all the histological section by using Image Pro-Plus 7.0 software (Media Cybernetics, USA). Tumor area was measured in mm². TVP index was calculated as TVP/area.

Results: Photoacoustic signal was detected in 30 lesions out of 42 at the depth of 26.8 ± 12.8 mm from which 80% were located superior to nipple. CA IX positive cases in comparison with CA IX negative cases significantly showed higher TVP index (p-value = 0.028 Mann-Whitney Test) suggesting more angiogenic profile of hypoxic tumors. However, lesions without any detectable signal were reported to have only a bigger mass size histologically (26.6 vs. 14.8 mm, p-value 0.28, Mann-Whitney Test) regardless of their TVP index or CA IX expression level. Moreover, SO2 was calculated 70.9% for signals located inside tumors and 85.5% for signals associated with subcutaneous vessels in the same breast (p-value <0.0001 Wilcoxon Test) compatible with the SO2 data from normal counterpart at tumor depth in the other breast (81.9%) (p-value 0.0001 Wilcoxon Test). Tumors bigger than 2 cm also tended to have lower SO2 (65.6%) compared with tumors smaller than 2 cm (70.1%) in accordance with higher TVP index in bigger tumors (p-value <0.001 Student T-test).

Conclusion: This is the largest clinical study of PAM till today and the correlation between histological profile of hypoxia and tumor microvasculature with PAM signal visibility as well as tissue SO2 seems promising. However, the improvement of techniques and resolution is necessary to develop a more clinically applicable non-invasive functional breast imaging modality for analyzing breast tumor vasculature and hypoxia. Some of these improvements have taken place for the second generation PAM (PAM-02) which is now under a clinical evaluation study.
Title: Are more frequent early follow up mammogram protocols necessary after breast conserving surgery and radiation therapy?

Chavi Kaufman¹, Tamara Fulop¹, Susan K Boolbol¹, David Lucido¹, Suzan Naam¹, Alyssa Gillego¹ and Manjeet Chadha¹. 'Beth Israel Medical Center and Roosevelt Hospital, NYC.

Body: Background: In an era of choosing wisely in healthcare there are many initiatives in radiology and oncology that are being evaluated for appropriateness of practice based on clinical evidence-base. More frequent follow up mammogram protocols in the first 3 years after BCT are a widely accepted practice. However, such breast imaging schedules have no strong clinical evidence-base to support the added testing every 6-months, nor a rationale to justify patient anxiety and added unnecessary biopsy procedures. The goal of our study was to evaluate the frequency of BIRADS score 4 on short follow up mammograms in a population of patients treated with breast conserving surgery and RT (BCT).

Materials and Methods: This is an IRB approved study. From 2001- 2007, we identified 681 patients who underwent BCT and who also underwent follow up mammograms at our cancer center. We reviewed short follow up mammograms defined as those obtained within the first 3-years after BCT. The BIRADS score was tabulated in all cases. Further, it was determined to study the frequency of BIRADS 4 score only, because this was deemed a clinically significant finding that routinely warranted additional evaluation.

Results: Median age of the study group is 51 years (31- 80 years). Among the 681 patients a total of 3648 follow up mammogram sessions were obtained. The median number of follow up mammogram sessions per patient during the observation period of 3-years was 6 (range 2-6 mammogram sessions). In 85% of patients followed the mammogram scores were BIRADS 1 to 3. In 15% of the patients the BIRADS score 4 was reported at least once. Among the BIRADS 4 category of patients, 56% had this score reported in the ipsilateral breast following BCT for breast cancer, and 44 % had BIRADS 4 reported in the contralateral breast. Specifically, in the ipsilateral breast the frequency of BIRADS 4 was 8.2%. We also observed a significant trend with a higher frequency BIRADS 4 reported within the first year of follow up in the ipsilateral breast as compared with the contralateral breast. In the 2nd and 3rd year the frequency of BIRADS 4 was < 10% and comparable between the treated ipsilateral breast and the normal contralateral breast. The pathology correlations showed a significantly lower yield of cancer in the BIRADS 4 subset of patients.

Conclusions: Similar to the observations of the ACRIN 6666 study ¹ on BIRADS 3 we observed a very low yield of cancer from frequent follow up protocol for the ipsilateral breast using the BIRADS 4 category. In promoting responsible medical care it is important to establish appropriate follow up guidelines and selection of schedules for groups of patients individualized by risk. Annual follow up mammograms might be adequate frequency for the ipsilateral breast. This study warrants further evaluation and standardized protocols.

¹ ACRIN 6666 study, Radiology 2013

This study is funded by the Ellen Blair Grant.
Mammographic changes after oncoplastic reduction mammoplasty

Anne Warren Peled¹, Merisa Piper¹, Laura J Esserman¹, Robert D Foster¹, Hani Sbitany¹ and Elissa R Price¹. ¹University of California, San Francisco, CA.

BACKGROUND:
Reconstruction of partial lumpectomy defects with reduction mammoplasty techniques can improve aesthetic outcomes. However, the impact of the significant tissue rearrangement on post-operative mammographic findings and subsequent recommendations for biopsy has not been well-studied.

METHODS:
A retrospective review of 50 patients who underwent partial mastectomy with immediate oncoplastic reduction mammoplasty reconstruction from 2001 to 2008 was performed. Mammography reports at 6 months, 1 year, 2 years, and 3 years post-operatively were reviewed for Breast Imaging Reporting and Data System (BI-RADS) scores, predominant findings, and recommendations for subsequent imaging or biopsy.

RESULTS:
At six months post-operatively, 49 patients (98%) had benign findings of post-surgical changes, while one patient had microcalcifications and underwent subsequent surgical re-excision with residual DCIS on pathologic analysis. At one year, 94% of patients continued to have benign mammograms; of these mammograms, 94% reported only post-surgical scarring, while 6% described benign-appearing scattered or dystrophic calcifications. Of the three patients with suspicious mammograms at one year, all underwent core biopsies with benign results. At two years, rates of fat necrosis (2%) and scattered or dystrophic calcifications (10%) increased, though all mammographic findings were considered benign and none required additional imaging or biopsies. By three years post-operatively, an additional two patients (4%) developed suspicious findings and underwent biopsies confirming local recurrence (one invasive, one in situ). The remaining 96% continued to have benign mammographic findings and were subsequently followed with routine biannual mammographic surveillance.

Overall, 88% of patients required no additional intervention in the three-year period following oncoplastic reduction mammoplasty. Of patients recommended for biopsy, malignancy was discovered in 50%, including two local recurrences (4% of total patients). This data is similar to a previously published large study (1841 women) of mammographic surveillance after partial mastectomy without oncoplastic reconstruction at our institution, which found a 6% malignancy rate at 5 years.

CONCLUSIONS:
Although substantial tissue rearrangement is performed at the time of oncoplastic reduction mammoplasty, our results demonstrate low rates of abnormal post-operative mammograms and subsequent biopsies over the first three years following the procedure. These findings support the use of oncoplastic reduction mammoplasty as a strategy for improving reconstructive outcomes in patients undergoing partial mastectomy.
Title: The aggregate number of false-positive recalls and biopsies performed under different breast cancer screening strategies in the US

Carlie K Thompson¹, Cristina O'Donoghue², Elissa M Ozanne³, Martin Eklund¹ and Laura J Esserman¹. ¹University of California, San Francisco, CA; ²University of Illinois at Chicago, Chicago, IL and ³Dartmouth Institute for Health Policy and Clinical Practice, Lebanon, NH.

Body: Introduction

The ideal screening program optimizes benefits while reducing harms. The balance of benefits and harms with breast cancer screening is debated. This study aims to inform breast cancer screening policies by calculating the aggregate number of false-positive recalls and false-positive breast biopsies under different screening strategies.

Methods

We created a model to estimate the number of screening mammograms, false-positive recalls and false-positive biopsies performed per year in the US based on current practice. The percent of women that participate in screening under current practice was estimated from the 2010 CDC Behavioral Risk Factor Surveillance System. The model also enabled the comparison of 3 screening strategies: annual, biennial, and United States Preventive Services Task Force (USPSTF) guidelines, using a target participation rate of 85%. The number of women at risk in each age group was taken from the US Census and excludes women who have had breast cancer in the past five years. False-positive recall rates and biopsy rates were obtained from Hubbard et al. (Ann Intern Med, 2011). Analyses were performed using R statistical software. Outcomes for this analysis were the total number of false-positive recalls and biopsies. Monte Carlo methods were used to compute 95% confidence intervals.

Results

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<th>False-positive recall rate (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>9.2</td>
<td>10.4</td>
</tr>
<tr>
<td>False-positive biopsy rate (%)</td>
<td>Annual</td>
<td>Biennial</td>
</tr>
<tr>
<td></td>
<td>0.85</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Formulas

Number of screening mammograms = Women at risk x percent women screened

Number of false-positive recalls = Number of screening mammograms x false-positive recall rate
<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Current practice</th>
<th>Annual strategy</th>
<th>Biennial strategy</th>
<th>USPSTF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of screening mammograms</td>
<td>4.6x10^7 (4.3x10^7-5.0x10^7)</td>
<td>6.1x10^7 (5.6x10^7-6.5x10^7)</td>
<td>1.6x10^7 (1.4x10^7-1.7x10^7)</td>
<td>2.1x10^7 (1.9x10^7-2.2x10^7)</td>
</tr>
<tr>
<td>Number of false-positive recalls</td>
<td>4.2x10^6 (3.4x10^6-5.3x10^6)</td>
<td>5.4x10^6 (4.1x10^6-6.9x10^6)</td>
<td>1.5x10^6 (1.1x10^6-1.9x10^6)</td>
<td>2.1x10^6 (1.5x10^6-2.6x10^6)</td>
</tr>
<tr>
<td>Number of false-positive biopsies</td>
<td>5.3x10^5 (4.1x10^5-6.4x10^5)</td>
<td>6.5x10^5 (4.8x10^5-8.2x10^5)</td>
<td>2.0x10^5 (1.5x10^5-2.5x10^5)</td>
<td>2.7x10^5 (2.1x10^5-3.5x10^5)</td>
</tr>
</tbody>
</table>

Conclusion
Compared to annual screening, we estimate that following the USPSTF guidelines would result in 62% fewer false-positive recalls and 58% fewer false-positive biopsies. The absolute magnitude is in the range of 374,000 biopsies annually. Given that these screening strategies have been projected to be of equal benefit, more widespread adoption of the USPSTF guidelines could decrease the patient risk and anxiety associated with recall and biopsy without impacting the benefits. Developing tools for personalized risk assessment would facilitate adoption of the USPSTF guidelines. These tools can be tested by comparing the personalized and annual screening strategies prospectively and can inform screening policies.
Title: Impact of breast density notification laws on radiology practices: A survey of 110 radiology facilities

Lina Nayak¹, Kanae K Miyake¹, Yueyi Irene Liu¹, William R Thomas¹, Edward A Sickles², Bonnie N Joe², Karen Lindfors³, R J Brenner⁴, Stephen Feig⁵, Lawrence W Bassett⁶, Jessica W Leung⁷, Haydee Ojeda-Fournier⁸, Jonathan Hargreaves³, Elissa Price⁸, Jafi A Lipson¹, Allison W Kurian¹, Elyse Love⁸, Donna D Walgenbach³, Lauren Ryan², Meg Durbin⁹, Bruce L Daniel¹, Linda Garcia⁶ and Debra M Ikeda¹. ¹Stanford University, Stanford, CA; ²University of California, San Francisco, CA; ³University of California, Davis, CA; ⁴Alta Bates Summit Medical Center, Berkeley, CA; ⁵University of California, Irvine, CA; ⁶University of California, Los Angeles, CA; ⁷California Pacific Medical Center, San Francisco, CA; ⁸University of California, San Diego, La Jolla, CA and ⁹Palo Alto Medical Foundation, Palo Alto, CA.

Body: Purpose: Breast Density Notification laws, passed in 15 states as of April 2014, mandate that breast density information be given to patients often without guidance on modalities, patient selection or funding for supplemental screening. The purpose of this study is to assess the impact of breast density notification laws on radiology practices, specifically regarding breast cancer risk assessment and supplemental screening studies.

Methods: We performed an anonymous 20-question web-based survey to Society of Breast Imaging radiologists using a Qualtrics Survey Tool between 8/2013-3/2014, with questions on radiology practices, breast cancer risk assessment, breast density measurement, supplemental screening tests, and support for referring physicians and patients. We compared survey results between groups using Fisher’s exact test.

Results: 121 radiologists from 110 facilities (48 academic, 43 large private hospital, 15 small private hospital and 4 other) representing 34 USA states and 1 Canadian site responded. 49% of facilities (54/110) were in states with an enacted breast density notification law. 37% of facilities (40/109) performed risk assessment, 26% (28/109) did not perform risk assessment, and 38% (41/109) did not but reported family history/other risk factors, with no significant difference in performing risk assessment between facilities with or without an enacted law (p-value 0.71). Of the 37 facilities performing risk assessment, 60% used the Gail model, 22% used the Tyrer-Cuzick model and 11% used the modified Gail model (multiple answers allowed [m.a.a.]). Of the 15 facilities performing risk assessment, 40% answered "yes" when asked whether performing risk assessment is a new task because of the density law. Breast density was estimated by only visual assessment in 98% of facilities (103/105), and by computer-based determination with or without visual assessment in 2% (2/105). Supplemental screening studies offered included magnetic resonance imaging (MRI) (88%, 92/105), handheld whole breast ultrasound (HHWBUS) (48%, 50/105), tomosynthesis (39%, 41/105), and automated WBUS (8%, 8/105) (m.a.a.). There was no significant difference in supplemental screening studies offered between facilities with or without an enacted law (p-value 0.26). In anticipation of the law, facilities implemented HHWBUS (33%, 16/48), tomosynthesis (6%, 3/48), automated WBUS (6%, 3/48) or none (60%, 29/48) (m.a.a.). Facilities with the enacted law prepared for the law with referring physician discussions (69%, 34/49), website (49%, 24/49), educational talks for referring physicians (43%, 21/49) or patients (31%, 15/49) (m.a.a.).

Conclusion: Our survey showed variations in available supplemental screening modalities and policy implementation at each facility. There was no significant difference in performing risk assessment and supplemental screening studies between facilities with or without an enacted breast density notification law.
Title: Breast cancer screening by women from BRCA1 & BRCA2 mutation positive families

Melanie Wuttke¹, Roger Milne², Prue Weideman¹, Sandra Picken¹, Charmaine Smith¹, Michael Friedlander³, kConFab Investigators¹, Sue-Anne McLachlan⁴, John L Hopper² and Kelly-Anne Phillips¹. ¹Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia; ²University of Melbourne, Melbourne, Victoria, Australia; ³Prince of Wales Hospital, Sydney, New South Wales, Australia and ⁴St Vincent's Hospital, Melbourne, Victoria, Australia.

Body: Purpose: Breast screening may detect breast cancer (BC) at an early stage but optimal screening depends on a woman’s level of risk and age. This study estimates the contemporary prevalence of BC screening by Australian women from families with a BRCA1/2 mutation identified by Family Cancer Clinics.

Methods: Subjects were carriers and true non-carriers from families with a BRCA1/2 mutation enrolled in the nationwide kConFab cohort. They are followed up every 3 years with a questionnaire which includes use of breast ultrasound, mammography (MMG), magnetic resonance imaging (MRI) and clinical breast examination (CBE) over the previous 3 year period. Data from each woman’s most recent questionnaire (completed Oct 2009- March 2014) were used in the analysis. All knew their mutation result. Those who had risk-reducing mastectomy, previous cancer, were evaluated for benign breast disease, pregnant or breastfeeding or received their mutation result in the most recent follow-up round were excluded. Screening behaviour was categorized based on current national guidelines (see table). Associations with underscreening and overscreening were assessed using unconditional logistic regression to estimate odds ratios and 95% confidence intervals.

Definitions of overscreening and underscreening for carriers & true non-carriers

<table>
<thead>
<tr>
<th>Groups</th>
<th>Overscreening*</th>
<th>Underscreening*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carriers</td>
<td>Breast imaging (MMG +/- MRI) more often than annually</td>
<td>Breast imaging less often than annually if age ≥30</td>
</tr>
<tr>
<td>True non-carriers</td>
<td>Age &lt;40: any screening MMG; age ≥40: MMG more often than every 2 years OR any MRI or ultrasound screening</td>
<td>Age 50-70: MMG less often than every 2 years</td>
</tr>
</tbody>
</table>

*Definitions derived from Cancer Australia Guidelines which do not recommend for or against CBE in any group and differ from US guidelines.

Results: Of 372 eligible participants, there were 92 mutation carriers (42 BRCA1, 50 BRCA2) and 280 true non-carriers. 1% of carriers were overscreening, 86% were screening annually while 13% were underscreening. MRI, which is funded for mutation carriers aged 30-50 years, was used by only 52% (13/27) of carriers aged <50 years and by 7 carriers aged ≥50. Underscreening carriers were more likely to be single (OR=2.78, 95%CI=1.28-5.88, P=0.009) while those with a first degree cancer affected relative were less likely to underscreen (OR= 0.40, 0.19-0.85, P=0.017). CBE was undertaken by 79% of carriers at least yearly; 15% had no or irregular CBE.

115/280 (41%) non-carriers were overscreening, 55% were screening appropriately and 5% were underscreening. Underscreening non-carriers were more likely to be single (OR 3.85, 95%CI=1.00-14.9, P=0.05). Predictive of overscreening in non-carriers was having a cancer affected first degree relative (OR=2.92, 1.55-5.51, P=0.001). Non-carriers were less likely to utilise CBE (54%).

Conclusion: Most mutation carriers in kConFab are having regular screening MMG, but MRI is underutilised even when funded. The reasons for low usage of MRI requires further research and is concerning given its increased sensitivity over MMG. Overscreening is common in true non-carriers of BRCA mutations. Family cancer history and marital status may predict inappropriate screening behaviour.
The contribution of the national health system to mammographic screening in Brazil

Ruffo Freitas-Junior¹, Danielle CN Rodrigues¹, Rosangela S Correa², João-Emilio Peixoto³ and Rosemar MS Rahal¹. ¹Program of Mastology, Federal University of Goias, Goiania, Goias, Brazil; ²CNEN, Comissao Nacional de Energia Nuclear, Goiania, Goias, Brazil and ³National Cancer Institute, Rio de Janeiro, Brazil.

INTRODUCTION: In Brazil, access to mammography is provided through the National Health System (SUS), the Supplemental Health System, or is paid for directly by the patient. SUS is the official government system and was established to conform to the constitutional requirement that health is a right of all Brazilian citizens and a duty of the state. In recent years SUS breast cancer control policies have advanced and government strategies for early detection have strengthened. However, evaluations are needed to monitor the effectiveness of these actions. OBJECTIVE: To describe the coverage of mammography in breast cancer screening conducted by the National Health System in Brazilian macro-regions and states in 2012. METHODS: An ecological study, where the estimate of coverage was the number of exams performed expressed as a percentage of the number of exams expected in the target population of women 50-69 years old. The exams performed refer to target population mammography production data from the Outpatient Information System (CIS) of DATASUS. To calculate the expected number of exams for this population, the biennial screening recommendations of the National Cancer Institute (NCI) and an estimate of the female population based on the census of the Brazilian Institute of Geography and Statistics (IBGE) were used. RESULTS: The coverage estimate for SUS-performed mammography in Brazil in 2012 was 26.6%. Stratified by macro region, the lowest coverage was in the northern region (12.1%) and highest in the South (34.6%).

<table>
<thead>
<tr>
<th>Macro-regions</th>
<th>Coverage</th>
<th>Minimum</th>
<th>Maximum</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>North Region</td>
<td>12.1</td>
<td>0.2</td>
<td>27.2</td>
<td></td>
</tr>
<tr>
<td>Northeast Region</td>
<td>23.9</td>
<td>10.3</td>
<td>37.2</td>
<td>0.001</td>
</tr>
<tr>
<td>Southeast Region</td>
<td>28.5</td>
<td>15.5</td>
<td>32.5</td>
<td></td>
</tr>
<tr>
<td>South Region</td>
<td>34.6</td>
<td>30.3</td>
<td>41.0</td>
<td></td>
</tr>
<tr>
<td>Midwest Region</td>
<td>14.8</td>
<td>10.3</td>
<td>19.7</td>
<td></td>
</tr>
</tbody>
</table>

* Chi-square test

Units of the Federation an estimated coverage ranged from 0.2% to 41%, the lowest in the state of Amapa and the largest in Santa Catarina. 48% of states were in the range of 10.1% to 20% coverage. CONCLUSION: The results imply that the contribution of SUS to mammography screening in Brazil is higher in both macro-regions and states with higher income and better organised health system.
Title: Qatar experience of Standard risk breast cancer screening program (BCSP)

Salha Bujassoum¹, Reena Alassam¹, Hekmat Bugrein¹ and Mufid Elmistiri. ¹Hamad Medical Corporation, Doha, Qatar.

Body: Background
Qatar has one of the highest age-adjusted breast cancer incidences in the Arab world. Although this is much lower than the incidence in the West. Breast cancer incidence in Qatar was 45 per 100,000 in 2003-2007 & 53 per 100,000 in 2008-2011. These higher incidence rates in Qatar are mainly due to the growing population. The prevalent age group, for Qatari and non-Qatari patients, was 40-50 years old. This suggests that the age-specific incidence of breast cancer in Qatari women is unlike the pattern usually seen in Western nations where median age at diagnosis is 61 years, moreover the diagnosis is often at advanced stages of breast cancer. These factors led to establishment the first hospital based (BCSP) in Qatar. It uses a distributed model of mammography service. The program launched 2008, accepts eligible asymptomatic women at ages 40 - 69 years.

Methods
A retrospective study was done during the period from April 2008-December 2013. Our aim is to describe our experience of (BCSP) in Qatar and to monitor performance indicators. Our (BCSP) includes an office call and recall as well as triple assessment. We also discuss positive cases in multi-disciplinary meeting. Supplement women satisfaction survey conducted along with screening for 100 women showed highly overall satisfaction reached 90%.

Results
Total number of screened women was 4264 with an increasing participation, year by year. Out of these, Qatari patient's accounts for 1145, and non Qatari for 3119. The age group of cases was (43-51). Total breast biopsies were 82, of which 45 were positive of breast carcinomas, (37) invasive ductal carcinoma, (8) noninvasive ductal carcinoma. The Invasive cancer detection rate was 8.2 %. The positive predictive value (PPV) was 46%. Sensitivity value has improved from 51% in 2008 to 70% in 2012 as well as specificity value that has increased from 77% in 2008 to 83% in 2012.

Conclusion
Public acceptance of the breast cancer screening program in Qatar gradually increased and women highly satisfied about the services, incidence rate higher in age group (43-52) years. Invasive detection rates were higher compare to the western counters, this indicate either aggressive behavior of the disease or women come forward to screening without knowing that they are symptomatic, in this part of the world. We have a unique population of multinationals that merits tailored screening tools .and intensify the public awareness and early deflection screening program.
Title: Diagnostic performance of PET/CT and bone scintigraphy in breast cancer patients with suspected bone metastasis

Naoki Niikura¹, Jun Hashimoto², Toshiki Kazama², Jun Koizumi¹, Rin Ogiya¹, Mayako Terao¹, Risa Oshitanai¹, Toru Morioka¹, Banri Tuda¹, Takuho Okamura¹, Yuki Saito¹, Yutaka Imai¹ and Yutaka Tokuda¹. ¹Tokai University School of Medicine, Japan and ²Tokai University School of Medicine, Japan.

Body: Introduction

Previous retrospective studies suggest that 18FDG PET/CT (PET/CT) has superior sensitivity and specificity to bone scintigraphy (BS) in detecting breast cancer bone metastases, but the difference in efficacy between these techniques has not been confirmed. Potentially, PET/CT may detect bone metastases more accurately than BS does. To test this hypothesis, this prospective study compared the diagnostic efficacy to detect bone metastases between PET/CT and BS in breast cancer patients. We also compared the response of bone metastases assessed by the PET/CT or BS with the bone metastases.

Method

This single-institution prospective study included consecutive patients with breast cancer diagnosed by biopsy and suspected bone metastases at the Breast Diseases Unit at Tokai University Hospital, Kanagawa, Japan between September 2011 and March 2014. Inclusion criteria were as follows: bone pain, elevated alkaline phosphatase, elevated tumor marker, and suspected bone metastases on BS. Two nuclear medicine physicians interpreted the PET/CT and BS images. Bone involvement was confirmed by biopsy, especially in the case of oligometastasis. If biopsy proved difficult to perform, conventional imaging and additional directed radiological studies and follow up were helpful. This study was approved by the Institutional Review Board of the Tokai University School of Medicine and is registered with UMIN, number 000006003. All patients provided informed consent.

Result

Thirty patients were initially enrolled, but two patients were excluded from analysis because they declined further follow-up imaging. The median patient age at diagnosis was 59 years (range, 31–74 years). Among the 28 patients, bone pain was observed in 6 patients, elevated alkaline phosphatase in 4, elevated tumor marker in 17, and suspected bone metastases were detected on BS in 7 patients. Among 10 patients were diagnosed bone metastases, PET/CT detected 10 of 10 bone metastases, however BS detected 7 of 10 bone metastases. PET/CT and BS were not highly concordant in detecting osseous metastases; among 19/28 paired studies (68%), 2 (10%) were positive for metastasis, and 17 (90%) were negative. Nine occurrences (32%) were discordant; of these, 2 of 9 were PET/CT positive and BS negative; 5 of 9 were PET/CT positive and BS equivocal; one case was PET/CT negative and BS equivocal; and one was PET/CT equivocal and BS negative.

Conclusion

This study supports the use of PET/CT for detecting suspected osseous metastases. A large prospective study is needed to determine whether PET/CT could replace bone scintigraphy in detecting suspected bone metastases.
Title: Whole transcriptome analysis of AR+ ER/PR- metastatic breast cancers treated with bicalutamide on TBCRC011

Tiffany A Traina¹, Ayca Gucalp¹, William Polkinghorn¹, Steven Isakoff¹, Sara Tolaney¹, Lisa Carey¹, James Ingle¹, Lisle Nabell¹, Andres Forero¹, Hope Rugo¹, Kimberly Blackwell¹, Minetta Liu¹, Matthew Soloway¹, Lisle Mose¹, Dilip Giri¹, Agnes Viale¹, Clifford Hudis¹, Charles Sawyers¹ and Joel Parker¹. ¹Translational Breast Cancer Research Consortium.

Body: **Background:** The heterogeneity of TNBC includes a subtype that is androgen dependent and whose growth is inhibited by antiandrogen therapy. We conducted a multicenter phase II trial which established the activity of bicalutamide for the treatment of patients (pts) with metastatic ER/PR-negative breast cancer TBCRC011 (Gucalp CCR 2013). We now report results of whole genome, next generation sequencing of the 26 evaluable pts to molecularly classify the study population and to identify potential biomarkers of response to bicalutamide. We hypothesized that those pts whose tumors express increased AR output are most likely to benefit from anti-androgen therapy.

**Methods:** Archival formalin-fixed, paraffin-embedded (FFPE) samples were collected from either primary or metastatic site and RNA was isolated using standard techniques. Whole transcriptome sequencing was performed using the Illumina HiSeq 2000 platform. This data set was compared to that of TCGA, the PAM50 and the Lehmann TN subtypes for molecular classification. Bioinformatic analysis defined a model of AR transcriptional output based on androgen stimulated AR+ ER/PR- breast cancer cell lines (HCC202, SUM185, MDA453) that was applied to the TBCRC011 data set as a predictor of response. Gene set analysis was performed to test secondary hypotheses.

**Results:** 21/26 pts provided adequate gene expression estimates for further analysis. Principal component analysis in comparison to whole transcriptomes from breast cancers in the TCGA show that TBCRC011 tumors associate with the basal like (BL) intrinsic subtype, and this was confirmed by PAM50 classification. Relative AR expression of TBCRC011 cases was similar to that of BLBC in the TCGA. There is a weak but statistically significant positive correlation between AR expression by IHC and transcriptome (r=0.395, p=0.034). An expression-based model of AR activity was derived from AR+ ER/PR- breast cancer cell lines exposed to androgen. TBCRC011 samples segregate with the TCGA samples predicted to be AR responsive by this model. However, the AR output signature did not predict clinical benefit (CR+PR+SD>24 weeks) as defined in TBCRC011. Unexpectedly, by the Lehmann TNBC subtype criteria TBCRC011 tumors were not consistently molecularly classified as luminal AR (BL1-1, BL2-1, Immunomodulatory-5, Luminal AR-4, Mesenchymal-2, Mesenchymal Stem Like-2, Unclassified-6). None of the 4 pts with clinical benefit were classified as BL by the Lehmann model. Gene set analysis revealed that estrogen signal pathways were associated with the number weeks on therapy, and that samples with high scoring AR by IHC exhibited expression patterns of medullary breast cancer.

**Conclusions:** The majority of samples demonstrated expression variation consistent with TCGA basal like breast cancer. Although cell line models predict enrichment of AR activity in both the TCGA basal like and TBCRC011 cohorts, this AR output signature did not predict clinical benefit of bicalutamide. Molecular classification found clinical benefit beyond the luminal AR subtype but exclusive of basal like 1 and 2. Whether IHC or molecular subtype is the optimal biomarker for pt selection for antiandrogen therapy remains uncertain.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-04-02
Average Grade: 5.20

Title: Multiple subtypes of triple negative breast cancer are dependent on androgen receptor

Valerie N Barton¹, Nicholas C D'Amato¹, Michael A Gordon¹, Britta M Jacobsen¹ and Jennifer K Richer¹. ¹University of Colorado Anschutz Medical Campus, Aurora, CO.

Body: Triple negative breast cancer (TNBC) constitutes 10-20% of invasive breast carcinomas and has the lowest five-year survival rate. Currently, there are no targeted therapies for TNBC. Recent studies demonstrate that the androgen receptor (AR) is expressed in up to a third of TNBC. AR is highly expressed in the "luminal AR (LAR)" molecular TNBC subtype, but is also present in other TNBC subtypes and may present an opportunity for targeted therapy. We hypothesized that AR+ TNBC critically depend on AR and that AR inhibition will decrease tumor burden in preclinical models of breast cancer. To determine the extent to which AR+ TNBC depend on AR, we inhibited AR activity with the AR antagonist enzalutamide (ENZ) and shRNAs against AR in multiple TNBC subtypes. Treatment with ENZ prevented AR nuclear localization in response to DHT in multiple TNBC cell lines, reduced baseline proliferation in 2D culture (p<0.05), and decreased anchorage independent growth in soft agar (p<0.01). AR knockdown significantly reduced proliferation (p<0.001) and increased apoptosis (p<0.001) compared to cells transduced with a non-targeting control. In addition to reduced proliferation, AR knockdown or treatment with ENZ altered cellular morphology from stellate to round in Matrigel and significantly decreased migration (p<0.05) and invasion (p<0.001) of cell lines spanning multiple TNBC subtypes. Microarray profiling and ELISA of TNBC lines treated with DHT and ENZ suggested that AR regulation of the EGFR ligand amphiregulin (AREG, p<0.05) is a mechanism by which AR influences proliferation, migration and invasion in TNBC. Indeed, treatment with exogenous AREG rescued decreased proliferation, migration and invasion of AR knockdown cell lines (p<0.05). In vivo, ENZ significantly decreased tumor viability (p=0.008) and increased necrosis (p=0.009) in SUM159PT xenografts. Our findings suggest that AR influences both proliferation and invasion of AR+ TNBC cells representing multiple TNBC subtypes and provide promising preclinical data on the efficacy of ENZ in AR+ TNBC. Thus, inhibition of AR by anti-androgens such as ENZ may represent an effective targeted therapy for TNBC.
PIK3CA mutations in androgen receptor-positive triple negative breast cancer confer sensitivity to the combination of PI3K and androgen receptor inhibitors

Brian D Lehmann¹, Joshua A Bauer¹, Johanna M Schafer¹, Christopher S Pendleton¹, Luojia Tang¹, Kimberly C Johnson¹, Xi Chen¹, Justin M Balko¹, Henry Gomez², Carlos L Arteaga¹, Gordon B Mills³, Melinda E Sanders¹ and Jennifer A Pietenpol¹.
¹Vanderbilt-Ingram Cancer Center, Nashville, TN; ²Instituto Nacional de Enfermedades Neoplásicas, Lima, Peru and ³University of Texas MD Anderson Cancer Center, Houston, TX.

Triple negative breast cancer (TNBC) is a heterogeneous collection of biologically diverse cancers, which contributes to variable clinical outcomes. Previously, we identified a TNBC subtype that has a luminal phenotype and expresses androgen receptor (AR+). TNBC cells derived from these luminal AR+ tumors have high frequency PIK3CA mutations. The purpose of this study was to determine if targeting PI3K alone or in combination with an AR antagonist is effective in AR+ TNBC.

Methods: We determined the frequency of activating PIK3CA mutations in AR+ and AR- TNBC clinical cases. Using AR+ TNBC cell lines and xenograft models we evaluated the effectiveness of PI3K inhibitors, used alone or in combination with an AR antagonist, on tumor cell growth and viability.

Results: PIK3CA kinase mutations were highly clonal, more frequent in AR+ vs. AR- TNBC (40% vs. 4%), and often associated with concurrent amplification of the PIK3CA locus. PI3K/mTOR inhibitors had an additive growth inhibitory effect when combined with genetic or pharmacological AR targeting in AR+ TNBC cells. We also analyzed the combination of bicalutamide +/- the pan-PI3K inhibitor GDC-0941 or the dual PI3K/mTOR inhibitor GDC-0980 in xenograft tumor studies and observed additive effects.

Conclusions: While approximately one third of TNBC patients respond to neoadjuvant/adjuvant chemotherapy, recent studies have shown that patients with androgen receptor positive (AR+) TNBC are far less likely to benefit from the current standard of care chemotherapy regimens and novel targeted approaches need to be investigated. In this study, we show that activating PIK3CA mutations are enriched in AR+ TNBC; and, the growth and viability of AR+ TNBC cell line models is significantly reduced after treatment with PI3K inhibitors used in combination with an AR antagonist. These results provide rationale for pre-selection of TNBC patients with a biomarker (AR expression) to investigate the use of AR antagonists in combination with PI3K/mTOR inhibitors.
The prognostic effect of a negative progesterone receptor (PgR) by immunohistochemistry (IHC) in luminal HER-2 negative breast cancer (BC) by age at diagnosis: 10 years follow-up study

Siel JAR Olbrecht¹, Kathleen Van Asten¹, Annouschka Laenen¹, Chantal Remmerie², Wildiers Hans², Floris Guiseppe², Christiaens Marie-Rose², Vergote Ignace² and Neven Patrick². ¹KU Leuven, Leuven, Belgium and ²University Hospitals, Leuven, Belgium.

Background
After menopause, an absent PgR predicts distant metastases (DM) in patients with ER positive HER-2 negative invasive BC. Yamamoto et al. (JCO 2013) questioned the prognostic effect of PR in premenopausal patients, as ovarian estrogens might affect PgR expression. We investigated the effect of PgR on DM free interval (DMFI) and BC specific survival (BCSS) by age at diagnosis as surrogate marker for menopause.

Patients and methods
Retrospective data of consecutive patients with a primary operable ER positive HER-2 negative BC (1/1/2000 - 31/12/2009) were retrieved from our prospectively managed database. Cases that received neo-adjuvant chemotherapy or were operated in another hospital, were excluded. BC with missing values for PgR, grade or lymph node status and/or lost to follow-up were excluded in some subgroup analyses. A multivariate (MV) competing risk model for DMFI and BCSS was established considering age at diagnosis (age ≤50 yrs vs >50 yrs), PgR status, tumor grade (1-3), tumor size (mm) and lymph node status (neg/pos). Steroid receptors were considered positive if ≥1% stained on IHC. Differential prognostic effects of these variables according to PR or age were tested by means of interaction effects. Only significant interactions were included.

Results
We included 3326 BCs (8 with missing PgR status); 2911 (87.5%) PgR positive [870 (26.2%) ≤50 yrs and 2041 (61.4%) >50 yrs] and 407 (12.5%) PgR negative [68 (2.0%) ≤50 yrs and 339 (10.2%) >50 yrs]. In absolute numbers and compared to PgR positive BCs, PgR negative cases >50yrs had more DM if grade 3 and more BCSS for all grades. In BC ≤50 yrs these differences were not found, but only 7.2% were PgR negative (Tab.1). Results from the MV models showed a significant interaction with PgR on DMFI and BCSS (respectively p=0.01 and p=0.002) resulting in higher risk of DM (HR, 1.9; 95% CI, 1.4-2.7; p=0.001) and BCSS (HR, 2.4; 95% CI, 1.6-3.7; p<0.001) when PgR was absent for BCP >50 yrs. This difference in DMFI and BCSS according to PgR status was not found in BC ≤50 yrs (Tab.2).

Table 1. Number of BCP with distant metastasis and BCSS according to age (yrs), PgR status(+/−) and tumor grade.

<table>
<thead>
<tr>
<th>Grade Metastatic Disease</th>
<th>Breast Cancer Specific Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤50 yrs</td>
<td>&gt;50 yrs</td>
</tr>
<tr>
<td>PgR+</td>
<td>PgR-</td>
</tr>
<tr>
<td>≤50 yrs</td>
<td>&gt;50 yrs</td>
</tr>
<tr>
<td>PgR+ PgR-</td>
<td>PgR+ PgR-</td>
</tr>
<tr>
<td>Grade 1-2</td>
<td></td>
</tr>
<tr>
<td>45/607 (7.4%)</td>
<td>5/42 (11.9%)</td>
</tr>
<tr>
<td>66/1512 (4.4%)</td>
<td>11/226 (4.9%)</td>
</tr>
<tr>
<td>28/607 (4.6%)</td>
<td>2/42 (4.8%)</td>
</tr>
<tr>
<td>33/1511 (2.1%)</td>
<td>7/226 (3.1%)</td>
</tr>
<tr>
<td>50/263 (19.0%)</td>
<td>4/26 (15.4%)</td>
</tr>
<tr>
<td>69/529 (13.0%)</td>
<td>35/113 (31.0%)</td>
</tr>
<tr>
<td>31/263 (11.8%)</td>
<td>2/26 (7.7%)</td>
</tr>
<tr>
<td>44/529 (8.3%)</td>
<td>26/113 (23.0%)</td>
</tr>
</tbody>
</table>

Table 2. Results of MV model including significant main effects and interaction effects between tumor characteristics, age and PgR status on DMFI and BCSS.
### 95% Confidence interval

<table>
<thead>
<tr>
<th>Interactions</th>
<th>Hazard ratio</th>
<th>Lower limit</th>
<th>Upper limit</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>A. BMFI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PgR Neg. vs Pos.</td>
<td>-50yrs</td>
<td>0.784</td>
<td>0.395</td>
<td>1.556</td>
</tr>
<tr>
<td>PgR Neg. vs Pos.</td>
<td>+50yrs</td>
<td>1.937</td>
<td>1.386</td>
<td>2.709</td>
</tr>
<tr>
<td><strong>B. BCSS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PgR Neg. vs Pos.</td>
<td>-50yrs</td>
<td>0.575</td>
<td>0.208</td>
<td>1.588</td>
</tr>
<tr>
<td>PgR Neg. vs Pos.</td>
<td>+50yrs</td>
<td>2.439</td>
<td>1.620</td>
<td>3.672</td>
</tr>
</tbody>
</table>

**Conclusions**

A negative PgR is only prognostic for DMFI and BCSS in women aged >50 at diagnosis. There was no difference of PgR for these endpoints in patients ≤50 yrs at diagnosis but PR negativity is rare in this age group.
Title: Invasive lobular carcinoma cell lines utilize WNT4 signaling to mediate estrogen-induced growth

Matthew J Sikora¹, Amir Bahreini¹, Caroline M Alexander² and Steffi Oesterreich¹. ¹University of Pittsburgh, Pittsburgh, PA and ²University of Wisconsin, Madison, WI.

Body: Invasive lobular carcinoma (ILC) is a histological subtype of breast cancer representing 10-15% of newly diagnosed breast tumors. Over 90% of ILC are ER-positive, however, endocrine response and estrogen signaling are not well described in ILC. Retrospective analyses suggest that ILC patients treated with endocrine therapy have poorer outcomes than similar invasive ductal carcinoma (IDC) patients, and that ILC patients may not benefit from adjuvant tamoxifen. Additionally, we recently identified ILC-specific ER-target genes and de novo tamoxifen resistance driven by ER in ILC model systems. Based on these observations, we hypothesize that ILC-specific signaling pathways driven by ER mediate growth and endocrine resistance in ILC cells.

Among ILC-specific estrogen-regulated genes in the ILC cell lines MDA MB 134VI (MM134) and SUM44PE (SUM44), Wnt signaling genes were highly differentially expressed. The secreted ligand WNT4 was the most strongly estrogen-induced gene in ILC cells. The frizzled receptor FZD7 is also strongly induced in ILC cells, but only transiently induced in the ER-positive IDC cell line MCF-7. Among IDC cell lines, either WNT4 or FZD7 is over-expressed in ER-positive or ER-negative cells, respectively. Conversely, MM134 and SUM44 over-express both WNT4 and FZD7. Also, we identified an ILC-specific ER binding site at WNT4; located in intron 1, this site contains a predicted estrogen response element. Direct WNT4 regulation and parallel regulation of pathway genes suggests that ER controls a WNT4 signaling pathway in ILC cells. In samples from the Cancer Genome Atlas, WNT4 and FZD7 are each over-expressed in ER-positive ILC versus IDC; co-expression is also enriched only in ILC. These observations suggest that a WNT4 signaling pathway may be specifically active in ILC.

To assess whether WNT4 is necessary for estrogen-induced growth, we used siRNA to knock down WNT4. Using either of two siRNAs, WNT4 knockdown completely blocks estrogen-induced growth in ILC cells, but not IDC cells. Consistent with this, WNT4 knockdown abrogated estrogen-regulation of a subset of ER-target genes in MM134 cells; induction or repression was inhibited by WNT4 knockdown prior to estrogen treatment. Thus, a subset of estrogen-induced gene expression changes is mediated by WNT4 signaling. Though Wnt signaling typically acts via the canonical, β-catenin-dependent pathway, we observed that β-catenin signaling is dysfunctional in ILC cells. Additionally, WNT4 over-expression or recombinant protein cannot activate canonical Wnt signaling in breast cancer cell lines. This suggests that WNT4 signaling mediates estrogen-induced growth in ILC cells via a novel non-canonical signaling pathway.

Wnt signaling pathway genes including WNT4 are uniquely regulated in ILC cell lines, and are over-expressed in ILC tumors, suggesting that a WNT4-driven pathway may be active specifically in ILC. WNT4 is necessary for estrogen-mediated growth in ILC cells, and likely signaling via a novel non-canonical signaling pathway. Targeting WNT4 signaling represents a novel approach to modulate endocrine response specifically for ILC patients. Future studies will focus on identifying the signaling pathway controlled by WNT4 in order to identify novel therapeutic targets.
Title: Inhibiting androgen receptor nuclear localization decreases estrogen receptor (ER) activity and tumor growth in ER+ breast cancer

Nicholas C D'Amato¹, Britta M Jacobsen¹, Dawn R Cochrane¹, Nicole S Spoelstra¹, Beatrice L Babbs¹, Anthony Elias¹ and Jennifer K Richer¹. ¹University of Colorado Anschutz Medical Campus, Aurora, CO.

Body: Background: Androgen receptor (AR) is widely expressed in breast tumors, but the role of AR in estrogen receptor (ER)+ tumors is controversial. In the absence of estradiol (E2), dihydrotestosterone (DHT) increases growth of ER+ breast cancer cells in vitro and in vivo. Anti-androgens such as bicalutamide (Bic) and enzalutamide (ENZ) inhibit this DHT-mediated proliferation. Surprisingly we have found that ENZ, which impairs nuclear entry of liganded AR, also inhibits E2-mediated proliferation of ER+ breast cancer cells, while Bic does not.

Hypothesis: We hypothesize that nuclear localization of AR is necessary for maximal E2-mediated proliferation in ER+/AR+ breast cancer cells, and targeting AR with ENZ or other agents that impede AR nuclear entry or cause AR degradation will inhibit growth of ER+/AR+ human breast cancer cell lines and decrease tumor burden in preclinical models.

Methods: ER+/AR+ MCF7, BCK4, and ZR-75-1 cells were treated with E2 plus or minus ER and AR antagonists and proliferation was measured by crystal violet staining or Incucyte live cell imaging. Nuclear AR was assessed by immunocytochemistry or nuclear/cytoplasmic fractionation. For in vivo experiments, 1x10^6 luciferase-expressing MCF7 cells were injected into the mammary fat pad of nu/nu mice and tumor growth monitored by caliper and IVIS imaging.

Results: ENZ blocked E2-induced proliferation and showed synergistic activity with the ER antagonists 4-hydroxy-Tamoxifen (OH-Tam) and Fulvestrant (Fulv) in vitro. E2-induced expression of ER target genes including PR and SDF1 was also inhibited by ENZ, but not by Bic. Similarly, AR knockdown decreased baseline and E2-induced proliferation of MCF7 cells and E2-induced ER target gene expression. Both DHT and E2 treatment induced nuclear translocation of AR, which was decreased by ENZ. Nuclear translocation of AR in response to E2 occurs only in ER+ cell lines, further supporting a role for nuclear AR in E2-induced ER activity. In vivo, ENZ inhibited E2-induced growth of MCF7 tumors as effectively as Tamoxifen (Tam), and the combination of ENZ plus Tam was more effective than either drug alone. ENZ also inhibited growth of Tam-resistant MCF7 cells in vitro.

Conclusions: Our results suggest that nuclear localization of AR plays a previously-unrecognized role in E2-mediated ER activity in ER+/AR+ breast cancer cells. Because of its ability to inhibit nuclear entry of liganded AR, ENZ may serve as an effective therapeutic in ER+/AR+ breast cancers. Importantly, ENZ may be particularly useful in combination with current anti-estrogen therapies (Tam or Fulv) since it affects ER, but via an indirect mechanism acting through AR. ENZ may also be effective in tumors resistant to ER-directed therapy based on in vitro data and published clinical data indicating that many such tumors express more AR protein than ER.

Funded by: DOD BCRP Clinical Translational Award BC120183 to JKR, American Cancer Society Postdoctoral Fellowship PF-13-314-01 – CDD to NCD.
**Title:** Androgen receptor expression in triple negative breast cancer

Hannah Gilmore¹, Vinay Varadan¹, Nicole Williams¹, Cheryl L Thompson¹, Stephanie Kim¹, Peter Hsu¹, Kristy Miskimen¹, Aditi Palkar¹, Shaveta Vinayak¹, Robert Lindner² and Lyndsay Harris¹. ¹Case Western Reserve University, Cleveland, OH and ²Yale University, New Haven, CT.

**Body:** Background: Patients with androgen receptor (AR) positive triple-negative breast cancer (TNBC) may derive a significant benefit from anti-androgen therapy. While AR positivity is often defined by protein expression by immunohistochemistry (IHC), the benefit of anti-androgen therapy in patients who have the Luminal Androgen Receptor (LAR) molecular subtype as defined by genomic profiling is unclear. Our aim was to study the clinical, pathologic and molecular profiles of AR+ tumors by IHC in a diverse set of TNBC.

Methods: Tissue microarrays from two separate, well-annotated institutional cohorts (Case Western Reserve University, Yale University) of early-stage TNBC were evaluated for AR expression by IHC (clone SP107, Ventana Benchmark Ultra). AR positivity was defined as greater than or equal to 10% staining in tumor nuclei. AR expression was correlated with clinical and pathologic features such as age, race, grade, and stage within and between the two cohorts. Gene expression was analyzed with the DASL assay (Illumina) on RNA extracted from formalin-fixed paraffin-embedded material using the Ambion RecoverAll kit (Applied Biosystems AM1975). Pietenpol TNBC molecular subtypes were calculated using the online TNBC type tool. Co-expression of AR with other hormone-related proteins including gross cystic disease fluid protein-15 (GCDFP-15, clone EP1582Y, Ventana Benchmark Ultra) and GATA transcription factor 3 (GATA3, clone L50-823, Bondmax Leica) were also assessed.

Results: Overall, 22% of cases (n=192) were AR+ by IHC. There was no association between AR expression and age, race, grade or stage within or between the two cohorts. Gene expression data was available on 88 tumors with AR staining results and demonstrated that AR positivity by IHC was not exclusive for the TNBC LAR molecular subtype. Three of five (3/5) tumors that were classified and LAR were in fact AR+ by IHC. Tumors that were AR+ by IHC were also identified in the basal-like 2 (BL2), mesenchymal stem-like (MSL), immunomodulatory (IM), and unstable molecular TNBC categories with low frequency. Notably, of cases that were rejected as ER+ by gene expression profiling despite being clinically TNBC, three of five (3/5) were in fact AR+ by IHC. Examination of other hormone-related proteins in a subset of these patients revealed that AR expression was significantly associated with GCDFP-15 expression (p=0.0007) and was borderline significant for GATA3 expression (p=0.068).

Conclusions: AR protein expression levels by IHC were similar in two separate institutional archival cohorts of TNBC and did not correlate with age, race, grade or stage. Comparison of AR IHC data with genomic data suggests that the AR+ phenotype is not unique to LAR subtype of TNBC by gene expression profiling. AR protein expression may be seen in clinically TNBC patients who have a more luminal subtype as evidenced by co-expression of GCDFP-15 and GATA3. Our results suggest that AR staining by IHC may be necessary to capture all patients who may benefit from anti-androgen therapy.
Title: Discordance with estrogen receptor alpha status estimated by immunohistochemical and immunofluorescent method associated with flowcytometry in breast cancer tissue

Maria Rodionova1, Tatiana Bogush1, Evgeniy Dudko1, Shestakova Elena1, Bogush Elena1, Vorotnikov Igor1 and Davidov Michael1. 1N.N. Blokhin Russian Cancer Research Center, Moscow, Russian Federation.

Body: Background.
Estrogen receptors alpha (ER) are one of the most important breast cancer prognostic and predictive markers but routine used immunohistochemical (IHC) method does not allow accurate ER determination because of semi quantitatively assay and tumor heterogeneity. That is why in studying of different tissue paraffin blocks the molecular diagnosis is often controversial. To avoid these problems an accurate quantitative immunofluorescent method associated with flowcytometry (FC) that allows the investigation up to 10,000 or more cells from several parts of surgical breast cancer samples was developed by us and used for the comparison of IHC and FC approaches in study of ER expression.

Materials and methods.
55 breast cancer surgical biopsy specimens were analyzed by FC. Single-cell suspensions were incubated with primary monoclonal antibody (SP1, ab27614) overnight and with secondary DyLight650-conjugated antibody (ab98510, Abcam) for 1.5h. DNA-specific dye Hoechst 33258 (Sigma) was used for the separation of debris and cellular aggregates. Cell fluorescence data were analyzed with FlowJo 7.6.1 software and Kolmogorov-Smirnov statistical approach. The routine IHC indexes of ER status were obtained from the history of cases of the same patients (pts). The pts were divided into 3 groups according to the levels of ER expression: ER–, low ER+and high ER+ that correspond respectively to IHC – <1%; 1-40%; >40% and to FC – <15%; 15-40%; >40%.

Results.
1. Approximately the same proportion of ER– and ER+ tumors, about 30 and 70% respectively, was detected using either IHC or FC method but discordance with the results of individual pts was revealed. 2. Differences in repeated FC estimations of ER status in the same tumors did not exceeded 10%.
3. Differences between IHC and FC indexes in the same tumors were significant. It was demonstrated that, when the tumors estimated as ER– by IHC were analyzed with FC, about 40% of cases were classified as ER+ tumors, and opposite, in the group of tumors estimated as ER+ by IHC, 30% were determined as ER– tumors by FC.
4. There were small differences between IHC indexes in high level of ER+ group (65-100%) but in this group of tumors analyzed by FC there were 15% of ER– ones, 35% –ER+ with low and 50% – ER+ cases with high level of the marker expression. So, accurate quantitative indexes of ER+ in IHC and FC groups were similar in half of cases only.

Conclusion.
Discordance with breast cancer ER status analyzed either with IHC or FC methods in various tissue paraffin blocks as well as between paraffin blocks of the same surgical sample supports a real contribution of tumor heterogeneity in inexactitude of molecular diagnosis of ER breast cancer status. Solely IHC investigation is not accurate enough for the final conclusion and repeated analysis of different tumor paraffin blocks using IHC in association with FC investigation of the same patient surgical tumor sample is needed to characterize ER tumor status in a more precise way.

Supported by RFBR grants (13-04-01004-a, 12-04-00028–a, 14-04-31734–mol-a), scholarship of the President of Russian Federation (376.2012.4.) and RAS grant (FIMT-2014-205).
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P3-04-10  
**Average Grade:** 4.60

**Title:** Progesterone receptor (PR) blockade by antiprogestin, CDB4124 in hormone receptor positive breast cancer cells leads to significant inhibition of G2/M cell cycle genes

Akash Gupa¹, Mi Ran Choi¹, Susan E Clare¹, Jun Wang¹, Oukseub Lee¹, David Z Ivancic¹, J Julie Kim² and Seema A Khan¹.  
¹Feinberg School of Medicine, Northwestern University, Chicago, IL and ²Feinberg School of Medicine, Northwestern University, Chicago, IL.

**Body:** Background: Several lines of evidence suggest that progesterone signaling is important in the breast cancer development, particularly in young women. Therefore, we sought to establish the effects of the antiprogestin CDB4124 (telapristone), and to identify PR related signature genes in hormone receptor positive (ER+ PR+) breast cancer cells.

Methods: The PR expressing breast cancer cell line T47D was used to evaluate responses to PR ligands (P4, MPA and R5020, 10nM) alone or in combination with estradiol (E2, 1nM). The effects of the antiprogestin CDB4124 (0.1 or 1µM) were tested using varying hormonal conditions. The effect on (a) PRE promoter activity by dual luciferase assay; (b) cell proliferation using the MTT assay; (c) cell cycle by flow cytometry; (d) determination of gene expression signatures related to active PR responses. For the gene array experiment, cells were treated with either vehicle or R5020 (10nM) or R5020 (10nM) plus CDB4124 (1µM) in triplicate for 24hr. Total RNA was isolated and converted to cDNA and human Illumina chip microarray was performed. Data obtained from the microarray was further analyzed by Metacore Gene GO and Ingenuity Pathway Analysis. Real time PCR was performed in triplicate to confirm the expression of those genes related to the cell cycle and proliferation. ANOVA analysis and post-hoc Sidak test were used to determined the statistical significance of the data.

Results: The PRE reporter activity resulting from P4, MPA and R5020 stimulation was inhibited by 80-90% in the presence of CDB4124 at 10 to 1000nM (p< 0.001). Cell proliferation was increased by PR ligands (P4, MPA and R5020) in the presence of E2; the addition of CDB4124 caused 50% inhibition of proliferation (p< 0.01) at 72 hours. Cell cycle analysis of T47D cells treated with P4, MPA and R5020 alone or in combination with E2 showed significant increases in S and G2/M phase and decreases in G0/G1. These were blocked by CDB4124 at 0.1 or 1.0µM (p<0.05 for all). Gene GO metacore analysis of genes identified in the microarray revealed significant enrichment of cell cycle pathways (FDR, p< 1.0X10-11 ) upon treatment with R5020. The addition of CDB4124 to R5020 treated samples showed inhibition of the same cell cycle pathways (FDR,p<1.0X10-14). A 16-gene panel related to G2/M phase of cell cycle was selected based on >1.5 fold upregulation (p<0.001) during treatment with R5020,10nM and blockade by CDB4124. Real time PCR confirmed upregulation of this 16 gene panel ≥2.0 (p<0.05) in the presence of PR ligands alone or in combination with E2 which were significantly blocked by the addition of CDB4124.

Conclusion: These data demonstrate that PR mediated cell proliferation occurs upon treatment with three different ligands of PR (P4, MPA and R5020); that PR actively engages key genes involved in the G2/M phase of the cell cycle to drive proliferation of ER+ and PR+ cells; and that the antiprogestin, CBD4124 is a potent transcriptional inhibitor for blockade of PR mediated cell proliferation in hormone receptor positive breast cancer cells.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-04-11
Average Grade: 0

Title: Thyroid hormone up-regulates estrogenic and pro-carcinogenic signaling, inducing a basaloid and more motile phenotype in only steroid receptor positive breast cancer cells

Ann D Thor¹, Zeying Fan¹, Reema Wahdan-Alaswad¹ and Susan M Edgerton¹. ¹University of Colorado Anschutz Medical Center, Aurora, CO.

Body: Background: Co-morbidity of thyroid disease and breast cancer has been recognized for over a century. Up to two-thirds of women with breast cancer have clinical or occult thyroid disease, as compared to one fifth of unaffected females. Thyroid disease typically proceeds breast cancer by at least a decade, and many (nearly half) are on long term thyroid hormone supplementation at the time of diagnosis. The exogenous administration of thyroid hormone, for hypothyroidism or status post thyroidectomy for neoplasia, has been associated with an increased risk of breast cancer and in some studies, a worse prognosis. Causality, however, has been controversial.

Results: We studied over 800 consecutive node negative invasive breast cancer patients diagnosed between 1976 and 1993 from a single institution. Of these, 92% were chemo-naive and only 14% received tamoxifen, reducing interactions with treatment for the study design. We showed untoward significant interactions with both univariate and multivariate analyses, between thyroid hormone supplementation, disease free and disease specific survival only in patients with steroid receptor positive tumors. These interactions were strongest in pre-menopausal, as compared to post-menopausal patients. We thus hypothesized that thyroid hormone (TH), either indirectly or directly, promoted carcinogenesis via the steroid receptors (SR) for estrogen and/or progesterone and have interrogated mechanisms of these interactions in vitro. Estrogen (E2) induce significant cellular proliferation, whereas TH alone (at concentrations of T₄ 1 x 10⁻⁶:T₃ 2.5 x 10⁻⁷ M) induced only mild cell proliferation in SR⁺ (MCF-7 and T47D), but not SR⁻ breast cancer cell lines (MDA-MB-468 and SKBR3). In contrast, TH induced significant proliferation in SR⁺ cells at significantly lower doses (down to T₄ 1 x 10⁻¹²: T₃ 2.5 x 10⁻¹³ M) if administered with low dose estrogen (E2: 1 x 10⁻¹⁰). These mitogenic effects were demonstrated by MTS and DNA quantification assays, as well as flow cytometry, which revealed the induction of G2/M arrest and an increase in the percentage of cells in S phase with TH alone (at higher doses) or TH + E2 (at lower doses). Tamoxifen partially mitigated the pro-growth effects of TH + E2, whereas ICI 182,780 completely abrogated these effects. Using Western blots we have shown that that TH (with or without E2) upregulated ERα expression and activation, Cyclin A, B,D1, and E, E2F1, MAPK/ERK1/2 but not AKT signaling. TH +/- E2 also promoted clonogenicity, motility, a more basal phenotype (CD24+/CD44+) and mammosphere formation in cells grown under non-adherent conditions.

Conclusions: We have demonstrated in Stage I breast cancer patients with minimal systemic treatment, that the administration of TH was associated with a significantly shortened disease free and disease specific survival, only in patients whose tumors expressed SR. TH administered to SR⁺ cell lines, particularly when co-administered with E2 at therapeutic/physiologic doses, upregulates and activates ER, cell proliferation, a more aggressive and motile basaloid phenotype.
Title: Pathological and molecular effects of lack of PR expression in ER positive breast tumors

Rachel E Ellsworth³, Allyson L Valente¹, Matthew T Hueman² and Craig D Shriver². ¹Windber Research Institute, Windber, PA; ²Walter Reed National Military Medical Center, Bethesda, MD and ³Henry M Jackson Foundation for Military Medicine.

Body: Background: Evaluation of hormone receptor status is standard in breast cancer diagnosis; however, prognosis and treatment decisions are frequently based on the status of the estrogen receptor (ER) but not the progesterone receptor (PR). To determine how PR status affects etiology and outcome of ER positive tumors, pathological and molecular characteristics of ER+PR+ and ER+PR- tumors were assessed.

Methods: ER and PR positivity were defined as >10% staining and all patients with ER+PR+ and ER+PR- were identified. Differences in pathological characteristics were evaluated using Chi-square tests with P<0.05 defining significance. RNA was isolated from primary tumor cells after laser-microdissection and hybridized to U133A 2.0 arrays. Gene expression data was analyzed by ANOVA using a false-detection rate <0.5 and >2.0-fold difference to define significance.

Results: Of the 1,139 ER+ tumors, 21% were PR-. Age at diagnosis, ethnicity, tumor size, lymph node and Ki67 status did not differ between groups; however, patients with PR- tumors were significantly more likely to be diagnosed at an advanced stage, with high-grade, HER2 positive tumors, and more likely to die of disease. Thirty genes were differentially expressed including significantly higher expression levels of APOBEC3B and significantly lower expression of GREB1, MAPT and SCUBE2.

Discussion: Expression of PR significantly alters tumor biology and clinical outcomes of ER positive tumors. Genes with altered expression in PR negative tumors are associated with more aggressive characteristics, such as increased invasion, kataegis, regulation of ER and response to tamoxifen. Thus, PR status is critical in the diagnosis and treatment of patients with ER positive breast tumors.
Title: Progesterone receptor activation downregulates GATA3 by transcriptional repression and increased protein turnover promoting breast tumor growth

Franco Izzo¹, Florencia Mercogliano¹, Leandro Venturutti¹, Gloria Inurrigarro², Mauro Ezequiel Cenciarini¹, Roxana Schillaci¹, Leandro Cerchietti³, Patricia Virginia Elizalde¹ and Cecilia Jazmín Proietti¹. ¹Instituto de Biología y Medicina Experimental, Buenos Aires, Argentina; ²Hospital Mater Dei, Buenos Aires, Argentina and ³Weill Cornell Medical College, New York, NY.

Body: The master transcription factor GATA3 is involved in mammary gland development and is crucial for the maintenance of the differentiated status of luminal epithelial cells. The role of GATA3 in breast cancer as a tumor suppressor has been widely established, although insights into the mechanism of GATA3 expression loss are still required. In the present work, we demonstrate that progestin-activated progesterone receptor (PR) reduces GATA3 expression through regulation at the transcriptional and post-translational levels in breast cancer cells. In the former mechanism, the histone methyltransferase enhancer of zeste homolog 2 (EZH2) is co-recruited with activated PR to a consensus progesterone response element in GATA3 proximal promoter, increasing the tri-methylation of lysine 27 of histone 3 (H3K27me3) and inducing chromatin compaction, resulting in decreased GATA3 mRNA levels. This transcriptional regulation is coupled with increased GATA3 protein turnover through progestin-induced GATA3 phosphorylation at serine 308 followed by 26S proteasome-mediated degradation. Both molecular mechanisms converge to accomplish decreased GATA3 expression levels in breast cancer cells upon PR activation. In addition, we demonstrated that decreased GATA3 levels are required for progestin-induced upregulation of cyclin A2, which mediates the G1 to S phase transition of the cell cycle and was reported to be associated with poor prognosis in breast cancer. Finally, we showed that downregulation of GATA3 is required for progestin stimulation of both in vitro cell proliferation and in vivo tumor growth.
Title: Prognostic impact of Her2 and hormone receptor expression in inflammatory breast cancer: A SEER database analysis

Anna Maria Affan¹, Babu P Mohan¹, Kali Praveena Iruku¹ and Keyvan Ravakhah¹. ¹St Vincent Charity Medical Center, Cleveland, OH.

Body: INTRODUCTION: Inflammatory breast cancer (IBC) is known to be one of the most aggressive forms of breast cancer. Patients with IBC generally present with early metastases and are frequently resistant to conventional chemotherapies. By using the SEER database, we explored the clinical relevance of Her2 and hormone receptor (HR) expression in IBC tumors and the possible impact it may have on management.

METHODS: Data for patients with IBC was extracted from the Surveillance, Epidemiology, and End Results (SEER 18) database from 2010 to 2011. A sub-analysis with the available receptor status data for the same period was analyzed using CanSurv survival software. The Actuarial method was used to construct survival curves according to the following receptor variables; Her2+/HR+, Her2+/HR-, Her2-/HR+, and triple negative IBC groups. Demographic data including race and age was also extracted.

RESULTS: Of 354 cases of IBC reported, 5% (n=18) of the tumors were Her2+/HR+, 7.9% (n=28) Her2+/HR-, 15.3% (n=54) Her2-/HR+, and 12.4% (n=44) triple negative. Highest 1 year survival rates were seen in those patients with Her2-/HR+ tumors at >95% while Her2+/HR+ had the lowest 1 yr survival rates at 65%.

Caucasian patients above 30 years represented the highest racial group at approximately 70% (n=105). Analysis of this subgroup showed 38% (n=40) of these patients having HER2-/HR+. Despite more than 75% (n=33) of these patients presenting with advanced disease, the 1 year survival remained highest among all the receptor combinations analyzed. This observation held true across all racial groups even with those population numbers for IBC being less.

CONCLUSION: The Her2-/HR+ receptor combination group showed the greatest 1 year relative survival when compared to the other groups. These results suggest that HR expression in the absence of Her2 is associated with an increased survival. This can play a role in the management of IBC by incorporating hormonal therapy into the treatment protocols for patients with IBC to possibly improve survival.
Title: Ethinylestradiol treatment downregulates ER and upregulates PgR; Immunohistochemical analysis in postmenopausal breast cancer tissues after prior long-term estrogen-deprivation therapy

Hirotaka Iwase¹, Yoko Omoto¹, Takashi Takeshita¹, Mutsuko Yamamoto-Ibusuki¹, Mitsuhiro Hayashi¹, Aiko Sueta¹, Saori Fujiwara¹ and Yutaka Yamamoto¹. ¹Kumamoto University, Kumamoto, Japan.

Body: **Background:** Estrogen receptor (ER) positive breast cancer can often be treated by hormone therapy; however a certain population of ER-positive patients becomes resistant to hormone therapy after long-term hormone treatment. Ethinylestradiol (EE2) is a derivative of estrogen which has shown promising effects on in these patients. **Methods:** We successfully obtained tissue samples from 6 patients undergoing EE2 treatment and examined 13 well-known breast cancer-related factors by analyzing their gene expression and by immunohistochemistry. Of the 6 patients, 5 responded but one patient did not. **Results:** Before EE2 treatment, staining for both ER and androgen receptor (AR) was strong in the nucleus, with weak staining of the progesterone receptor (PgR). EE2 treatment significantly down-regulated ER and up-regulated PgR while nuclear and cytosolic AR were oppositely down- and up-regulated, respectively, by EE2. Cytosolic staining of BRCA1 was significantly up-regulated by EE2 whereas nuclear staining tended to decrease. Individual comparisons suggested less induction of PgR and decreasing AKT but increasing pAKT in the non-responder following EE2 treatment. **Conclusion:** Our observations revealed that EE2 activated ER downstream genes, although it did not stimulate cell growth. This suggests that hormone resistant cells might receive growth signals from a non-genomic pathway and this may be reflected in their sensitivity to EE2 treatment.
Title: Estrogen receptor β agonists reduce breast cancer tumor growth in syngeneic mouse models

Cathy Samayoa¹, Naveen K Krishnegowda¹, Ratna K Vadlamudi¹ and Rajeshwar R Tekmal¹. ¹University of Texas Health Science Center, San Antonio, TX.

Body: The estrogen receptors (ER) play a significant role in breast cancer, with the majority of breast cancers expressing estrogen receptor alpha (ERα) and depending on its signaling. ERα has proliferative function, however, estrogen receptor beta (ERβ) has anti-proliferative functions. Recently, several selective ERβ agonists have been identified. The objective of this study was to determine the effectiveness of ERβ agonists in inhibiting the growth of distinct breast cancer cells both in-vitro and in-vivo, and to determine the mechanisms involved. The mouse mammary tumor cell lines, D2A1 and MM51, express both ERα and ERβ. Specifically D2A1 cells are dependent on estrogen for growth and have increased expression of aromatase. Additionally, D2A1 cells are very aggressive and highly metastatic; representing a good model of breast cancer progression. MM51 cells are Her2/neu positive and are an adequate model to study the crosstalk between growth factors and ER signaling. Syngeneic mouse tumor models were used to incorporate an intact immune system which plays a role in the tumor’s microenvironment. Our in-vitro studies demonstrate that ERβ agonists significantly inhibit cell growth in a dose dependent manner. Our syngeneic studies show that, in-vivo, ERβ agonists effectively reduce tumor volume and inhibit tumor progression. This study reveals that in addition to acting on ERβ, these agonists reduce the expression of ERα at both the mRNA and protein level, therefore modulating the ratio of ERα to ERβ. Additionally, treatment with ERβ agonists results in increases apoptosis through increased p53 expression, and cell cycle arrest through p27 and cyclin D1. Together, these studies demonstrate the therapeutic potential of ERβ agonists for the treatment of breast cancer.
**Title:** Triple negative breast carcinomas clinical and pathology associated with androgen receptor expression

Marcia Graudenz¹, Mirian Pedron¹, João Maximiliano¹, Diego Uchôa¹ and Sídia Jacques¹. ¹UFRGS, Faculty of Medical Sciences, Porto Alegre, Rio Grande do Sul, Brazil.

**Body:**

**INTRODUCTION:** Triple negative breast carcinomas (TNBC) are a heterogeneous group of tumors characterized by poor patient survival and lack of targeted therapies. Androgen receptor (AR) has been described in TNBC but the prognostic impact of the expression in this subgroup of tumors is not clear. **OBJECTIVE:** To investigate the association of AR expression status by immunohistochemistry in TNBC cases with clinical (age, survival) and pathological variables (tumor size, tumor grade).

**METHODS:** 62 TNBC were analyzed by automated immunohistochemistry for androgen receptor. Immunohistochemistry was scored by two investigators and biomarker expression was assessed by H-Score (intensity plus the percentage of staining). Kaplan–Meier was used to evaluate overall survival, where differences in distributions were evaluated based on marker expression. **RESULTS:** 26% of TNBC were AR-positive (n=16) and 74% AR negative (n=46). All AR-positive cases occurred in women ≥ 40 years, while 13% of AR-negative cases were seen in women ≤ 40 years. 93% (52/56) of all TNBC were infiltrating ductal carcinomas of no special type (ICNST) and 14/15 (93%) of AR-positive cases were ICNST. Tumor size varied from 2-4.9 cm in the majority of AR-positive cancers (n=8/13; 62%). 60% (n=9/15) of AR-positive cases showed histological grade 3 tumors, followed by 27% of grade 2 tumors. No differences were observed between AR-positive and AR-negative patients when compared for age, tumor size, tumor grade and tumor type (all p>0.17). AR immunohistochemical positivity was also not associated with better overall survival (p=0.737, N = 51) or disease-free survival (p = 0.552; N = 45) in TNBCs. **Conclusion:** From the prognostic point of view, AR immunoreactivity has been associated with better overall patient survival. This result was not confirmed in our series. This could be related to the relatively small series of analyzed samples and to the low prevalence of AR-positive cases in this specific breast cancer subtype. Further studies with bigger samples are needed to investigate this biomarker in TNBC.

**Key Words**

Breast Cancer, Immunohistochemical markers, Androgen receptor Triple negative.
**Title:** Development for clinical utility of a validated predictive test of clinical response to aromatase inhibitors

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**Body:** Background: Aromatase inhibitors (AIs) have an established role in the treatment of estrogen receptor alpha positive post-menopausal breast cancer. Response rates are only 50-70% even in patients with ER-rich cancers in the neoadjuvant setting and are lower in advanced disease. Recently we developed and validated a microarray-derived 4-gene test to predict response to AIs in the neoadjuvant setting. Whole-genome expression analysis is impractical for clinical utility. There is a need to translate and validate any test utilising clinically accessible and reproducible material and technologies such as polymerase chain reaction (PCR) and immunohistochemistry (IHC).

Methods: The original microarray experiment used pre- and on-treatment (at 14 days and 3-months) biopsies from 89 post-menopausal women with ER-rich breast cancer receiving 3 months of neoadjuvant letrozole. Dynamic response was based on periodic 3D ultrasound measurements performed during treatment. The derived 4-gene model was independently validated in a cohort of 44 post-menopausal women with ER-rich breast cancer treated with neoadjuvant anastrozole [table 1]. RNA was extracted from the original biopsies for RT-qPCR analysis using validated primers for the 4 genes with SYBR-green technology normalised to the geometric mean of 3 housekeeping genes. Matched formalin-fixed paraffin embedded (FFPE) tissue sections were used for IHC with optimised antibodies against 3 of the 4 proteins (where validated antibodies were available) using Envision technology.

Results: PCR: Microarray and PCR expression levels for each of the four genes were well correlated (Pearson r=0.87-0.65, p<0.0001). Application of the 4-gene model, using PCR expression levels, to a cohort of patients from the initial training set (n=26) resulted in prediction of response with 96% accuracy [table 1]. IHC: stained FFPE tissue sections for proteins corresponding to the 3 most informative genes in the model were independently scored using a histoscore approach (0, 1+, 2+, 3+) in a sample of patients where tissue was available (n=28). Increasing histoscores were associated with increasing gene expression for all 3 proteins (r=0.72, 0.73, 0.60). Each sample was assessed and histoscore determined, then a positive and negative cut-off histoscore was determined for each protein to maximise response prediction. This was then applied to an IHC decision tree, based on the original 4-gene microarray model, and was able to categorise patients as responsive or non-responsive with 89% accuracy.

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<th>N</th>
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Sensitivity and specificity of model in training (microarray, PCR, IHC) and validation datasets (microarray). PPV/NPV = postivie/negative predictive value.

**Conclusion:**
- A 4 gene model has been developed and validated to predict response to neoadjuvant aromatase inhibitors.
- This model has been shown to work with a high degree of accuracy using both PCR and IHC technologies. Further independent validation is currently underway.
- This new test has the potential to predict accurately the benefit of endocrine therapy and has huge potential clinical value.
Title: Global characterisation of the SRC-1 transcriptome and rational drug design results in the identification of a novel peptide targeting ADAM22 in endocrine resistance

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Body: In spite of therapeutic advances, up to 25% of luminal breast cancers will eventually develop resistance to endocrine therapy and develop metastatic disease. The underlying mechanism causing ER-positive, steroid responsive tumours to develop a resistant, metastatic phenotype remains unresolved. Previous work from our group and others has identified the P160 protein SRC-1 as a significant predictor of recurrence on endocrine therapy. The purpose of this study is to further examine downstream SRC-1 targets in the context of endocrine resistant breast cancer.

We adopted a global approach to define the transcriptional targets of SRC-1. SRC-1 ChIP sequencing in endocrine resistant luminal B breast cancer cells was combined with SRC-1 gene expression array analysis. This identified a number of pathways significantly elevated following tamoxifen treatment, including a number involved in cellular adhesion. From these pathways, A Disintegrin And Metalloproteinase-22 (ADAM22) was selected for further study.

Knockout studies confirmed ADAM22 as a tamoxifen dependent SRC-1 target gene. Functional assays including migration, three dimensional cell culture and adhesion independence growth assays confirmed a role for ADAM22 in promoting a migratory, aggressive phenotype. Samples from two separate TMAs comprising over 1,000 patients confirmed that ADAM22 is associated with poor disease free survival in breast cancer patients.

LG1 is a naturally occurring neuropeptide which acts on an inhibitory manner on ADAM22 in the central nervous system. Using molecular modelling, a novel peptide mimetic targeting the disintegrin binding domain of ADAM22 was designed. Treatment with this peptide mimetic restored endocrine resistant cells to a less aggressive, sensitive phenotype, similar to the effect seen with knockdown of ADAM22. Moreover in an endocrine resistant xenograft model, treatment with the LG1 mimetic significantly reduced primary and metastatic tumour burden in tamoxifen treated animals.

We have used next-generation sequencing techniques to identify a novel therapeutic target in endocrine resistant, metastatic breast cancer. Rational drug design has been used to manufacture a therapeutic peptide against ADAM22. A combination of in vitro, in vivo and patient studies has confirmed a role for ADAM22 in metastatic breast cancer. Our novel peptide mimetic may form a future basis for targeting ADAM22 in endocrine resistant disease.
Title: Reversal of endocrine therapy resistance with inhibitors of AKT, mTOR, or MEK as single agents or in combination

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Body: Background: Crosstalk between estrogen receptor (ER) and growth factor receptor (GFR) downstream signaling pathways [PI3K/AKT/mTOR and MEK\p42\44MAPK (MAPK)] has been associated with endocrine resistance. Single downstream inhibitors like everolimus partially reverse endocrine resistance. However, more than one downstream escape pathway may contribute to endocrine resistance. Furthermore, disruption by a single downstream inhibitor of the negative feedback loops that balance the amplified signals from GFRs may result in compensatory pathway activation. Therefore we investigated whether dual downstream signaling inhibitions are required to more effectively overcome tamoxifen-resistant growth, using in vitro and in vivo models.

Materials and Methods: The effects of different kinase inhibitors (i) AZD2014 (mTORi), AZD5363 (AKTi), and AZD6244 (MEKi) on endocrine therapy [tamoxifen (Tam) and fulvestrant (Fulv)] were tested in ER+ MCF7 and T47D Tam-resistant derivatives (TamR). In vitro growth and apoptosis were assessed using methylene blue and c-PARP, respectively. Western blot analysis was used to analyze the effect of each inhibitor or combination on their respective pathway substrates. Nude mice with transplantable MCF7 TamR xenografts at a size of 200 mm3 were randomized to continued Tam, continued Tam + (mTORi, AKTi, MEKi, mTORi+MEKi, AKTi+MEKi) or Fulv ± the inhibitor combinations.

Results: We found that in two ER+ models MCF7 TamR and T47D TamR in vitro, both mTORi and AKTi were effective in restoring growth inhibition, and effective in inhibiting their respective pathways. Interestingly, inhibition of mTOR and AKT resulted in upregulation of pMAPK. However, while MEKi did inhibit its pathway; it did not restore growth inhibition by the antiestrogens. On the other hand, dual inhibition, adding the MEKi to either mTORi or AKTi, resulted in a more potent reduction of cell growth as well as of downstream signaling. In the TamR derivative of MCF7, ER is still maintained and plays a role in resistance, unlike the T47D model, where there is little to no ER expression after TamR develops. Thus, as might be expected, combining downstream inhibitors with potent ER blockade by Fulv enhances the effect of single and dual downstream signaling inhibitors mainly in the MCF7 TamR model. Finally, Fulv in addition to dual downstream inhibitions (mTORi+MEKi or AKTi+MEKi) delayed MCF7 TamR xenograft growth significantly more than Fulv with single downstream inhibitors.

Conclusion: This study provides evidence that dual inhibition of GFR downstream pathways is needed to overcome activation of escape mechanisms that are up-regulated with acquired endocrine resistance and after resistance to single pathway inhibitors. Although the downstream inhibitors alone significantly inhibit TamR growth, combination with Fulv robustly slowed growth of TamR tumors in vivo. Based on these results further studies combining MEK inhibition with inhibitors of the PI3K pathway and ER downregulators are warranted.
Title: AKT antagonist AZD5363 targets estrogen receptor (ER) function in endocrine resistant breast cancer (BC) and synergises with fulvestrant in vivo

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Body: AIM: To evaluate the efficacy and functional consequences of combining AZD5363 with endocrine therapy in pre-clinical models of endocrine-sensitive and resistant ER+ BC.

BACKGROUND: The PI3K/AKT/mTOR signalling pathway plays an important role in BC. Its close interaction with ER signalling becomes more complex and inter-dependent with acquisition of endocrine resistance. Targeting the mTOR pathway in combination with endocrine therapy has shown clinical utility. However, a negative feedback loop exists downstream of the PI3K/AKT/mTOR pathway with mTOR inhibition leading to increased activation of IGFR1-dependent AKT activity potentially negating long-term benefit. Direct blockade of AKT in combination with endocrine therapy, may provide a better rationale for treatment of endocrine-resistant BC, impacting on both cell survival/apoptosis and ER ligand-independent signaling. In this investigation, we assessed the efficacy of AZD5363, a pan-AKT inhibitor with endocrine therapies in pre-clinical models of endocrine sensitive and resistant ER+ BC and its impact on molecular and cellular response.

METHODS: Inhibition of AKT using AZD5363 was examined in 5 ER+ BC lines before and after adaptation to long-term estrogen deprivation (LTED) or tamoxifen (TAMR). The effects of AZD5363 on cell proliferation were determined alone and in combination with endocrine treatment and feedback upregulation and activation of receptor tyrosine kinases (RTKs) was examined by western blotting. ER-transactivation was measured with an estrogen-response element (ERE)-linked luciferase reporter construct and confirmed using chromatin-immunoprecipitation. Global gene expression analysis was used to identify pathways associated with response. Xenografts were treated with AZD5363 ± fulvestrant to determine in vivo effects.

RESULTS: AZD5363 caused a dose-dependent decrease in proliferation in all cell lines tested (GI50<500nM) with the exception of HCC1428-wild-type and LTED. Of note both T47D-LTED and ZR75-LTED, which lose expression of ER were exquisitely sensitive (GI50∼100nM). AZD5363 re-sensitised the TAMR cell line to tamoxifen and acted synergistically with fulvestrant in MCF7-Wt and MCF7-LTED (CI<1). AZD5363 decreased phosphorylation of AKT/mTOR substrates PRAS40, p70S6 and S6 in all cell lines tested and caused a significant decrease in phosphorylation of ERα with an associated 50% reduction in ERα-mediated transcription and decrease in recruitment of ER and CBP to ERE on the TFF1 promoter. Furthermore, AZD5363 reduced Rb and cyclinD1. Inhibition of AKT with AZD5363 resulted in upregulation and activation of RTKs, including IGF-IR, EGFR, ERBB2 and ERBB3, which was cell line specific. Global gene expression and pathway analysis of MCF7 and MCF7-LTED treated with AZD5363 highlighted the relevance of ERBB2-ERBB3, ERK5 and IGF1 signalling as potential feedback loops. Combined treatment with AZD5363 and fulvestrant showed strong synergy in an MCF7 xenograft.

CONCLUSION: These data suggest that AZD5363 plus fulvestrant may be effective in BC that is sensitive or resistant to E-deprivation or tamoxifen and that activated AKT is a determinant of response. These data strongly support the need for clinical evaluation.
A nude mouse model of diet-induced-obesity to study the mechanisms of resistance to aromatase inhibitor letrozole in MCF-7Ca xenografts

Amanda Schech¹, Stephen Yu¹, Preeti Shah¹, Olga Goloubeva¹, Angela Brodie¹, Saranya Chumsri¹ and Gauri Sabnis¹. ¹University of Maryland School of Medicine, Baltimore, MD.

Body: Obesity has been identified as one of the risk factors for breast cancer progression. However, the mechanisms underlying this link are not completely understood. Specifically, breast cancer patients who are overweight, obese or have excess abdominal fat have increased risk of local or distant (metastasis) recurrence and cancer related death. Furthermore, breast cancer patients gain weight during treatment, especially those who receive hormone depletion therapies, are at increased risk of developing obesity and metabolic syndrome as survivors. Importantly, the presence of obesity may influence the resistance of breast cancer to existing treatments, such as aromatase inhibitors (AIs). In an effort to understand the effect of obesity on the growth of hormone-dependent breast cancer tumors, we fed ovariectomized athymic nude mice a diet containing 45% kcal fat (45% of the total calories coming from the fat in the diet) and low fat (10% kcal fat) diet. We also used a standard chow diet to compare with our previous results. We saw that mice fed 45% kcal fat diet had a higher rate of tumor growth (p=0.04) compared to mice that were fed 10% kcal fat containing diet or chow diet. In addition, the mice fed 45% kcal fat had higher body weight, fasting insulin (as measured by C-peptide ELISA) and high fasting glucose levels. Glucose tolerance test (IP-GTT) demonstrated that mice fed high fat diet exhibited reduced glucose tolerance as measured by high AUC_{glucose} in high fat versus low fat or chow diet. There was no statistically significant difference between the chow diet and the low fat diet.

Next, we examined the effect of insulin on the growth of MCF-7Ca cells in response to E₂ or letrozole. When co-treated with 2µM of exogenous insulin (in otherwise serum starved condition), the growth of MCF-7Ca cells was not stimulated by E₂ at 1nM. However, at lower doses, the combination of insulin with E₂ stimulated cell growth more than E₂ alone. This suggests that insulin makes MCF-7Ca breast cancer cells hypersensitive to mitogenic effects of E₂. Hypersensitivity to E₂ is one of the suggested mechanisms of resistance to endocrine therapy. Furthermore, response to anti-proliferative effects of letrozole was also abrogated in presence of insulin (2µM). This was more pronounced at lower dose of letrozole. These results suggest that the presence of insulin makes MCF-7Ca cells less responsive to E₂ and letrozole. Furthermore, tumors of mice fed with high-fat diet also had higher activation of MAPK and Akt, suggesting induction of growth factor receptor pathways. Exogenous insulin treatment of MCF-7Ca cells also resulted in reduction of ERα protein levels and increase in p-IR, p-IGFR and p-Akt. These results suggest that diet-induced obesity may result in acquisition of resistance to letrozole due to development of hyperinsulinemia. We hypothesized that the presence of obesity can augment adaptation of cancer cells and impact the mechanism. This may be the result of a dramatically different hormonal milieu upon development of obesity.
2014 San Antonio Breast Cancer Symposium

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Title: Determining the role of somatic ERα mutations in acquired hormone (or SERM) resistance

Sean W Fanning¹, Christopher Mayne², Weiyi Toy³, Yang Shen⁴, Abhishek Sharma², Srinivas Panchamukhi¹, Jason Nowak⁵, Kendall W Nettles⁵, Sarat Chandarlapaty³, John A Katzenellenbogen² and Geoffrey L Greene¹. ¹University of Chicago, Chicago, IL; ²University of Illinois, Urbana-Champaign, IL; ³Memorial Sloan Kettering Cancer Center, New York, NY; ⁴Toyota Technological Institute, Chicago, IL and ⁵Scripps, Jupiter, FL.

Body: The estrogen receptor alpha (ERα) is a member of the nuclear hormone receptor (NHR) family and is critical for the etiology and treatment of breast cancer. Approximately 70% of breast cancers express ERα and many of these are sensitive to anti-estrogen therapies. Selective estrogen receptor modulators (SERMs), such as tamoxifen and raloxifene, are approved to treat or reduce the risk of ER-dependent breast cancers. SERMs act by competitively binding to the ERα ligand-binding domain (LBD). Unfortunately, metastatic breast tumors recur in approximately half of patients and become SERM resistant while remaining ER-positive in many cases. Recently, conserved somatic mutations in the ERα LBD were identified in patients who received SERM/aromatase inhibitor (AI)/selective estrogen receptor disruptor (SERD) therapy for an average of five years. Because these mutations were observed in approximately 25% of tumors and the most frequent mutations (Y537S or D538G) were located in or just prior to helix 12 (H12), the molecular switch that controls AF-2 activity, they represent a possible mechanism for acquired SERM insensitivity for a significant population of patients. Further studies revealed that these mutations conferred hormone-independent ERα activity and that the inhibitory efficacy of currently approved SERMs was reduced. Our goal is to understand the effects of these mutations on the structure and function of ERα in these tumors to guide the generation of novel compounds which bypass the effects of these somatic mutations. Here, we employ x-ray crystallography, molecular dynamics simulations, biochemical assays in addition to breast cancer cell proliferation assays to dissect the role of somatic mutation in acquired hormone/SERM resistance. X-ray crystal structures of the ERα LBD D538G mutant in the unliganded (apo), agonist and SERM-bound states, combined with molecular dynamics simulations, reveal a stabilized loop between H11 and H12 that allows the receptor to preferentially adopt an agonist conformation versus an antagonist conformation. The biochemical and breast cancer cell proliferation assays reveal structural insights that may explain mutant ERα function within the tumor. Further, we use these methods to explore the utility of next generation SERMs and SERDs to inhibit these mutant ERs as well as to guide the synthesis of additional novel compounds. Importantly, our work is expected to yield more potent and effective SERMs/SERDs that overcome the impact of acquired activating mutations and result in improved patient survival.
Title: Effect of a novel selective progesterone receptor modulator EC312 for menopausal hormone therapy (MHT) in comparison with Bazedoxifene in vitro

Hareesh B Nair¹, Bindu Santhamma¹, Walter Elger¹ and Klaus J Nickisch¹. ¹Evestra, Inc, San Antonio, TX.

Body: Significant increase in the breast cancer risk reported from the Women’s health initiative (WHI) study and other clinical studies in postmenopausal women lead to a significantly reduced usage of estrogen/ progestin based HRT treatment in women. A new and interesting approach to provide women with an effective treatment for vasomotor symptoms without an increased breast cancer risk has been reported in preclinical and clinical studies. A combination of conjugated estrogens (CE) + selective estrogen receptor modulator (SERM), bazedoxifene (BZA) offers an effective treatment for overcoming vasomotor symptoms but preventing the proliferation of breast tissue. However, this treatment also has some drawbacks. A higher rate of induced endometrial hyperplasia due to unopposed estrogenicity with BZA+ CE treatment regimen has been noted in recent randomized clinical trials. In addition, the risk of venous thromboembolic events has been shown not avoided completely in women who were older at the time of BZE+CE treatment compared with those who were younger. Therefore an unmet need for new HRT regiments that combine effective treatment of symptoms without increasing the risk of breast cancer in postmenopausal women.

A potential approach is the combination of estradiol with a partial agonistic antiproprgestin (mesoprogstin or SPRM). In order to test the hypothesis we have compared the combinatory effect of BZA with estrogen (E2) as well as a novel SPRM, EC312+ E2 in a cell culture model of postmenopausal breast cancer. In our model, we have found that the antiestrogenic effect of BZA on T47D cell growth was comparable that of EC312. Both EC312 and BZA increased apoptosis dose dependently in the presence of E2 as well as reduced E2 mediated BrdU incorporation as a read out of cell proliferation. E2 (1nM) treatment increased the expression of anti-apoptotic genes whereas 100nM treatment of EC312 and BZA decreased the levels of anti-apoptotic genes. Expression of E2 stimulated effect on target genes such as cMyc, pS2 and Cyclin D1 were reduced with the treatment of EC312 or BZA. It is evident from our study that EC312 as a novel SPRM exerted no agonistic effects on human breast cancer cells and effectively blocked the stimulatory actions of E2 or conjugated estrogens. The in vitro molecular characterization of EC312 was performed using gene transactivation assays and confirmation of EC312 as a PRM was determined in cycling guinea pig model. Our future animal model with breast cancer xenografts will underscore the possibility of using EC312 as a safer SPRM that antagonize the growth promotional effect of estrogens as well as eliminating the increased breast cancer risk of progestin component of MHT. Further rationale for choosing SPRM/mesoprogestin such as EC312 would stabilize the uterine tissue and endometrium to minimize the oligo-menorrhea and endometrial hyperplasia associated with MHT.
Title: Glucocorticoid and aldosterone mimic progestin-induction of a therapy-resistant cytokeratin-5 positive cell population in estrogen receptor-positive breast cancer through a Bcl6-dependent mechanism

Hallgeir Rui¹, Takahiro Sato¹, Amy Peck¹, Melanie A Girondo¹, Chengbao Liu¹, Albert J Kovatich², Jeffery A Hooke², Craig D Shriver², Edith Mitchell¹, Terry Hyslop³ and Chelain Goodman¹. ¹Thomas Jefferson University, Philadelphia, PA; ²Walter Reed National Military Medical Center, Bethesda, MD and ³Duke University, Durham, NC.

Body: Resistance to anti-estrogen therapy remains a significant problem in patients diagnosed with estrogen receptor-α (ERα) positive breast cancer. Recent progress has defined a "lumino-basal" subclass of ERα-positive breast cancer characterized by mosaic presence of a minor population of ERα-negative cells expressing the basal cytokeratin-5 (CK5). The CK5-positive cells are therapy-resistant and have increased tumorigenic potential. Initial studies have suggested that progestins but not other steroids expand this CK5+ cell population. Unexpectedly, we discovered that at least two 3-ketosteroids other than progestins, glucocorticoids and mineralocorticoids, are capable of inducing the CK5+/ERα- cell population. CK5+ cells induced by glucocorticoid or aldosterone showed increased clonogenicity in soft agar, expressed the stem cell marker CD44, showed loss of ERα and PR expression, and demonstrated therapy-resistance with reduced apoptosis in response to chemotherapy, and were further enriched following adjuvant antiestrogen or chemotherapies. Induction of CK5+ cells by 3-ketosteroids was consistently preceded by induction of Bcl6, a transcriptional repressor implicated in breast cancer progression. Suppression of Bcl6 by shRNA or the Bcl6 suppressor, prolactin, abolished 3-ketosteroid-induction of CK5+ cells. Prolactin also blocked 3-ketosteroid-induced colony formation in vitro and suppressed progesterin-induction of the CK5+ cell population in T47D xenograft tumors in vivo. Survival analyses with recursive partitioning revealed that CK5 and Bcl6 transcripts or protein levels in ERα+ breast cancer identify patients at high or low risk for tumor in two independent cohorts. The observations provide a mechanism by which stress-related or pharmacologic elevation of glucocorticoids may adversely affect patients with ERα+/CK5+ breast cancer, and may justify further exploring of inhibitors to 3-ketosteroid receptors or Bcl6 for therapeutic benefit.
Title: Glutamine metabolism promotes survival through the unfolded protein response in endocrine resistant breast cancer

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Body: About 70% of all breast cancers are estrogen receptor alpha positive (ER+). Anti-hormone therapy such as antiestrogens (e.g., Tamoxifen; TAM) are often used to treat ER+ breast cancer but breast cancer cells can develop resistance to these drugs (endocrine resistance). Unfortunately, ~50% percent of all antiestrogen treated tumors eventually develop endocrine resistance, and therefore, there is an urgent need to find ways to treat this incurable disease. Endocrine resistant cells survive antiestrogen treatment by initiating a pro-survival pathway mediated by an evolutionary conserved process call the unfolded protein response (UPR) within the endoplasmic reticulum. Cellular stress induced by therapeutics can be initiated by the UPR leading to a pro-survival response in the short-term and a pro-death response in the long-term, depending on the magnitude of the stress signal. Our studies show that antiestrogens can decrease glucose uptake in ER+ breast cancer cells, and in glucose-deprived conditions, presence of glutamine in the media can trigger the UPR through GRP78-IRE1α. Subsequently, a glutamine-dependent pathway can induce cell death via GRP78-IRE1α-JNK-CHOP and survival in others via GRP78-IRE1α-XBP1. Glutamine metabolites such as glutamate (essential substrate for many vital cellular processes) and proline (substrate for collagen production) are significantly elevated in endocrine resistant cells. Knockdown of glutaminase (GLS1) with siRNA or small molecule inhibitor, CB-839, significantly decreased cell proliferation in endocrine resistant cells compared with sensitive cells within 48 h. Moreover, cell lines derived from endocrine resistant cells that are grown in glucose-free media are significantly more sensitive to CB-839 compared with their respective controls, indicating an increased dependency on glutamine. Glutamine may offer an alternate source of energy and raw materials in periods of glucose deprivation, and in endocrine resistant cells, this adaptive pro-survival cellular metabolic mechanism is up-regulated. Thus, CB-839 may be a potent anti-cancer agent in treating antiestrogen resistant breast cancers.
**Title:** Circulating oxysterol metabolites as potential new surrogate markers for hormonotherapy in patients with hormone receptor-positive breast cancer? A pilot study

Florence Dalenc¹, Luggi Iuliano², Thomas Filleron³, Maud Voisin⁴, Henri Roché¹, Sandrine Silvente-Poirot⁴ and Marc Poirot⁴.
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**Body:**

**Background:** Clinicians need new predictive biomarkers of response to hormonal therapy in patients (pts) with hormone receptor-positive (HR+) breast cancer (BC). Current treatments used the selective estrogen receptor modulator tamoxifen (Tam) or aromatase inhibitors (AI). Several data from the literature and our preclinical results indicate that cholesterol metabolism pathway is involved in BC oncogenesis and sensitivity/resistance to Tam. Our team has established that Tam modulates cholesterol oxidative metabolism and modulate oxysterol (OS) levels *in vitro* and *in vivo* on BC cell lines. Tam is a potent inhibitor of the cholesterol-5,6-epoxide hydrolase (ChEH), which led to the accumulation of cholesterol epoxides (CEs) and the inhibition formation of cholesterol triol (CT). Importantly, we found that CEs mediated the cytotoxicity of Tam in BC. Therefore, an increase in CEs, and no increase in CT could be markers of the efficacy of Tam reflecting its inhibition of ChEH in pts. On the other hand 27-hydroxycholesterol (27HC), another OS, is an estrogen receptor ligand with tumor promoter properties in BC. The 27HC level could constitute a new risk factor for BC development that has to be measured. The impact of AI on cholesterol metabolism is totally unknown, it is thus important to study its impact on OS levels in pts.

**Methods:** We have conducted a monocentric, prospective, clinical trial in pts who must received Tam or AI in adjuvant or metastatic setting for a HR+ BC. The primary end point was the feasibility of detection of circulating OS (CEs, CT and 27HC) in the serum of patients before the first administration of hormonal therapy and at 1 month. Key secondary end points were to measure variations in the concentration of OS according to patients and treatments. 12 different OS including CEs, CT and 27HC were quantified by GC/MS.

**Results:** bCE relative concentration significantly increased in the entire population (p=0.0109) while no increase in CT was measured under HT treatment establishing that Tam inhibited ChEH in pts. It should be noted that an important inter-individual variability in pts was observed according the OS species considered. AI stimulated the accumulation of βCE (p=0.0022) suggesting that they modulate CEs metabolism. We found that AI were not direct inhibitors of ChEH in BC cells suggesting that they modulate CEs level through a different mechanism than Tam. Importantly, we found that letrozol, but not exemestane or Tam, increased the blood level in 27-HC. This suggests that letrozol increased a factor of BC risk since 27HC is a tumor promoter which may be involved in BC recurrence.

**Conclusion:** This pilot study provides the first evidence that circulating OS could be measured in the blood of pts with BC. The clinical utility of OS as biomarkers of sensibility/resistance to hormone therapy needs further clinical investigations. Based on the present study the CE/27HC ratio should be more specifically investigated. The mechanisms involved in the modulation of OS by AI deserve further studies.
Title: Overexpression of insulin receptor substrate 4 can mediate acquired resistance to lapatinib-containing regimens in HER2+ breast cancer cells

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Body: Background: The HER2 pathway can be inhibited by potent targeting agents such as lapatinib (L), trastuzumab (T), or their combination (LT), but acquired and de novo resistance still occur. Resistance to these drugs remains a major hurdle in the management of HER2+ breast cancer. Consequently, elucidation of mechanisms of acquired therapeutic resistance to HER2-directed therapies is of critical importance.

Methods: To obtain clues to the mechanisms for resistance we developed a panel of HER2+ breast cancer cell lines resistant to L, T, or LT. Parental cells and resistant derivatives of the HER2+ BT474 cell line were characterized by RNA-seq. Genes that were overexpressed in resistant compared to parental cells were confirmed by RT-PCR, Western blotting, and immunohistochemistry (IHC). Cell growth and cell signaling were assessed in parental and resistant cell lines after down-regulation (by siRNA) or overexpression (via an inducible cDNA) of IRS4 in the presence or absence of treatment. The effect of IRS4 overexpression on L resistance was assessed in a BT474 xenograft model. The proteins that interact with IRS4 were identified by co-immunoprecipitation with IRS4 followed by separation of the associated proteins by SDS-PAGE and microsequencing by mass spectrometry.

Results: RNA-seq analysis revealed that IRS4 was the most up-regulated gene in BT474 L or LT resistant derivatives in which HER2 signaling is effectively inhibited, but not T alone, where HER2 signaling is reactivated. Western blotting and IHC validated this result and identified membrane localization of IRS4. Knockdown of IRS4 in L- or LT-resistant cells reversed resistance and restored growth inhibition. IRS4 knockdown also inhibited downstream signaling, with a reduction in pAKT but not in pMAPK. Induction of the cell cycle regulator p27 and down-regulation of survivin were observed after IRS4 knockdown. Overexpression of IRS4 cDNA in parental BT474 and SKBR3 cells led to resistance to L/LT, increased pAkt, and decreased the apoptotic marker cleaved PARP in the presence of L or the LT combination. The BT474 xenograft model showed that IRS4 overexpression in the absence of treatment had no effect on tumor growth but it significantly reduced the inhibitory effect of lapatinib (p=0.002). A group of proteins that interact with IRS4 in BT474 L-resistant cells were identified by mass spectrometry. The roles of these proteins in IRS4-mediated resistance to lapatinib-containing regimens are under investigation.

Conclusion: IRS4 overexpression is a critical factor in causing resistance to lapatinib-containing regimens in BT474 cells. Investigation of IRS4 and its signaling partners in HER2+ human tumors resistant to lapatinib will be important to determine if this mechanism is also operative in patients.
Modeling chemoendocrine therapy for ER+/p53wt luminal breast cancer

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Body: Background
ER+ breast cancers are predominantly p53 wildtype (wt). Despite this genotype, these cancers are relatively resistant to chemotherapy-induced apoptosis in the presence of estrogen. Thus, current clinical practice is to administer hormonal therapy and chemotherapy sequentially rather than concurrently. Using the ER+/p53wt cell line MCF-7, we previously demonstrated that ER bound by either the ER agonist estradiol or partial antagonist tamoxifen inhibits the expression of a set of proapoptotic p53 target genes, whereas the full ER antagonist fulvestrant, a selective ER downregulator (SERD), removes the ER-mediated suppression of these genes, sensitizing breast cancer cells to p53-mediated cell death. We hypothesize that effective chemoendocrine therapy requires complete ER antagonism resulting in ER downregulation, increased p53 activity, and ultimately apoptosis.

Methods:
To model mechanisms of tumor sensitivity and resistance to therapy in vivo, therapeutic experiments were performed in immunodeficient mice bearing ER+/p53wt tumor xenografts derived from a 61-year-old African-American female with grade 2 invasive ductal carcinoma. The mice were randomly assigned to the following treatment groups: A) fulvestrant B) tamoxifen, C) doxorubicin, D) fulvestrant plus doxorubicin, E) tamoxifen plus doxorubicin, and F) control. Tumors were measured weekly for 42 days to determine treatment effects on growth. In addition, RNA was harvested from tumors at an early time point for RNA sequencing to determine genes regulated by the treatments.

Results:
Combination therapy comprising doxorubicin plus fulvestrant resulted in tumor regression compared with single-agent or doxorubicin plus tamoxifen treatment, which inhibited the growth of tumors but did not lead to their regression.

Conclusions:
Current paradigms for the treatment of ER+ breast cancer either in the adjuvant or advanced setting have involved the sequential use of endocrine therapy and chemotherapy. This protocol is partially based on evidence of potential antagonism between tamoxifen and chemotherapy as observed in early studies. Our published work in vitro and the preclinical study presented here suggest that complete ER antagonism with SERDs such as fulvestrant is required to overcome the ability of ER to block p53-mediated apoptosis. Our results suggest treatments involving fulvestrant concurrent with chemotherapies involving p53 activation should be considered for the treatment of patients with ER+/p53wt breast cancer.
**Title:** Divergent activation of AKT1 and AKT2 isoforms downstream of PI3K mutation impacts response of breast cancer cells to estradiol and PI3K inhibitors

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**Body:** Background: PI3K mutations are observed in 30% of breast cancers, which are more common in estrogen receptor (ERα) positive breast cancers compared to ERα-negative breast cancers. AKT, a the major kinase downstream of PI3K, modulates ERα activity. It is unknown whether PI3K mutation leads to preferential activation of specific AKT isoform with ability to modulate ERα function.

Results: We report elevated AKT1 but not AKT2 mRNA in breast cancers with PI3K mutation. Breast epithelial cells with targeted substitution of PI3K with PI3K-E545K or PI3K-H1047R demonstrated elevated AKT1_pS473 compared to AKT2_pS474, distinct AKT substrate phosphorylation, and enhanced Estrogen Receptor (ERα) activity. AKT1 had a dominant role in ERα:estradiol (E2)-dependent gene expression and proliferation. We have identified an unique gene expression signature in ERα-positive breast cancers that is dependent on ERα, estradiol (E2), AKT1, and the pioneer factor FOXA1. Elevated expression of this signature in ERα-positive tumor was associated with better response to endocrine therapy and outcome. In addition, AKT1 determined the sensitivity to PI3K-specific inhibitor BYL719 and pan-PI3K inhibitor BKM120. In contrast, AKT2 controlled global gene expression and elevated levels of an AKT2-directed protein signature predicted poor outcome in breast cancer patients.

Conclusions: PI3K mutation favors AKT1 activation leading to imbalance in AKT isoform-specific kinome/proteome and an effect on specific signaling pathways, particularly ERα:E2 signaling network. Knowledge gained from this functional dissection of AKT isoform activity downstream of PI3K mutation can be exploited for therapeutic stratification, particularly for anti-estrogen and PI3K inhibitor-based therapies.
Title: The effect of HER-2/neu inhibition on prolonging clinical benefit with fulvestrant treatment for metastatic estrogen receptor positive breast cancer patients treated with trastuzumab

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Body: Background: Fulvestrant is a well-established treatment for postmenopausal patients with estrogen receptor (ER) positive metastatic breast cancer, and some patients experience prolonged clinical benefit exceeding one year. HER2 activation is a major cause of endocrine resistance, and cross-talk between HER2/neu and ER coactivator MED1 regulates tamoxifen resistance in breast cancer cells. (Cancer Res 2012 1;72(21):5625-34.). In a xenograft mouse model, suppression of MED1 enhanced tumor growth inhibition by fulvestrant in HER2/neu overexpressing breast cancer cells (PLoS One 20123 30;8(7)).

Objective of study: To determine if blocking the HER2/neu receptor with trastuzumab can improve response to fulvestrant.

Methods: This was an IRB approved record review of patients from three medical oncologists with biopsy-proven ER+ metastatic breast cancer treated with fulvestrant. Demographic data collected included age at diagnosis, type and stage of cancer, original and metastatic ER, progesterone receptor (PR), and HER2/neu biomarkers, and site(s) of metastasis, and primary local and systemic treatment. All patients with HER2/neu positive primary tumors received trastuzumab. The duration of fulvestrant therapy was calculated. Time to clinical disease progression on fulvestrant was measured as a surrogate for duration of clinical benefit. Based on the median duration of therapy of 425 days, patients were divided into two groups: Short Treatment (< 425 days) versus Prolonged Treatment (>425 days). Results: One hundred metastatic ER+ fulvestrant treated breast cancer patients with documented duration of therapy were identified. There was no difference between the Short and Prolonged Treatment Groups in regards to age, sites of metastases, or use of adjuvant endocrine or chemotherapy. Eighty five patients had recorded HER2/neu tumor status. All 11 of 85 (13%) patients with documented HER2/neu positive primary tumors received trastuzumab. Patients with HER2/neu positive tumors tended to have longer durations of fulvestrant therapy (772 (51-1911) days (median (range)) compared to HER2/neu negative patients (360 (60-2739) days, p=0.059). Only 2 of 45 (4%) tumors from the Short Treatment Group were HER2/neu positive, while 9 of 40 Prolonged Treatment Group patients with documented HER2/neu status were positive (Fisher’s exact test p<0.021). Patients with HER2/neu positive tumors were more likely to experience prolonged responses to therapy with an odds ratio of 6.2 (1.26 to 30.92 95% confidence interval, p=0.0249). Conclusion: Overexpression of HER2/neu in tumors from ER+ metastatic breast cancer patients treated with trastuzumab was associated with a prolonged response to fulvestrant therapy.
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Title: Haddow’s paradox revisited: Anti-estrogen withdrawal induces ER-driven apoptosis in anti-estrogen-resistant cells

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Body: Anti-estrogen therapies that antagonize ER transcriptional activity have improved survival in many patients. However, resistance to anti-estrogen therapies is common, resulting in disease recurrence in ∼20% of patients within 10 years of follow-up. The current clinical strategy for managing ER+/HER2- breast cancer is treatment with anti-estrogens until disease progression, followed by subsequent lines of anti-estrogens and chemotherapies. However, before tamoxifen was approved in the 1970s, estrogens were used for treatment of breast cancer with response rates similar to those elicited by anti-estrogens in the advanced setting. Similarly, withdrawal of anti-estrogen therapy has shown anti-tumor effects in some cases. In 1970, Alexander Haddow summarized decades of observations that longer time intervals after menopause were associated with increased clinical benefit from estrogen therapy. These studies suggest that the return of ER signaling after a prolonged period of ER suppression (via menopause, or anti-estrogen therapy) may inhibit tumor growth. Preclinical studies have provided some insight on the mechanism underlying estrogen-induced apoptosis, but the anti-tumor effects of estrogen remain unclear, and biomarkers to identify patients that would benefit from estrogen therapy have not been identified.

We found that MCF-7 cells with acquired resistance to the selective ER downregulator fulvestrant (MCF-7/FR) retain ER expression and harbor ESR1 (ER) gene amplification. Upon withdrawal of fulvestrant, these cells engage ER as demonstrated by increased luciferase transcriptional reporter activity and re-expression of select proteins encoded by ER-inducible genes. Following 8 days of fulvestrant withdrawal, MCF-7/FR cells show drastically decreased proliferation and increased apoptosis, concomitant with re-engagement of ER activity. Temporal cell cycle analysis revealed a reduction of cells in G0/G1 phase following 11 days of fulvestrant withdrawal, which paralleled decreases in cell cycle proteins E2F1 and phospho-Rb, as well as the anti-senescence protein FoxM1. Transcriptomic analyses confirmed that fulvestrant withdrawal progressively induces gene expression patterns indicative of stress and senescence. Similar effects were observed in long-term estrogen-deprived (LTED) MCF-7 cells treated with 17b-estradiol. In contrast, withdrawal of fulvestrant from T47D/FR cells, which do not retain ER expression, does not induce cell death or re-engage ER activity. Thus, re-engagement of ER in anti-estrogen-resistant cells may be required for anti-tumor effects of anti-estrogen withdrawal. We postulate that in MCF-7/FR cells relieved of fulvestrant, high ER levels drive aberrantly regulated transcription through ER hyperactivation, and altered ER/DNA binding patterns result in estrogen-induced apoptosis. Gene expression signatures suggest that fulvestrant withdrawal from MCF-7/FR cells induces downregulation of the transcription suppressive activity of the Polycomb Repressive Complex 2 (PRC2) that contains EZH2 methyltransferase. Ongoing studies are characterizing the roles of EZH2 and ER to determine therapeutic potential for anti-estrogen-resistant breast cancer.
Title: RNA-seq reveals lncRNAs associated with hormonal influences and endocrine therapy


Body: Background: Long non-coding RNAs (lncRNAs) are pervasively transcribed in the genome yet their role in human disease is not well understood. LncRNAs can have regulatory effects on coding mRNAs through a number of mechanisms, including repressing their sense-strand protein-coding partners. There is also emerging evidence that hormone signaling affects the expression of a wide variety of lncRNAs. However, the role of lncRNAs in the response mechanisms to endocrine therapy remains mostly unknown. We investigated MCF7 and T47D cell-line RNA-seq data to characterize changes in lncRNA expression and evaluate their response to estradiol (E2), progesterone (P4) and to their antagonists, tamoxifen (TAM) and mifepristone (RU486).

Methods: RNA-seq data from MCF7 cell-lines with response to E2 and TAM and T47D cell-lines with response to P4 and RU486 were obtained from publicly available short read archive (SRA: ERP000992). Expression values after hormonal or antagonist treatments were compared to non-treated cells. We independently sequenced total RNA samples of T47D cell-lines with or without E2 treatment and created a robust list E2-responsive lncRNAs for further analysis. The RNA-sequencing data was aligned to a lncRNA database containing 14,572 unique lncRNAs. Significant log-fold changes in relative abundance of lncRNA transcripts were selected using percentile-p-value. ER-responsive binding sites on or near the ER-associated lncRNAs were investigated in a ChIP-seq study in MCF7 cells following estrogen treatment. Finally, we identified the subset of hormonally-responsive lncRNAs that are also evolutionarily conserved.

Results: On average, in each cell-line experiment, 5000 lncRNAs were detectable. Expression of 55 lncRNAs were associated with differing response to E2 and TAM (p<0.01) while 120 lncRNAs were associated with differing response to P4 and RU486 (p<0.01). To probe whether similar lncRNA-related response mechanisms are at play for both types of hormonal influences, we examined the overlap between the two sets of significantly responsive lncRNAs (>3-fold change between response to agonist and antagonist). Less than 10% of the lncRNAs were common among the two sets suggesting that while there might be some cross-talk, E2 and P4 are generally regulating unique sets of lncRNAs. For example, XLOC_013954, a bona-fide E2-responsive lncRNA shows 5-fold difference between response to E2 and TAM, but has a fold change of mere 1.1 between response to P2 and RU486. From the MCF7 ChIP-seq data we found that >50% of the ER-associated lncRNAs had ER binding site either overlapping or neighboring the lncRNA. Less than 30% of the response-associated lncRNAs were evolutionarily conserved suggesting they might be under different selection pressures.

Discussion: We have shown that lncRNA expression levels are associated with hormonal influences and endocrine therapy. We have further established that the lncRNAs impacted by the different hormonal influences are quite exclusive and suggest separate regulatory networks. We are also exploring lncRNA-mRNA expression for coding partners of the response-associated lncRNAs. Understanding the regulatory effects of lncRNA expression opens up new opportunities for stratification and management of breast cancers.
Title: A new cell panel to study oestrogen receptor loss in acquired endocrine resistant breast cancer

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Body: Background: Oestrogen receptor positive (ER+) breast cancer patients can acquire endocrine resistance and 10-20% tumours have lost ER at relapse. While growth factor pathway hyperactivity and ER promoter methylation contribute to de novo ER negativity, ER loss in acquired resistance is largely unexplored. We have recently developed 11 lines from MCF7, T47D, BT474 & MDAMB361 cells to model acquired resistance emerging with prolonged (3 year) endocrine treatment both in ER+/HER2- and ER+/HER2+ disease. Here we establish prevalence of acquired ER loss in the panel, determine any association with aggressiveness, and explore ER loss mechanisms in acquired endocrine resistance.

Methods: Authenticated acquired resistant models derived from endocrine responsive lines cultured for 3 years with 10^-7 M tamoxifen (TamR), 10^-7 M fulvestrant (FasR) or oestrogen deprivation (5% charcoal-stripped foetal calf serum SFCSR) were profiled for ER & PR by PCR, immunocytochemistry and Western blotting (+/- 1-2wk antihormone withdrawal), for 2nd-line endocrine responsiveness and for migration using Boyden chamber assays vs. time-matched controls. Src kinase, EGFR, HER2, MAPK & AKT activity were examined and whether their respective inhibition using saracatinib or gefitinib (1µM), trastuzumab (100nM), U0126 or LY294002 (5µM) for 1wk restored ER. ESR1 promoter methylation was examined by bisulfite modification & MethyLight PCR.

Results: Substantial ER mRNA & protein loss occurred in 7/11 long-term acquired endocrine resistant lines. This was irreversible by antihormone withdrawal and paralleled by complete PR loss and endocrine growth-insensitivity. While seen in all fulvestrant resistant lines, ER loss was less frequent with tamoxifen (in MCF7TamR & MDATamR) and only seen in oestrogen deprived resistant T47DSFCSR cells. Increased migration accompanied acquired ER loss in ER+/HER2- derived MCF7TamR, MCF7FasR & T47DSFCSR cells and was saracatinib-sensitive. Src and further growth factor pathway activity increased in several acquired resistant models, and ER loss associated with increased EGFR/HER2 in the MCF7- & MDA-derived cells and with increased MAPK activity in all lines. Weak ER recovery was seen in antioestrogen resistant models treated with saracatinib (MDATamR, MCF7FasR), gefitinib (MDATamR, BT474FasR, MCF7FasR/TamR) or trastuzumab (MCF7TamR). ESR1 DNA methylation was only prominent in MDATamR and MCF7TamR cells. No inhibitor restored ER in the T47D-derived cells, including T47DSFCSR which also lacked ESR1 methylation.

Conclusions: Although ER loss is very prominent in this acquired resistant cell panel, it demonstrates there is capacity of prolonged antihormones, chiefly antioestrogens, to promote receptor loss independent of initial HER2 status. Acquired ER loss clinically would be expected to confer endocrine insensitivity and poorer prognosis given the panel findings. Where ER loss emerged with antioestrogens there was some mechanistic-overlap with de novo ER negativity, including ER promoter methylation for acquired tamoxifen resistance. Our future studies will use the panel to address if targeting these mechanisms can be optimized for ER recovery or if further mechanisms also drive ER loss in acquired resistance, notably for prolonged oestrogen deprivation.
Title: ESRP1 adds sp(l)ice to endocrine resistance

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Body: Introduction:
De novo or acquired resistance to endocrine therapy limits its utility in a significant number of estrogen receptor (ER) breast cancers. An increasing number of molecular assays predict the likelihood of distant recurrence in tamoxifen-treated patients with node-negative, ER+ breast cancer. However, these do not provide the mechanistic basis for endocrine resistance. It is crucial to identify novel targets and improve the success of endocrine therapies. We previously shown that epithelial splicing regulatory proteins 1 and 2 (ESRP1 and ESRP2), RNA binding proteins that promote splicing, are significantly elevated in cases with high Oncotype DX scores (innate resistance) and in ERα-positive cells with acquired tamoxifen resistance (MCF-7 LCC2 cells) and fulvestrant and tamoxifen resistance (MCF-7/LCC9 cells). The aim of this study was to investigate the ESRP1/ESRP2-regulated alternative splicing events leading to innate and acquired endocrine resistance.

Methods:
A combinatorial bioinformatics approach was employed to identify genes altered through alternative splicing by ESRP1/ESRP2 in endocrine resistance. Briefly, genes with ESRP1/ESRP2 motifs were screened in the human genome. This data was integrated with tamoxifen-treated datasets, and filtered using protein-protein interactions (PPI; BIOGRID) and clinical outcome (KM plotter. Potential target gene transcripts were further narrowed down using an algorithm based on motif binding location and the Cancer Genome Atlas (TCGA) breast cancer datasets with low and high ESRP1 cases. The alternative transcripts were further validated using splice variant specific-custom qRT-PCR in low and high RS Oncotype cases as surrogate for endocrine sensitivity and resistance.

Results:
Motif analysis in combination with tamoxifen-treated datasets identified 2212 differentially expressed genes. Further collective analysis of number of PPI (>50) and survival data from KM plotter (P<0.001) narrowed down 79 candidate genes that are associated with tamoxifen resistance. TCGA data confirmed presence of distinct transcripts (splicing variants) based on ESRP1 expression levels. Splice specific RT-PCR confirmed alternative splicing events involving cycle related genes such as AURKA, FZR1, and MDM2 in low ESRP1 (and low RS) and high ESRP1 (high RS) cases.

Conclusion:
ESRP1/ESRP2 by inducing alternative splicing play an important role in tamoxifen resistance and recurrence of ER+ breast cancer. Targeting alternative splicing may offer novel avenues for combating endocrine-resistance in breast cancer.
Title: Investigation into the oncogenic potential of the androgen receptor in aromatase inhibitor resistant breast cancer

Laura Creevey¹, Azlena Ali¹, Arnold DK Hill¹, Leonie Young¹ and Marie McIlroy¹. ¹Royal College of Surgeons in Ireland, Dublin, Ireland.

Body: The heterogenous nature of breast cancer, exemplified by a wide range of subtype classifications, has been well documented. The identification of the novel molecular apocrine breast tumours (ER-, PR-ve, HER2-ve, AR+), with a gene expression profile reflective of ER+ luminal breast cancer, has led to the proposition of an alternative role for the androgen receptor as a pseudo ER. This proposition was borne out of global expression profiling and AR recruitment of molecular apocrine cells, demonstrating that more than 50% of AR binding to the genome mirrors the pattern of ER binding suggesting that in the absence of ER, AR can mimic ER in its DNA binding capabilities.

Aromatase Inhibitor (AI) therapy is the gold standard first line therapy for postmenopausal breast cancer. AIs function by abrogating the activity of the enzyme Cyp 19 (aromatase), which is responsible for converting circulating androgens to estrogen. By preventing this conversion step, this eradicates the main steroid driving breast cancer growth and in turn results in a more androgenic environment. We hypothesise that AR may also act as a pseudo ER in our AI resistant (MCF7-letrozole resistant) cells. Preliminary data demonstrates that AR is recruited to some but not all classical ER target genes as well as some AR target genes in MCF7-LetR cells, therefore highlighting the potential of AR to act as an oncogene in this setting. Analysis of AR protein expression also demonstrates higher levels of AR in AI resistant cells versus sensitive cells. In the evaluation of the responsiveness of a range of breast cancer cell lines to the AR antagonist Bicalutamide, it was noted that our AI resistant cells demonstrated responsiveness to Bicalutamide treatment.

RNA sequencing experiments following AR knockdown in AI resistant cells also provide crucial information as to the key genes differentially regulated by AR. Given that resistance to AI therapy is an emerging clinical issue, further elucidation of the potential mechanisms of AI resistance and potential AR targets is highly warranted.
Title: PI3K pathway as a novel target in androgen-driven aromatase inhibitor resistant breast cancer

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Body: Breast cancer has the highest incidence and second highest mortality rate among all cancers in women worldwide (1). The gold standard treatment for post-menopausal breast cancer is aromatase inhibitor (AI) therapy. Unfortunately, resistance is inevitable in about 30-60% of these patients (2). Research from our lab has previously identified a transcription factor, HOXC11, to be associated with breast cancer resistance and metastases (3). Prosaposin (PSAP) was then identified as a putative HOXC11 target gene in the AI resistant setting. PSAP is a secreted protein that also plays a role in metastatic prostate cancer in both a ligand dependent and independent fashion (4,5).

We have previously shown PSAP to be an androgen responsive gene that can upregulate androgen receptor (AR) expression in a ligand dependent manner in AI resistance. An AR antagonist was effective at slowing down cell proliferation in AI resistant cells \textit{in vitro}. However, since PSAP can also potentially act in a ligand independent fashion, this current study is focused on evaluating the role of PSAP in PI3K pathway activation in the development of AI resistance. Treatment of cells with recombinant PSAP protein (rhPSAP) resulted in increasing expression of p-AKT in a dose-dependent manner. To target this pathway of resistance, we used a pan-class PI3K/mTOR inhibitor BEZ235 on our AI resistant cells. Functional studies with BEZ235 treatment showed significant reduction in both cell motility as well as cell proliferation. Treatment of AI resistant cells with BEZ235 resulted in decreased expression of p-AKT, with little or no effect on AR expression. These findings suggest that to prevent resistance to AI therapy, combination treatment regimens including AR antagonists plus a PI3K inhibitor may ensure sustained response to therapy.

References:
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Title: Novel, non-uterotrophic, selective estrogen mimics cause regression of tamoxifen-resistant breast cancer in 2D and 3D cultures and in mouse xenograft models

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Body: Prior to the advent of tamoxifen therapy, estradiol (E2) and the ER agonist, diethylstilbestrol, showed efficacy in the clinical in therapeutic treatment of breast cancer. The adverse effects of these therapeutic options led to discontinuation, despite tamoxifen itself sharing the adverse effect of uterine growth. The perceived therapeutic action of tamoxifen in countering the proliferative actions of E2 at the estrogen receptor (ER) in endocrine-sensitive breast tissues is not entirely compatible with the therapeutic actions of E2 itself in breast cancer, and points to a more complex role for ER. Tamoxifen is the standard of care for many patients with ER+ breast cancer; however significantly, 30-50% of women are resistant to tamoxifen therapy. Development of a Selective Estrogen Mimic (SEM) that is effective in breast cancer without uterotrophic and other side effects is a novel treatment option that we hypothesize will be effective in causing regression of tamoxifen-resistant breast cancer. We theorize that a rational approach would be an ER ligand with pharmacological partial agonist actions, thus able to mimic E2 in regressing breast cancer, but with antagonist actions in the face of excessive ER activation. Using the benzothiophene scaffold common to the clinical Selective Estrogen Receptor Modulators (SERMs), raloxifene and arzoxifene, we hypothesized that an ideal SEM could be designed by structural engineering. A library of over 30 compounds was developed and assayed in tamoxifen-resistant cell lines, including MCF-7 and T47D. One SEM was assayed in two mouse xenograft models and shown to cause tumor regression; impressively this SEM did not fuel growth of estrogen-dependent T47D xenografts and did not cause uterine growth. This SEM and several other compounds were observed to act as partial agonists at ERα, with variable actions at ERβ. To understand this phenomenon further, the contributions of apoptotic mechanisms and cell cycle arrest to SEM activity was studied in cell cultures. The results collected to date on SEMs that act as partial agonists at ERα indicate that this is a viable, new approach to breast cancer therapy.
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Title: Adaptation to AI therapy in breast cancer can induce dynamic alterations in ER activity resulting in estrogen independent metastatic tumours

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Body: Acquired resistance to aromatase inhibitor therapy is a major clinical problem in the treatment of breast cancer. The detailed mechanisms of how tumour cells develop this resistance remain unclear. Here, discovery of pro-survival adaptations to the estrogen receptor (ER) signalling pathway in response to AI treatment is reported. Global ChIPseq analysis shows that the ER retains binding activity in AI resistant cells under steroid-depleted conditions. In AI treated patients, evidence of steroid independent adaptive ER signalling was demonstrated by the ER target gene Early Growth Response 3 (EGR3). Expression of EGR3 decreased initially upon AI treatment but subsequently recovered following as little as 12 weeks of AI treatment. In vitro data indicates that this increased expression of EGR3 may enhance cell growth and motility of tumour cells. Finally, evidence from established metastatic tumours suggests that the ER signalling network may undergo further adaptations with disease progression as EGR3 expression is routinely lost in the established metastatic tumour. Overall, these data provide evidence of a dynamic ER response to AI treatment which may provide vital clues for overcoming the clinical issue of AI resistance.
Title: Nuclear β-catenin negativity predicts for late relapse in ER+, tamoxifen-treated breast cancer

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Body: Background: The annual recurrence rates of post-menopausal ER-positive breast cancers persist beyond the first 5 years of diagnosis and treatment and the mortality rates in this period are higher versus ER-negative cancers. Extended endocrine therapy past 5 years has been shown to be of benefit but is associated with increased toxicity and cost thus the ability to predict patients who are at highest risk of late relapse would be of significant benefit in clinical decision making in this context. β-catenin is an intracellular protein that undergoes Wnt-mediated nuclear translocation where it transactivates genes implicated in tumour development and progression. We have also previously reported that β-catenin can play a role in aggressive resistance that accompanies prolonged endocrine treatment in vitro. In this study, we thus investigated whether β-catenin expression in ER+ breast cancer was predictive of recurrence beyond 5 years in an analysis of three separate endocrine-treated cohorts.

Methods: Associations between β-catenin gene expression and relapse free survival (RFS) were performed using the online KMplotter tool. Immunostaining for total β-catenin was performed on tissue samples from 3 ER+ primary breast cancer series with long-term follow-up data: Nottingham 2000 (n=384, tamoxifen-only); ABC (n=570; tamoxifen only); transATAC (n=743; tamoxifen or anastrozole). The association between subcellular (nuclear or cytoplasmic) β-catenin expression and RFS was determined in (i) the entire cohort, (ii) the first 5 years of tamoxifen treatment versus post-5 years.

Results: KMplotter analysis of β-catenin in ER+, tamoxifen-treated patient samples (n=665) revealed a significant relationship with improved RFS in the post-five year cohort [HR: 0.48 (0.34-0.68); p=0.000019] versus the first 5 years [HR: 0.91 (0.61-1.36; p=0.64)]. No significant association was observed in untreated ER+ patients. In the Nottingham series, nuclear β-catenin positivity was significantly associated with improved survival in the 20-year follow-up for tamoxifen-treated patients (p=0.047) but not in the first five years (p=0.239). Cytoplasmic β-catenin did not associate with survival. Further analysis in the ABC series revealed an association between nuclear β-catenin positivity and improved survival for tamoxifen-treated patients in the entire cohort (HR: 0.52 (0.18, 0.55); p=0.00005). However, nuclear β-catenin was more strongly associated with improved outcome in the post-5 year treatment group (HR: 0.30 (0.14, 0.66); p=0.01) versus the first five years of treatment (HR: 0.33 (0.15,0.73); p=0.06). No significant associations were seen with cytoplasmic β-catenin. The transATAC trial material again revealed an association between presence of nuclear β-catenin and reduced distant recurrence in the post 5-years endocrine-treated cohort (HR: 0.54 (0.33, 0.91): χ²=5.52, p=0.018) versus years 1-5 (HR: 1.16 (0.67, 2.01); χ²=0.29, p=0.59).

Conclusions: This is the first study to demonstrate that nuclear β-catenin may represent a predictive biomarker for late relapse following tamoxifen treatment in ER+ breast cancer where, contrary to traditional hypotheses and pre-clinical data, its nuclear expression is strongly associated with good outcome post-five years of treatment.
Title: Exploratory analysis of single gene predictive biomarkers in TransHERA DASL cohort reveals that C8A mRNA expression is prognostic of outcome and predictive of benefit of trastuzumab

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Body: HERA is an international multi-center randomized trial comparing 1 or 2 years trastuzumab, given every 3 weeks, with observation in women with HER2+ breast cancer after standard neoadjuvant or adjuvant chemotherapy. From December 2001 to June 2005, 5102 patients were randomized. Comparing 1 year trastuzumab with observation at 8 years of follow-up, statistically significant differences in disease-free survival (DFS) and overall survival, despite crossover to trastuzumab of observation arm patients, were found. No benefit in DFS of 2 years compared to 1 year trastuzumab was found[Goldhirsch 2013].

To determine possible predictive single-gene biomarkers, an exploratory ITT analysis, with DFS as the primary endpoint, was conducted using mRNA expression data from 610 TransHERA FFPE samples profiled on Illumina Whole-Genome DASL cubic spline normalization was applied to the data. Outcome was obtained from the HERA database with 8 years median follow-up and clinical cut-off date April 12, 2012. Characteristics were well balanced between treatment groups. The exploratory analysis of 20,464 genes using DFS as the endpoint identified C8A as a possible biomarker that is prognostic and predictive of response to treatment. Cox regression was used to model DFS, with the interaction term between treatment and C8A as a continuous and a categorical variable split on the cohort mean. The observation arm consists of 199 samples with 66 events and the trastuzumab arm(1&2-year combined) of 411 samples with 108 events.

A statistically significant interaction between C8A mRNA and treatment was detected (p<0.001), indicating that C8A mRNA is predictive of response to trastuzumab treatment. For the C8A low subgroup (mRNA expression lower than the cohort mean) no significant treatment benefit is observed (p=0.73). On the other hand for the C8A high subgroup, patients in the trastuzumab arm experience a lower hazard of a DFS event by almost 75% compared to patients from the observation arm (HR=0.25; 95%CI:0.15-0.43, p<0.001). A significant prognostic effect of C8A mRNA is also observed (p<0.001) in the observation arm, where for the C8A high group the hazard of a DFS event is three times the respective hazard of the C8A low group (HR=3.27; 95%CI:2.01-5.32, p<0.001).

C8A is a member of the membrane attack complex and is part of the innate immune system. C8A inserts into the membrane of the target cell and binds with multiple copies of the pore-forming C9 leading to cell lysis. From the GeneAtlas, C8A is highly expressed in liver tissue suggesting an advantage for tumors with high expression of C8A and innate immune response. The Cancer Cell Line Encyclopedia indicates a wide range of C8A mRNA expression.

C8A as a single gene biomarker that is prognostic of DFS and predictive of benefit from trastuzumab has the potential to improve the standard of care in HER2+ breast cancer. Understanding the advantage of over expression of C8A related to the innate immune response can give insight into the mechanisms that drive cancer. We note with caution that this finding is the result of an exploratory analysis and is being pursued in additional trastuzumab cohorts for further validation.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-06-03
Average Grade: 3.50

Title: Quantitative p95HER2 and HER2 correlations with outcome in the FinHer trial

Jeff Sperinde¹, Weidong Huang¹, Aki Vehtari², Ahmed Chenna¹, Pirikko-Liisa Kellokumpu-Lehtinen³, John Winslow¹, Petri Bono⁴, Yolanda Lie¹, Jodi Weidler¹ and Heikki Joensuu⁵. ¹Monogram Biosciences, South San Francisco, CA; ²Aalto University, Aalto, Finland; ³Tampere University Hospital, Tampere, Finland; ⁴Helsinki University Central Hospital, Helsinki, Finland and ⁵Helsinki University Central Hospital & Helsinki University, Helsinki, Finland.

Body: Background: Expression of p95HER2 (p95), a truncated form of the HER2 receptor that lacks the trastuzumab binding site but retains kinase activity, appears to be a prognostic biomarker for poor trastuzumab treatment outcome in HER2-positive metastatic breast cancer. The impact of p95 expression on trastuzumab treatment efficacy in early HER2-positive breast cancer is less clear. In the current study, p95 expression levels were measured in HER2-positive patients from the phase III FinHer adjuvant trastuzumab trial and correlated with treatment outcome.

Methods: In the FinHer phase III trial, 232 HER2-positive early breast cancer patients were randomized to receive 9-weeks of trastuzumab treatment versus no trastuzumab treatment (control). Quantitative p95 protein expression was measured in formalin-fixed paraffin-embedded samples using the p95 VeraTag® assay (Monogram Biosciences), specific for the M611 form of p95. Quantitative HER2 protein expression was measured using the HERmark® assay (Monogram Biosciences). Time to distant recurrence (TDR) was used as the primary outcome measure.

Results: Sufficient tissue was available to measure p95 in 192 HER2-positive patients randomized to receive chemotherapy vs chemotherapy plus trastuzumab. The chemotherapy only (n=97) and chemotherapy plus trastuzumab (n=95) arms were first analyzed separately. In the chemotherapy only arm, increasing log(p95) correlated with shorter TDR (HR = 2.0; p = 0.02) when stratified by hormone receptor status, nodal status and chemotherapy regimen. In the chemotherapy plus trastuzumab arm, increasing log(p95) was not correlated with a shorter TDR (HR = 0.58; p = 0.19). Log(HER2) was not significantly correlated with TDR in either arm. In a combined analysis of both treatment arms, log(p95) was significantly correlated with trastuzumab treatment outcome in a multivariate model that included hormone receptor status, nodal status, chemotherapy regimen, log(p95) and treatment arm. Subset analyses of hormone receptor positive and negative groups indicated that the interaction of p95 expression with trastuzumab treatment was largely driven by the hormone receptor negative subset.

Conclusions: In the FinHer phase III adjuvant breast cancer trial, HER2-positive patients with elevated breast tumor p95HER2 expression experienced poor outcomes when treated with chemotherapy alone, whereas patients with elevated p95 expression experienced the most benefit when trastuzumab was added to chemotherapy. The different influence between hormone receptor subsets of p95 expression on trastuzumab response resembles the effect of HER2 expression on trastuzumab response in the NSABP B-31 trial (JNCI 105:1782, 2013).
Introduction:
The BOLERO-2 study showed significant doubling of PFS benefit with the addition of EVE to EXE in postmenopausal women with hormone receptor–positive advanced breast cancer progressing after non-steroidal aromatase inhibitor (NSAI) therapy. The BOLERO-2 bone sub study indicated an immediate positive influence of EVE on bone health after 6 and 12 weeks of treatment. To further investigate the longer term effect of mTOR inhibition on bone health, we included this exploratory objective in the phase IIIb, multi-center, open label study 4EVER for postmenopausal women with hormone receptor positive advanced breast cancer treated with EVE +EXE.

Methods:
From May 2012 to November 2012 bone biomarker samples of 247 postmenopausal women with metastatic or locally advanced, hormone receptor positive, HER2 negative breast cancer refractory to NSAI were collected within this phase IIIb study. Here, we report the results of the planned exploratory analysis of biomarkers of bone turnover. The objective of the exploratory biomarker analysis of bone resorption and formation was to assess the effect of EVE on changes of biomarker levels of bone metabolism (CTX, P-I-NP, osteocalcin, vitamin D, testosterone, estradiol, DHEAS, SHBG, PTH, TSH and FSH) from day 1 to weeks 4, 12 and 24 to confirm potential protective effects of EVE on bone via inhibition of bone osteoclast resorption. Descriptive statistics were used to summarize the single bone resorption markers by visit and difference to baseline.

Results:
Trial data base lock will occur in late June 2014, therefore, the final biomarker analysis of bone resorption and formation and their influence on ORR, PFS and safety will be presented at SABCS 2014.

The preliminary analysis on the changes of bone marker levels from baseline to week 24 included 247 patients. The measured changes from baseline to week 24 in biomarker levels revealed increasing levels of testosterone, DHEAS, and FSH, while SHBG and PTH were significantly decreased from baseline to week 24. The combination of EVE to EXE resulted in stabilization of bone health as documented by a decrease of absolute P-I-NP levels at week 24 compared to baseline as well as a stabilization of bone resorption as measured by CTX.

Conclusion:
Our first preliminary results (data cut off 15 Nov 2013) on the changes in bone marker levels supported the hypothesis of EVE having direct impact on bone health, suggesting a decrease of bone turnover and a reversal of the increase in bone resorption associated with aromatase inhibitor therapy. The final analysis of the 4EVER sub study on bone marker levels in correlation with the efficacy data will provide more detailed insights into possible benefits of the combination of EVE plus EXE.
**Title:** Evaluation of an *in vitro* derived signature of olaparib response (PARPi-7) as a predictive biomarker of response to veliparib/carboplatin plus standard neoadjuvant therapy in high-risk breast cancer: Results from the I-SPY 2 TRIAL

Denise M Wolf¹, Christina Yau¹, Ashish Sanil², Anneleen Daemen⁹, Laura Heiser⁸, Joe Gray⁸, Lamorna Brown-Swigart¹, Susan Flynn¹, Gillian Hirst¹, I-SPY 2 TRIAL Investigators⁴, Meredith Buxton¹, Angela DeMichele⁴, Nola Hylton¹, Fraser Symmans⁵, Doug Yee⁹, Melissa Paoloni³, Laura Esserman¹, Don Berry², Hope Rugo¹, Olufunmilayo Olopade⁷ and Laura van ’t Veer¹. ¹University of California, San Francisco, CA; ²Berry Consultants, LLC; ³QuantumLeap Healthcare; ⁴University of Pennsylvania, Philadelphia, PA; ⁵University of Texas MD Anderson Cancer Center, Houston, TX; ⁶University of Minnesota, Minneapolis, MN; ⁷University of Chicago, Chicago, IL; ⁸Oregon Health & Science University, Portland, OR and ⁹Genentech, San Francisco, CA.

**Body:**

**Background:** We developed a 7-gene DNA-repair deficiency signature (PARPi-7) that predicts breast cancer cell line sensitivity to the PARP inhibitor olaparib [PMID: 22875744]. We hypothesized that this signature would also predict response to other PARP inhibitors including veliparib. In the I-SPY 2 TRIAL, HER2- patients were randomized to receive standard chemotherapy or the oral PARP inhibitor veliparib in combination with carboplatin (V/C) and chemotherapy. V/C graduated in the triple-negative (TN) signature. Here we assess the PARPi-7 as a specific biomarker of V/C response.

**Methods:** 115 HER2- patients (V/C: 71 and concurrent controls: 44) were considered in this analysis. The PARPi-7 signature score is computed from Agilent 44K array data as published using expression levels of BRCA1, CHEK2, MAPKAPK2, MRE11A, NBN, TDG, and XPA. We assess association between PARPi-7 and response in the V/C and control arms alone (Wald p < 0.05), and relative performance between arms (biomarker x treatment interaction, likelihood ratio p < 0.05) using a logistic model. In an exploratory analysis, we dichotomized patients by the PARPi-7 score using the published in vitro derived cutpoint (0.037). To assess PARPi-7 in the context of the graduating signature, we added the PARPi-7 High patients to the graduating TN subset and evaluated the treatment effect in this ‘biomarker-positive’ group. Our study is exploratory with no claims for generalizability of the data. Statistical calculations are descriptive (e.g. p-values are measures of distance with no inferential content). Our analyses do not adjust for multiplicities of other biomarkers in the trial but outside this study.

**Results:** The PARPi-7 signature associates with patient response in the V/C arm (OR = 3.9, p=0.00056) but not in the control arm (OR = 0.87, p=0.68). There is a significant biomarker x treatment interaction (OR in V/C arm relative to control arm = 4.48, p=0.0028), which remains significant upon adjusting for HR status (p=0.0018). In an exploratory analysis, PARPi-7 dichotomized using the published in vitro derived cutpoint yields 62 PARPi-7 Low and 53 PARPi-7 High patients. 26% of PARPi-7 High patients are not TN. The distribution of pCR rates among PARPi-7 dichotomized groups are in Table 1.

<table>
<thead>
<tr>
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<th>V/C (n=71)</th>
<th>Control (n=44)</th>
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<tbody>
<tr>
<td></td>
<td>PARPi-7 Low (n=38)</td>
<td>PARPi-7 High (n=33)</td>
</tr>
<tr>
<td>TN (n=59)</td>
<td>5 / 13</td>
<td>17 / 25</td>
</tr>
<tr>
<td>HR+HER2- (n=56)</td>
<td>2 / 25</td>
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When the PARPi-7 High patients are added to the graduating TN subset, the OR associated with V/C is 5.12, which is comparable to that of the TN signature (OR: 4.29), while increasing the prevalence of biomarker-positive patients by ~12%. Evaluation of PARPi-7 in the context of the graduating signature under the I-SPY 2 Bayesian model is pending.

**Conclusion:** Our sample size is small. Our pre-specified analysis suggests the PARPi-7 signature shows promise for predicting response to veliparib/carboplatin combination therapy relative to control. If verified in a larger trial, this cell-line derived signature may contribute to the selection criteria of PARP inhibitor trials in the future.
Title: Prediction of benefit from endocrine therapy in ER+ early stage breast cancer: Correlative studies of the breast cancer index HoxB13/IL17BR (H/I) ratio, ER, PR, and HER2 expression in the randomized Stockholm trial

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Body: Background: Hormone receptor expression is an indication for endocrine therapy, however, receptor status is insufficient to account for the considerable heterogeneity of response, and additional predictive biomarkers are needed. The HoxB13/IL17BR (H/I) gene expression ratio has previously been shown to be predictive of benefit from extended endocrine therapy for women with estrogen receptor-positive (ER+) breast cancer treated in the MA.17 trial. In this correlative study, H/I was compared with ER, PR, and HER2 expression in assessing response to adjuvant tamoxifen (TAM) versus untreated (UNT) in ER+, node-negative patients from the prospective, randomized Stockholm trial.

Methods: Analysis included 600 ER+, LN- patients (317 TAM, 283 untreated) from the Stockholm cohort. Expression profiles were generated by RT-PCR for H/I, ER, PR, and HER2, with additional IHC studies for ER, PR, and HER2. Multivariate Cox models including age, tumor size and tumor grade, were used to assess the significance of the interaction between treatment and each biomarker as continuous variables. 10-year risk of distant recurrence, as a function of each continuous biomarker, was estimated from Cox model in each of the 2 treatment arms.

Results: The interaction between H/I and TAM treatment was significant (p = 0.003). Consistent with the significant interaction, the 10-year rate of distant recurrence as a function of continuous H/I demonstrated that the reduction in recurrence rates with TAM correlated with increasing H/I. Interaction P values for all other biomarkers were nonsignificant [ER (PCR), P=0.473; ER (IHC), P=0.371; PR (PCR), P=0.555; PR (IHC), P=0.475; HER2 (PCR), P=0.947, and HER2 (IHC), P=0.839]. The treatment effect of tamoxifen was unchanged across ER, PR, and HER2 expression levels.

Conclusions: Results of this study provide further support for HoxB13/IL17BR as a biomarker of endocrine response. The H/I gene expression ratio, but not ER, PR, or HER2 expression evaluated as continuous variables, was predictive of benefit from adjuvant TAM treatment. Findings indicate that H/I endocrine response activity is not strictly correlated with ER+ expression and provides independent predictive information regarding estrogen signaling-driven recurrences.
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Average Grade: 5.50

Title: Alterations of intratumoral signalling in breast cancer patients receiving pre-operative trastuzumab alone or combined with everolimus

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Body: Background: PI3K/AKT/mTOR and MAP kinase pathways are major signaling pathways involved in mammary tumorigenesis and are investigated as putative targets for therapy. Multiple cross-talks exists between these two pathways, allowing the regulation of one another by and inversely, depending on the cell conditions. It has been reported that trastuzumab acted differently when used pre-operatively or in neo-adjuvant setting with a lower implication of signaling blockade and a higher induction of ADCC when used alone in chemotherapy naive patients. Additionally, mTOR blockade has been experimentally reported to activate MAPK pathway through a feed-back loop effect. The purpose of this study was to retrospectively investigate the effect on MAPK signaling of adding everolimus to trastuzumab as preoperative therapy of HER-2 positive primary breast cancer amenable to surgery (Unicancer RADHER Phase II trial).

Patients and methods: Formalin-fixed paraffin embedded and frozen tumor samples of primary breast cancer (n=80), were obtained from 82 patients with infiltrating breast carcinoma randomized from July 2008 to April 2012 to receive trastuzumab alone (T arm) (loading dose 4mg/kg, then 2mg/kg/week), or combined with everolimus (T+E arm) (10 mg/day) for a 6 week pre-operative treatment. The median patient age at diagnosis (at the randomization) was 52.7 years. All patients had baseline biopsies taken before initiation of the treatment, at cycle 4 as an option and at surgery. FFPE samples were used for immunohistochemistry (pAKT, pS6K, elfF4E, LKB1), frozen samples were used for multiplex immunoanalysis of phosphorylated PI3K/AKT/mTOR and MAPKinase signaling proteins analysis (p-AKT, p-GSK3, p-P70S6K, p-MEK1, p-ERK1/2, p-P90RSK). Before being submitted to total protein extraction, all biopsies were controlled to ensure a tumor content >50%. 40 pairs associating baseline + surgery tumor specimens or baseline + cycle 4 biopsies were eligible for protein extraction.

Results: No statistically significant relationship was observed between the expression level of any of the phosphoproteins in the initial biopsies and neither the clinical nor the pathological response, overall. After treatment, as compared to the level of expression measured in the initial biopsies, no significant variation of expression of either PI3 kinase or MAP kinase related phosphoprotein was observed in T arm. In T+E arm, significant inhibition of PI3 kinase/mTOR pathway was only observed downstream mTOR protein with decreased expression of p-P70S6 kinase and p-4EBP1 together with a significant activation of MAPK pathway was detected with increased expression of p-MEK1, p-ERK1/2 was observed in T+E arm.

Conclusion: These results confirm that when used alone in chemotherapy naive patients, trastuzumab could not mainly act through the blockade of signaling and therefore when combined with mTOR inhibitors could lead to the suppression of negative feedback regulation of MAP kinase pathway.
Title: Plasma (p) biomarker results from the TANIA trial evaluating continued or reintroduced bevacizumab (BEV) after 1st-line BEV for HER2-negative metastatic breast cancer (mBC)

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Body: BACKGROUND: A potential predictive and prognostic effect of pretreatment pVEGF-A concentrations was suggested in exploratory analyses of phase III trials in HER2-negative mBC (AVADO), gastric cancer (AVAGAST), and pancreatic cancer (AViTA). This led to initiation of the MERiDiAN trial evaluating pVEGF-A prospectively in patients (pts) receiving 1st-line paclitaxel ± BEV in HER2-negative mBC. Potential predictive value was also noted for pVEGFR-2 in AVADO, AViTA, and in early BC in BEATRICE. We report prespecified pVEGF-A and pVEGFR-2 analyses in TANIA.

METHODS: TANIA is an open-label randomized phase III trial evaluating the addition of BEV (15 mg/kg q3w or 10 mg/kg q2w) to the investigator’s choice of chemotherapy (CT) in pts with HER2-negative mBC who experience disease progression (PD) on/after 1st-line BEV-containing therapy. Allocation to BEV or no BEV is continued with 3rd-line therapy after 2nd PD (ie no crossover permitted). The primary endpoint is PFS from randomization to 2nd-line PD/death. Pts consenting to the optional biomarker substudy provided 6 mL plasma samples in EDTA before drug administration at randomization, at wks 7 and 13, every 12 wks thereafter, and at 2nd PD. pVEGF-A and shedded pVEGFR-2 were measured using the IMPACT assay (v2.03). The median concentration for each marker before 2nd-line treatment was prespecified as the cut-off between low (≤ median) and high (> median) biomarker subgroups. 2nd-line PFS was analyzed in each subgroup.

RESULTS: The PFS benefit from BEV seen in the ITT population (N=494; stratified hazard ratio [HR] 0.75, 95% CI 0.61–0.93) was observed consistently within subgroups of the biomarker population (N=312). However, there was no differential BEV effect according to pVEGF-A or shedded pVEGFR-2 concentrations. The stratified HR for PFS was 0.69 (95% CI 0.46–1.04) in pts with low pVEGF-A (≤1010.6 pg/mL [median]) and 0.80 (95% CI 0.54–1.18) in pts with high pVEGF-A (interaction p=0.47). As all pts had received prior BEV, unlike earlier trials showing a potential predictive effect of pVEGF-A, we explored pVEGF-A concentrations according to BEV-free interval. The BEV-free interval was ≤12 wks in 117/150 pts (78%) in the CT arm and 136/162 pts (84%) in the BEV+CT arm. Median pVEGF-A was much lower in the subgroup of 59 pts with a BEV-free interval >12 wks (45.1 pg/mL) than in pts with a BEV-free interval ≤12 wks (1135.3 pg/mL). Analyses of 2nd-line PFS according to shedded pVEGFR-2 concentration showed stratified PFS HRs of 0.68 (95% CI 0.45–1.02) for low pVEGFR-2 (≤9.6 ng/mL [median]) and 0.90 (95% CI 0.60–1.34) for high pVEGFR-2 (interaction p=0.49).

CONCLUSIONS: The potential predictive effect of pVEGF-A in AVADO was not observed in TANIA, although high pVEGF-A concentrations in pts recently treated with BEV complicate interpretation. pVEGF-A is being evaluated prospectively in BEV-naïve pts in the ongoing MERiDiAN trial. Shedded pVEGFR-2 showed no predictive effect in TANIA and the suggested trend was in the opposite direction to the effect seen in AVADO and BEATRICE. Further analyses are planned to evaluate the impact of gene expression, cell-free DNA, protein expression, and DNA mutations on treatment effect in TANIA.
Title: Plasma biomarker analysis in patients with HER2-negative locally recurrent or metastatic breast cancer (LR/MBC) treated with first-line bevacizumab (A) and paclitaxel (T) without or with capecitabine (X)

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Body: Background

The phase II ATX trial aimed at evaluating safety and efficacy of first-line AT or ATX for HER2-negative LR/MBC (NTR1348; BOOG 2006-06). Plasma samples were collected for investigation of circulating proteins involved in angiogenesis and their possible association with therapy outcome. We here report the prognostic value of plasma VEGF-A, soluble (s)VEGFR-2, ANG2, sTIE2, IL6, IL8 and CA9 at baseline (C1D1) and their changes after cycle 1 (C2D1).

Methods

312 patients were randomized 1:1 to AT (T 90 mg/m² d1, 8, 15 & A 10 mg/kg d1, 15 q4w x 6 cycles → A 15 mg/kg d1 q3w for next cycles) or ATX (T 90 mg/m² d1, 8, A 15 mg/kg d1 & X 825 mg/m² bid d1–14 q3w x 8 cycles → A & X at same dose q3w for next cycles). Plasma proteins were measured by immunoassays (R&D Systems and MSD). The association of (continuous) protein levels on C1D1 and their changes on C2D1 with response, PFS and OS were evaluated by Mann-Whitney U test and univariate Cox regression analysis.

Results

The biomarker cohort (n=181) and trial cohort had similar baseline characteristics and clinical outcome. After a median follow-up of 46 months, there were 178 (98%) PFS events and 152 (84%) deaths.

In C1D1, levels of ANG2 and sTIE2 were significantly higher in patients with hormone-receptor positive disease compared to those with triple-negative disease (p=.025 and p=.001, respectively). A high level of VEGF-A was noted in patients with visceral metastases (p=.028) and those with an increasing number of metastatic sites (p=.017). High levels of ANG2, IL6, IL8 and CA9 were significantly associated with poor PFS and OS (Table 1). Protein levels were not associated with response.

Table 1. Association of circulating proteins at baseline with PFS and OS

<table>
<thead>
<tr>
<th>Protein</th>
<th>PFS HR (95%CI)</th>
<th>PFS p-value</th>
<th>OS HR (95%CI)</th>
<th>OS p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG2</td>
<td>2.3 (1.0-4.9)</td>
<td>.04</td>
<td>3.1 (1.4-7.0)</td>
<td>.007</td>
</tr>
<tr>
<td>IL6</td>
<td>1.6 (1.1-2.3)</td>
<td>.02</td>
<td>1.9 (1.3-2.8)</td>
<td>.001</td>
</tr>
<tr>
<td>IL8</td>
<td>1.9 (1.3-2.8)</td>
<td>.001</td>
<td>2.8 (1.8-4.3)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>CA9</td>
<td>1.8 (1.2-2.7)</td>
<td>.006</td>
<td>1.9 (1.3-2.9)</td>
<td>.002</td>
</tr>
</tbody>
</table>

On C2D1, levels of all proteins, except for IL6, had significantly changed. Whereas VEGF-A, ANG2, sTIE2 and IL8 decreased...
significantly, sVEGFR2 and CA9 showed a significant increase. The median relative change of both IL8 and sVEGFR2 was significantly different between patients having CR/PR vs. SD/PD (for IL8: -19.4% vs. 22.3%, \( p = .001 \) and for sVEGFR2: 6.5 vs. 2.1%, \( p = .01 \)). A large relative increase in CA9 level was associated with better PFS (HR = 0.36, 95% CI, 0.19 – 0.68, \( p = .002 \)) and OS (HR = 0.50, 95% CI, 0.27 – 0.94, \( p = .03 \)). All patients had very low levels of free VEGF-A on C2D1 (median 8 pg/ml).

**Conclusions**

In patients with HER2-negative LR/MBC receiving first-line bevacizumab-containing chemotherapy, high baseline plasma levels of ANG2, IL6, IL8 and CA9 indicate a high risk for poor PFS and OS. These proteins might be useful for stratification according to prognosis. Moreover, relative decrease in IL8 and increase in sVEGFR2 on C2D1 are associated with response, whereas a large relative increase in CA9 is associated with better PFS and OS. Changes in these proteins after one cycle might be early indicators of efficacy of bevacizumab combined with chemotherapy.

Financial support from Roche Netherlands.
Title: Hand-foot-syndrome (HFS) is a strong predictor for OS and PFS in HER2-negative metastatic breast cancer (mBC) treated with first-line capecitabine (CAP) + bevacizumab (BEV): Results of a subanalysis of the randomized phase III CECOG TURANDOT trial

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Body: Background:
The randomized phase III TURANDOT trial performed by the Central European Cooperative Oncology Group (CECOG) compared first-line BEV + paclitaxel (PAC) vs BEV + CAP in HER2-negative mBC in a prospective randomized trial [Lang, Lancet Oncol 2013]. In this trial, HFS was the most common adverse drug reaction in the BEV + CAP arm. In the present subanalysis, we investigated whether the occurrence of HFS constituted a predictor for improved efficacy of BEV + CAP in the study population randomized to this treatment arm.

Methods:
In the TURANDOT trial, patients with HER2-negative mBC who had received no prior chemotherapy for mBC were randomized to either BEV-PAC (BEV 10 mg/kg d1 & 15 + PAC 90 mg/m2 d1, 8, & 15 q4w) or BEV-CAP (BEV 15 mg/kg d1 + CAP 1000 mg/m2 bid d1-14 q3w). Only patients who were randomized to the BEV-CAP arm and received at least one dose of CAP were included in the present analysis. Cox proportional hazard models with time-dependent covariate "HFS of any grade" and "HFS grade" were used to analyze the association between HFS and OS or PFS. In addition, landmark (LM) analyses were performed analyzing the impact of HFS occurrence within the first 3 treatment months on PFS and OS.

Results:
Baseline characteristics of patients with HFS (n=154) versus no HFS (n=123) were well balanced and showed no significant differences. Table 1 shows that with the occurrence of HFS, the hazard for progression or death was reduced by > 40%, while the risk reduction (RR) was >55% for OS. Moreover, with increasing HFS-grade an increasing RR was observed for OS. In patients with and without HFS in the first 3 months, the median PFS after the 3 months LM were 10 and 6.2 months (logrank test p=0.0026), respectively, and the corresponding OS rates at 2 years after the 3 months LM were 63% and 44% (log rank test, p=0.0842), respectively. Updated OS results will be presented at the conference.

Cox Proportional Hazards Models for PFS and OS with HFS as Time-dependent Covariate

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>Univariate</th>
<th>Multivariate*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time dependent variable</td>
<td>Hazard ratio (95%CI)</td>
</tr>
<tr>
<td>PFS</td>
<td>HFS(Yes vs No)</td>
<td>0.577(0.431,0.772)</td>
</tr>
<tr>
<td></td>
<td>HFS Grade 1</td>
<td>0.639(0.440,0.927)</td>
</tr>
<tr>
<td></td>
<td>HFS Grade 2</td>
<td>0.481(0.321,0.720)</td>
</tr>
<tr>
<td></td>
<td>HFS Grade 3</td>
<td>0.641(0.411,0.999)</td>
</tr>
<tr>
<td>OS</td>
<td>HFS(Yes vs No)</td>
<td>0.417 (0.273, 0.637)</td>
</tr>
<tr>
<td></td>
<td>HFS Grade 1</td>
<td>0.568 (0.334, 0.968)</td>
</tr>
<tr>
<td></td>
<td>HFS Grade 2</td>
<td>0.417 (0.227, 0.765)</td>
</tr>
<tr>
<td></td>
<td>HFS Grade 3</td>
<td>0.220 (0.093, 0.520)</td>
</tr>
</tbody>
</table>
* Significant baseline factors in multivariate models: ECOG (0 vs 1-2) in all models, ER/PR status (Any positive vs Other) in both OS models.

Conclusion:
HFS is a strong predictor for prolonged PFS and OS in mBC patients receiving BEV + CAP first-line treatment. Early occurrence of HFS and HFS severity might be used for treatment modifications and patient motivation. Detection of biomarkers for HFS could strengthen this approach.
Homologous recombination deficiency (HRD) score predicts response to cisplatin neoadjuvant chemotherapy in patients with triple negative breast cancer

Andrea L Richardson¹, Daniel P Silver¹, Zoltan Szallasi²,³, Nicolai J Birkbak², Zhigang C Wang¹, J Dirk Iglehart¹, Erica L Mayer¹, Eric P Winer¹, Nadine M Tung⁴, Paula D Ryan⁸, Steven J Isakoff⁵, William T Barry¹, April Greene-Collozi¹, Alexander Gutin⁶, Julia Reid⁶, Chris Neff, Joshua Jones⁷, Kirsten Timms⁶, Anne-Renee Hartman⁷ and Judy E Garber¹. ¹Dana-Farber Cancer Institute, Boston, MA; ²Technical University of Denmark, Lyngby, Denmark; ³Children's Hospital Boston, Boston, MA; ⁴Beth Israel Deaconess Medical Center, Boston, MA; ⁵Massachusetts General Hospital, Boston, MA; ⁶Myriad Genetic Laboratories, Inc, Salt Lake City, UT; ⁷Myriad Genetic Laboratories, Inc, Salt Lake City, UT and ⁸Fox Chase Cancer Center, Philadelphia, PA.

**Body: Background** A significant proportion of triple negative breast cancers (TNBC) carry homologous recombination (HR) defects and are sensitive to therapeutics that target this pathway. Several clinical trials have demonstrated improvement in pathologic response with the addition of platinum to standard of care regimens but at a cost of increased toxicities. Recently 3 DNA based metrics (LOH, Abkevich et al.; TAI, Birkbak et al; LST, Popova et al.) have been shown to be highly associated with BRCA1 or BRCA2 (BRCA1/2) status or predictive of sensitivity to platinum chemotherapy. The HRD score was defined as the sum of LOH, TAI, and LST metrics, and a threshold separating tumors with high (≥42) and low HRD scores was predefined on independent datasets based on tumor BRCA1/2 mutation or methylation status. This study assesses the association of HR deficiency, defined as HRD score ≥42 or BRCA1/2 mutant, with response to cisplatin neoadjuvant chemotherapy in patients with TNBC.

**Methods** Archival tumor samples were obtained from 70 patients with TNBC from 2 clinical trials conducted at DFHCC under IRB approved protocols. One trial enrolled 28 patients who received neoadjuvant cisplatin therapy (Silver et al.). The second trial enrolled 51 patients who received cisplatin and bevacizumab therapy (Ryan, et al.). HRD score and tumor BRCA1/2 mutations were determined. Response was categorized by the residual cancer burden (RCB) class (Symmans et al.) with pathologic response (PR) defined as RCB0 or I and pathologic complete response (pCR) as RCB 0. Logistic regression was used to assess HR deficiency as a predictor of response to neoadjuvant therapy. All analysis was conducted according to a pre-specified statistical analysis plan.

**Results** 62 tumors provided adequate tissue and passed sequencing quality metrics. 31 (50%) tumors were HR deficient, 22 (35%) HR non-deficient, and 9 (15%) failed assays. The association of HR deficiency status with PR (RCB0/I) and pCR (RCB0) was tested by univariate logistic regression in 50 samples with complete HR status and clinical data. HR status predicted both PR and pCR at the 5% level (Table 1). In a multivariate model of PR, HR status retained statistical significance when combined with clinical variables (p=0.0017, OR=12.08 (1.96, 74.4)). In 51 subjects with known BRCA1/2 mutation status, BRCA1/2 mutation was not associated with either PR (p=0.17) or pCR (p=0.14). When restricted to BRCA1/2 non-mutated tumors (n=38), HR deficiency remained significantly associated with both PR (p=0.0039, OR=9.44 (1.69, 52.7)) and pCR (p=0.018, OR=14.79 (1.48, 2001)).

**Conclusions** This study demonstrates that the HRD score can be used as a tool to identify patients with breast tumors with underlying HR deficiency, including in BRCA1/2 non-mutated tumors, that will benefit from platinum therapy.
Title: Predicting residual risk of recurrence after neoadjuvant chemotherapy- a retrospective analysis of EndoPredict® in the GeparTrio trial

Body: Background: Patients (pts) with breast cancer who do not achieve a pathological complete response (pCR) after neoadjuvant chemotherapy (NACT) have a poor prognosis and might be candidates for post-neoadjuvant clinical trials investigating novel agents. The CPS-EG score (Mittendorf et al. JCO 2011) – a combination of clinical/pathological parameters - is currently used as criterion for selecting patients with highest risk of recurrence after NACT. Here, we examined whether the gene expression test EndoPredict (EP) performed on surgical specimen after NACT provides independent prognostic information for predicting the likelihood of recurrence in breast cancer patients with ER-positive, HER2-negative (ER+/HER2-) disease.

Methods: The molecular EP score was determined by qRT-PCR in 76 available surgical specimen classified as ER+/HER2- from breast cancer pts with residual disease after NACT participating in the neoadjuvant GeparTrio trial. The pre-specified clinical/molecular hybrid score EPclin was determined using ypT and ypN after NACT as clinical variables. Patients were classified as having low or high risk according to pre-defined cut-off values. CPS-EG score was calculated based on stage before and after NACT and pretreatment grade and ER status. Primary endpoint was disease-free survival (DFS). Associations were assessed with uni- and multivariate Cox proportional hazard models. The unbiased c-index was estimated for common clinical/pathological parameters and EP/EPclin scores.

Results: Among the 76 ER+/HER2- breast cancer pts evaluated, EP classified 50% of all pts (n=38) as low risk. EP high-risk patients had a significantly increased risk for relapse compared to the low-risk group (continuous EP HR 1.14 [95% CI 1.05-1.27] p-value 0.02; log-rank p=0.015). Multivariable Cox regression and unbiased c-index estimates (using EP and CPS-EG score as continuous variable) showed that the EP-score (HR 1.15 [95% CI 1.03-1.29 p=0.014] and CPS-EG (HR 1.51 [95% CI 1.07-2.13] p=0.019) provide independent prognostic information. Using the composite EPclin score 13 pts (17%) were classified as being EPclin low. The risk of relapse was significantly higher for EPclin high compared to low (continuous EPclin HR 1.78 [95% CI 1.29-2.46] p<0.001; log-rank p=0.047). Bivariate Cox regression analysis including the CPS-EG and EPclin score (both as continuous variables) showed that only EPclin (HR 1.63 [95% CI 1.14-2.31] p=0.0068) but not the CPS-EG (HR 1.25 [95%CI 0.85-2.31] p=0.26) was significantly associated with worse DFS. Results were similar for overall survival.

Conclusions: Our study shows that EPclin performed on surgical specimen of ER+/HER2- pts after NACT is an independent predictor of recurrence in pts not achieving a pCR after NACT. EP/EPclin low risk patients are probably sufficiently treated with (neo-)adjuvant chemo-endocrine treatment alone, whereas EPclin high risk patients have an increased risk of recurrence, despite receiving standard (neo-)adjuvant chemo-endocrine therapy. The identification of molecular luminal high-risk patients could help to identify high risk patients as candidates for novel drug-based approaches in addition to endocrine therapy to overcome resistance in post-neoadjuvant trials.
Title: Prospective cohort study using the breast cancer spheroid model as a predictor for response to neoadjuvant therapy – The SpheroNEO study

Kathrin ML Halfter\textsuperscript{1}, Nina Ditsch\textsuperscript{1}, Hans-Christian Kolberg\textsuperscript{2}, Holger Fischer\textsuperscript{3}, Tanja Hauzenberger\textsuperscript{4}, Franz Edler von Koch\textsuperscript{5}, Ingo Bauerfeind\textsuperscript{6}, Gunter von Minckwitz\textsuperscript{7}, Ilona Funke\textsuperscript{8}, Alexander Crispin\textsuperscript{9} and Barbara Mayer\textsuperscript{1}. \textsuperscript{1}University Hospital of the LMU Munich, Munich, Germany; \textsuperscript{2}University Hospital of the LMU Munich, Munich, Germany; \textsuperscript{3}Marien Hospital Bottrop, Bottrop, Germany; \textsuperscript{4}Klinikum St Marien Amberg, Amberg, Germany; \textsuperscript{5}Klinikum Dritter Orden, Munich, Germany; \textsuperscript{6}Klinikum Landshut, Landshut, Germany; \textsuperscript{7}GBG Forschungs GmbH, Neu-Isenburg and University Women's Hospital Frankfurt, Frankfurt, Germany; \textsuperscript{8}SpheroTec GmbH, Martinsried, Germany and \textsuperscript{9}Institute for Medical Informatics, Biometry and Epidemiology LMU, Munich, Germany.

Body: (1) PURPOSE
The aim of the prospective trial was to determine if cell survival in a breast cancer spheroid model following cytostatic treatment \emph{in vitro} predicts treatment response in breast cancer patients receiving equivalent neoadjuvant therapy.

(2) PATIENTS AND METHODS
Three-dimensional spheroids were directly generated from fresh tumor biopsy samples of 78 patients eligible for neoadjuvant therapy. Cell survival \emph{in vitro}, as well baseline clinical and pathological characteristics were correlated with the outcome following treatment of each patient to determine the factor(s) most highly associated with pathological complete response (pCR i.e. ypT0/ypN0) at surgery.

(3) RESULTS
Cell survival after treatment in the breast cancer spheroid model proved to be a sensitive and specific predictor for pCR in individual breast cancer patients. A mean cell survival of 21.8\% was found in the breast cancer spheroid model for 22 patients with pCR versus 63.8\% in 56 patients without pCR (P = .001). A receiver operator characteristic analysis determined an area under the ROC curve of 0.86 (95\% CI: 0.77 to 0.96) for cell survival compared to classic factors i.e. negative hormone receptor and positive Her2/neu status, and age \leq 50 years at primary diagnosis (AUC = 0.80, 95\% CI: 0.70 to 0.90). A cutoff of 35\% cell survival was proposed, which grouped patients according to likelihood for pCR. Out of the 32 patients with values below this threshold, 21 patients (65.6\%) and one patient (2.2\%) with a cell survival greater than 35\% achieved pCR respectively; (sensitivity 95.5\% (95\% CI: 0.86 to 1.00); specificity 80.4\% (95\% CI: 0.70 to 0.91)).The specificity was improved to 81\% (95\% CI: 58 to 95\%) if the patient was treated per-protocol.
A positive correlation was also found between cell survival \emph{in vitro} and residual tumor size (P = .021), indicating the possibility of the model to predict degree of response.

(4) CONCLUSION
The breast cancer spheroid model is a valuable predictor for treatment outcome in patients undergoing neoadjuvant chemotherapy for primary breast cancer. Preclinical selection of the most efficient drugs is a prerequisite to improve pCR.
Title: Serum activin A and response to the aromatase inhibitor (AI) letrozole versus tamoxifen in metastatic breast cancer

Meghan Jensen¹, Ashley Kang¹, Suhail M Ali¹,², Kim Leitzel¹, Ashwani Garg¹, Jaqueline Rogerio³, David Chen³, Raymond Hall³, Scott Hofsess³, Hilary A Chaudri-Ross⁴, Nicholas Bade⁵, Walter P Carney⁵ and Allan Lipton¹. ¹Penn State Hershey Medical Center, Hershey, PA; ²VA Medical Center, Lebanon, PA; ³Novartis Pharmaceuticals Corporation, East Hanover, NJ; ⁴Novartis Pharma AG, Basel, Switzerland; ⁵R&D Systems Inc, Minneapolis, MN and ⁶Nuclea Biotechnologies/Oncogene Science, Cambridge, MA.

Body: Introduction: The prognostic and predictive utility of pretreatment serum activin A (TGF-B superfamily ligand) was correlated with the response of first-line metastatic breast cancer patients (MBC) in the phase 3 randomized trial of letrozole vs. tamoxifen.

Methods: 555 ER+ first-line MBC patients had pretreatment serum available for activin A ELISA (R&D Systems). Clinical outcomes were analyzed using Cox proportional hazards modeling. Actin A levels were analyzed using continuous and categorical (median cutpoint) pretreatment serum activin A levels. A pretreatment serum activin A analysis was performed within the normal (not elevated, <15 ng/ml) pretreatment serum HER2 patient subgroup since tumor HER2 status was not available in this older clinical trial.

Results: Serum activin A had a median of 971 pg/ml and an interquartile range of 623 and 1751 pg/ml. In the total population with available serum (n=555), patients with higher serum activin A (> median) had significantly reduced objective response rate (ORR) (17.33% vs 34.6%, Odds Ratio (OR)=0.4; p<0.0001) and reduced clinical benefit rate (CBR) (33% vs 53%, OR=0.45; p<0.0001), as well as significantly shorter time to progression (TTP) [median 5.88 vs 10.98 mo, HR=1.69; p<0.0001], and reduced overall survival (OS) (median 22.78 vs 48.59 mo, HR=2.43; p<0.0001) compared to those with lower serum activin A (<median) (table). The results were similar in subgroup analyses within treatment arms (letrozole or tamoxifen), as well as within the subgroup of patients with normal (not elevated, <15 ng/ml) pretreatment serum HER2 levels (table).

| Outcome (Serum activin A by median cutpoint, high vs. low) |
|----------------------------------|-----------|----------|----------|
| Cohort                           | Patients  | TTP HR   | OS HR   |
| Total Population                 | 555       | 1.69     | 2.43     |
| Letrozole Arm                    | 274       | 1.58     | 2.24     |
| Tamoxifen Arm                    | 261       | 1.92     | 2.6      |
| Serum HER2 (not elevated)        | 395       | 1.55     | 2.45     |

In the total population with available serum (n=555), multivariate analysis for TTP revealed that high serum activin A was a significant adverse prognostic factor (HR=1.46, p<0.001). Multivariate analysis for OS also revealed that high serum activin A was an independent adverse prognostic factor (HR 1.78, p<0.0001).

Conclusions: Patients with high pretreatment serum activin A levels had a significantly reduced ORR, CBR, TTP and OS compared to patients with low serum activin A. The results were similar within the letrozole or tamoxifen treatment arms, and within the serum HER2 not-elevated subgroups. High pretreatment serum activin A level is associated with relative resistance to hormone therapy in first-line metastatic breast cancer.
Title: Evaluation of HER family protein signaling network as a predictive biomarker for pCR for breast cancer patients treated with neratinib in the I-SPY 2 trial

Julia D Wulfkuhle1, Christina Yau2, Denise M Wolf2, Rosa I Gallagher1, Ashish Sanil4, Lamorna Brown-Swigart3, Susan Flynn3, Gillian Hirst3, I-SPY 2 TRIAL Investigators5, Meredith Buxton3, Angela DeMichele6, Nola Hylton7, William F Symmans2, Laura van't Veer8, Douglas Yee8, Melissa Paoloni5, Laura Esserman3, Donald Berry4, Minetta C Liu9, John W Park2 and Emanuel F Petricoin III1.
1George Mason University, Manassas, VA; 2Buck Institute; 3University of California, San Francisco, CA; 4Berry Consultants, LLC; 5Quantam Leap Health; 6University of Pennsylvania, Philadelphia, PA; 7MD Anderson Cancer Center; 8University of Minnesota, Minneapolis, MN and 9Mayo Clinic.

Body: Background: We hypothesize that response to the pan-ERBB inhibitor, neratinib (N), may be predicted by pre-treatment HER2-EGFR signaling. In the I-SPY 2 TRIAL, N graduated in the HR-/HER2+ signature. All patients received at least standard chemotherapy. For HER2+ patients, N was administered in place of trastuzumab. We evaluated 18 HER family signaling proteins as biomarkers of N response using reverse phase protein microarray (RPMA) data from pre-treatment LCM purified tumor epithelium.

Methods: 168 patients (N: 106, concurrent controls: 62) had RPMA and pCR data. 18 biomarkers relating to HER family signaling were evaluated: AKT S473, AKT T308, EGFR, EGFR Y1068, EGFR Y1148, EGFR Y1173, EGFR Y992, ERBB2, ERBB2 Y1248, ERBB3 total, ERBB3 Y1289, ERK1/2 T202/Y204, Heregulin, mTOR, mTOR S2448, PI3K p85 Y458/p55 Y199, PTEN S380, and SHC Y317. We assessed association between biomarker and response in the N and control arms alone (likelihood ratio test), and relative performance between arms (biomarker x treatment interaction) using a logistic model. Analysis was also performed adjusting for HR/HER2 status. In an exploratory analysis, we selected the marker with the greatest interaction (phosphorylated EGFR Y1173) to dichotomize patients optimally based on the data and assessed it in the context of the graduating signature by adding the EGFR Y1173-High patients to the HR-/HER2+ subtype and evaluating the treatment effect in this ‘biomarker-positive’ group. Our study is exploratory with no claims for generalizability of the data and does not account for multiplicities. Statistical calculations are descriptive (e.g. p-values are measures of distance with no inferential content).

Results: 7 HER pathway markers (EGFR Y1068, EGFR Y1173, EGFR Y992, ERBB2 total, ERBB2 Y1248, ERBB3 Y1289, SHC Y317) are associated with response in the N but not the control arm. However, the difference in performance between arms did not reach significance by permutation testing. Adjusting for HR/HER2 status, EGFR Y1173 shows a significant biomarker x treatment interaction (p = 0.049). In an exploratory analysis, we dichotomized patients by their EGFR Y1173 levels and evaluated the distribution of pCR rates (Table 1).

<table>
<thead>
<tr>
<th></th>
<th>Neratinib (n=106)</th>
<th>Control (n=62)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGFR Y1173 Low</td>
<td>EGFR Y1173 High</td>
</tr>
<tr>
<td></td>
<td>(n=31)</td>
<td>(n=75)</td>
</tr>
<tr>
<td>HR-/HER2+</td>
<td>0 / 4</td>
<td>12 / 18</td>
</tr>
<tr>
<td>Not HR-/HER2+</td>
<td>3 / 27</td>
<td>24 / 57</td>
</tr>
<tr>
<td></td>
<td>EGFR Y1173 Low</td>
<td>EGFR Y1173 High</td>
</tr>
<tr>
<td></td>
<td>(n=29)</td>
<td>(n=33)</td>
</tr>
<tr>
<td>HR-/HER2+</td>
<td>1 / 1</td>
<td>5 / 5</td>
</tr>
<tr>
<td>Not HR-/HER2+</td>
<td>5 / 28</td>
<td>5 / 28</td>
</tr>
</tbody>
</table>

Table 1

OR between EGFR Y1173 groups in the N relative to control arm is 10.1. When EGFR Y1173 High patients are added to the graduating HR-/HER2+ subset, the OR associated with treatment is 3.2 and is comparable to that in the HR-/HER2+ signature (OR: 2.1), while increasing the prevalence of biomarker-positive patients by ~50%. Evaluation of EGFR Y1173 under the I-SPY 2 Bayesian model is pending.

Conclusion: Our sample size is too small to draw definitive conclusions. Our exploratory analysis reveals that HER family phosphoproteins associate with response to N, but only phosphorylated EGFR Y1173 appears to add value to the graduating signature. Given that this biomarker would expand the patient population that may benefit, it merits evaluation in other ongoing
trials of neratinib.
Title: Thrombocytopenia is associated with pathological complete response to neoadjuvant carboplatin/docetaxel chemotherapy in BRCA wild type triple negative breast cancer

Priyanka Sharma¹, Benjamin C Powers¹, Bruce F Kimler¹, Claire Ward¹, Jennifer R Kliemp¹, Carol S Connor¹, Marilee K McGinness¹, Joshua MV Mammen¹, Jamie L Wagner¹, Qamar J Khan¹, Roy A Jensen¹, Andrew K Godwin¹ and Carol J Fabian¹.
¹University of Kansas Medical Center, Westwood, KS.

Body: Introduction: Growing evidence demonstrates activity of neoadjuvant carboplatin in triple negative breast cancer (TNBC). Underlying germline and somatic Homologous Recombination (HR) repair deficiency may predict response to DNA damaging agents like platinum compounds in TNBC. Certain DNA repair machinery genes (Fanconi Anemia gene) are also involved in the maintenance of hematopoetic stem cell (HSC) function and impaired repair of DNA double strand breaks can lead to HSC and progenitor cell dysfunction. Thus, in presence of HR defects DNA damaging chemotherapy may lead to unique hematological toxicity. It is also possible that in presence of HR defects breast cancer response and hematological toxicity with DNA damaging agents will parallel each other.

Aim: To evaluate the impact of hematological toxicity on response to neoadjuvant Carboplatin/Docetaxel chemotherapy in patients with sporadic and BRCA associated TNBC utilizing clinical and BRCA mutation data from a prospective TNBC registry.

Methods: 288 patients with Stage I (T>1cm) II and III TNBC were enrolled on a multisite prospective registry between 3/2011 to 4/2014, out of which 49 patients received neoadjuvant Carboplatin AUC 6 + Docetaxel 75mg/m² every 21 days (4-6 cycles) and have undergone breast surgery. Carboplatin was dosed using the modified Cockcroft-Gault formula. Hematologic toxicity was graded using the CTCAE version 4.03. All patients received prophylactic pegfilgrastim on day 2. Pathological complete response (pCR) was defined as absence of invasive disease in the breast and axilla. All patients underwent comprehensive BRACAnalysis®(Myriad).

Results: For the 49 eligible patients median age was 50 years, median weight was 172 lbs, 18% were African American, and 37% had LN+ disease. 26% (13/49) of patients carried deleterious BRCA mutation (9 BRCA1, 4 BRCA2). pCR of the cohort was 65%. Overall 61%, 96%, and 12 % patients demonstrated > Grade1 thrombocytopenia (Tp), anemia, or neutropenia, respectively; 4%, 6%, and 6% patients demonstrated grade 3/4 thrombocytopenia, anemia, or neutropenia, respectively. There was no association between pCR and anemia or neutropenia. Carboplatin dose reductions/delays/omission was more common in patients with Tp compared to patients without Tp (33% vs. 5%; p=0.02). Patients with Tp were more likely to achieve a pCR compared to patients without Tp (85% vs. 47%; p=0.013). 77% of BRCA carriers and 64% of BRCA wild type TNBC demonstrated Tp (NS). pCR rates in BRCA wild type patients with and without Tp were 82% and 47%, respectively (p=0.041). pCR rates in BRCA mutation carriers with and without Tp were 89% and 50%, respectively (p=0.20). On multivariable platelet count was independently associated with pCR (p=0.001).Conclusions: Tp was associated with decreased dose delivery of carboplatin but an improved pCR in BRCA wild type TNBC. Comprehensive assessment of HR defects beyond germline BRCA mutation status may be required to elucidate the biological process that explains this observation. Tp may be a harbinger of underlying HR deficiency and further correlative studies exploring this association of Tp with pathological response to carboplatin in TNBC are warranted.
Title: Multiplatform molecular profiling of BC reveals significant differences in actionable targets from matched female breast carcinomas

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Body: Introduction
Male breast cancer (MBC) is rare, comprising ~1% of all breast cancers. While clinically characterized as being similar to postmenopausal ER+ BC, MBC has been much less characterized molecularly than female BC.

Methods
60 male (ages 37-84) and 5000 female (ages 27-98) breast cancer samples were evaluated for common gene mutations (Sanger or Illumina TruSeq), protein expression (immunohistochemistry), microarray, and/or amplification/rearrangement (CISH or FISH). The samples were analyzed for patterns within the MBC cohort and similarities/differences compared to the female (FBC) subtypes (TNBC, non-TNBC, HER2+, and ER+) evaluated at Caris Life Sciences.

Results
Within the MBC cohort, approximately 23% were negative for ER, PR, and HER2 (TNBC); of those 18% were also negative for AR; 72% were ER+; 51% were both ER+ and PR+. The incidence of high ER and PR protein expression was greater (72% vs. 56%, 54% vs. 40%) but incidence of HER2 overexpression (IHC, 3+) and amplification (FISH, HER2/CEP17 ratio higher than 2) was lower (8.8% vs. 11%, 5% vs. 14.9%) when compared to FBC overall. The rate of EGFR amplification (measured as ≥ 4 copies in 40% or more tumor cells by FISH) was not different from FBC (15%), while the percentage of MBC pts with AR protein expression (70%) was most similar to ER, PR positive FBC patients. Other biomarkers: the rate of ERCC1 overexpression was lower in MBC when compared to FBC (36% vs. 49), the rate of PTEN loss was lower (36% vs. 61%), and the rates of MGMT, TLE3, and RRM1 overexpression were higher (73% vs. 64%, 70% vs. 53%, and 47% vs. 30%, respectively). In the 14 MBC cases evaluated by NGS, no PTEN gene mutations were identified, although PIK3CA gene mutations were seen at a similar rate (41%) as in the >50yo ER+ FBC (37%), and TP53 gene mutations (21%) were seen slightly less frequently than in the >50yo ER+ FBC (27%). Comparison of the TN MBC to the ER+ MBC cohort identified differences in the mTOR pathway (PTEN loss of 13% vs. 28% and PIK3CA mutation rate of 13% vs. 50%, respectively), in P53 mutation rates (33% vs. 0%), and in AR protein expression (33% vs. 82% overexpression), TLE3 (14% vs. 83% overexpression), and ERCC1 (100% vs. 77% low).

Conclusions
The gene mutation, amplification, and protein expression profiles in MBC patients, including HER2 protein expression/amplification, AR and TLE3 protein expression and PIK3CA gene mutation, may inform standard and investigational therapeutic options for this rare cancer population.
Title: The structural hotspot mutation at amino acid 282 in the TP53 gene is overrepresented in residual disease of patients with invasive breast cancer following neoadjuvant chemotherapy with DNA damaging agents

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Body: The presence of residual disease after neoadjuvant chemotherapy (NAC) is an independent predictor of relapse and death in breast cancer patients, independent of subtype. Poor pathological response to DNA damaging agents in patients receiving NAC has previously been linked to p53 accumulation in residual disease. Previously considered a class of inactivating mutations, structural/functional studies show extensive biological heterogeneity among the common TP53 missense mutations for drug metabolizing enzymes, in vitro resistance to DNA damaging agents and patient outcomes.


Results: Among the patients, 76.4% had a least one p53 mutation with the majority of residual disease testing positive for multiple p53 somatic mutations post NAC (Table 1). Approximately 30% (35 tumors) of the residual disease was positive for > 5 mutation types. Unexpectedly, we found that among the p53 mutation positive residual disease, the majority (88/96; 93.6%) screened positive for the structural variant at hotspot R282W. In the few tumors without mutation at R282, structural hot spot mutations, as opposed to DNA binding mutations, predominated. Mutations in one of the two DNA binding hotspots at R248 comprise the second most common mutation. In patients with ≥2 mutations, the majority had mutations detectable at R248.

Number and Major Types of TP53 mutations in residual disease

<table>
<thead>
<tr>
<th># mts</th>
<th>% of Tumors</th>
<th>R282W</th>
<th>Major 2nd Mutations</th>
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<tbody>
<tr>
<td>0</td>
<td>24</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>21</td>
<td>20/26 (76.9%)</td>
<td>4 structural (R175H (2), G245S, H179R); 2 DNA binding R248Q/L; R273H/L</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>14/14 (100%)</td>
<td>R248Q/L (28%), R248W (14%)</td>
</tr>
<tr>
<td>3-5</td>
<td>16</td>
<td>19/19 (100%)</td>
<td>R248Q/L (84%), R248W (74%), V157F (53%), R249S (42.1%)</td>
</tr>
<tr>
<td>&gt;5</td>
<td>28</td>
<td>35/35 (100%)</td>
<td>R248W (100%), R248Q/L (100%), R273C/S (94%), V157F (91.4%), R249S (91.4%), G245S (60%), C176F (40%), Y163C (28.6%), Y220C (22.9%)</td>
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</table>

Conclusion: To our knowledge, this is the first report of p53 mutation profiles in post NAC residual breast tumors. We observed a significant overrepresentation of the structural p53 mutation hotspot R282W with evidence for complex additional p53 mutations in residual disease. Our results require replication given the high observed prevalence of p53 mutations in residual disease and the potential to significantly improve the survival of breast cancer patients through active drug development efforts in targeting mutated p53 proteins.
Title: Gene expression profiles accompanying phenotypic changes during non-malignant breast epithelial cells acini formation to explain MRI phenotypes

Marcia V Fournier, Alan Derr, Alex Margulis, Kevin Reid, Sara Brumbaugh, Richard Anderson, W Fraser Symmans, Laura Esserman and Nola Hylton. BIOARRAY Therapeutics Inc, Farmington, CT; Ceres Analytics, Redwood City, CA; Symmetric Computing, Boston, MA; University of Texas MD Anderson Cancer Center, Houston, TX and UCSF Helen Diller Family Comprehensive Cancer Center, San Francisco, CA.

Body: Magnetic resonance imaging (MRI) captures the three dimensional way in which tumors are organized in the breast, defined as imaging phenotypes in the I-SPY 1 trial. We developed a gene set based on the way in which breast epithelial cells aggregate and organize in three dimensional cultures. We investigated whether these organizational genes correspond to the imaging phenotypes.

MRI phenotypes have been shown to correspond to the degree of response to neoadjuvant chemotherapy, and are used to predict the ability to achieve breast conservation treatment (Mukhtar et al, Ann Surg Oncol, 20: 3823–3830, 2013). We hypothesized that the molecular profile accompanying phenotypic changes occurring during the organization process of non-malignant acini may explain the molecular basis of MRI tumor phenotypes.

We have developed prediction models for MRI phenotypes based on expression profiles identified during the organization process of non-malignant breast epithelial cells in three-dimensional laminin-rich extracellular matrix (Fournier et al. Cancer Res, 66, 7095-102, 2006). We analyzed a subset of 324 organizational genes in 147 samples from stage II-III breast cancer in the I-SPY 1 trial cohort with Agilent microarrays and MRI annotation (CALGB 150007/150012; ACRIN 6657). MRI phenotype of the index lesion was assessed by the site radiologists using the following radiographic criteria: A) well defined unicentric mass; B) well-defined multilobulated mass; C) area enhancement with nodularity; D) area enhancement without nodularity; E) Septal spreading. The distribution of phenotypes in I-SPY 1 was: A: 16%, B: 33%, C: 30%, D: 15%, E: 7%. We developed predictors for MRI phenotypes dichotomized as either "well-defined" (A and B) or "non-well-defined" (C, D and E). Using patent-pending algorithms we selected a subset of the organizational genes with the greatest predictive power to identify MRI "well-defined" phenotypes in the I-SPY 1 cohort. Then prediction models were developed using the 50 top ranking genes and logistic regression methods. We followed Monte-Carlo cross validation method to make sure that the performance of a model is not a result of over-fitting the model to the sample data using separate datasets to support the modeling. The samples were randomly partitioned 10,000 times in a 85% training, and 15% test ratio to test models performance. The resulting performance of predictive models in the test set had an average classification accuracy of greater than 80% (ROC statistics AUC>0.8) using gene expression measurements from between 16 to 20 genes. For random lists of 16-20 genes, the accuracy was approximately 50% (ROC AUC≈0.5). Amongst the MRI models were several genes that are known to regulate key cellular pathways such as cell division, metabolism, and migration using MetaCore pathway analysis. Taken together, the results suggest that the MRI phenotypes may be a manifestation of the organization genes that determine behavior in three dimensional culture. Future research includes confirming these results using an independent dataset, defining the potential drivers of MRI phenotypes, and determining if putative drivers provide a key contribution to tumor subtypes.
Title: A seven-gene signature can predict distant recurrence in patients with triple-negative breast cancers (TNBCs) who receive adjuvant chemotherapy following curative surgery of the primary breast cancer

Yeon Hee Park¹, Hae Hyun Jung¹, In-Gu Do¹, Eun Yoon Cho¹, Insuk Sohn¹, Sin-Ho Jung¹, Won Ho Kil¹, Seok Won Kim¹, Jeong Eon Lee¹, Seok Jin Nam¹, Jin Seok Ahn¹ and Young-Hyuck Im¹. 'Samsung Medical Center.

Body: BACKGROUND: Women with triple-negative breast cancers (TNBCs) represent a significant treatment challenge as they have a relatively poor prognosis and no effective targeted therapy exists. Although TNBCs are often discussed as a single disease entity of breast cancers, in fact they are very heterogeneous. The aim of this study was to investigate candidate genes that might function as biomarkers to differentiate TNBCs among patients, who received adjuvant chemotherapy after curative surgery, into those with high or low risk for distant recurrence.

METHODS: We tested whether the results of a NanoString expression assay that targeted 245 prospectively selected genes and used mRNA extracted from paraffin wax-embedded tumor tissues would predict distant recurrence in patients with TNBC. The levels of expression of seven genes were used in a prospectively defined algorithm to allocate each patient to a risk group (low or high).

RESULTS: NanoString expression profiles were obtained for 203 tumor tissue blocks. Increased expressions of the five genes (SMAD2, HRAS, KRT6A, TP63, and ETV6) and decreased expression of the two genes (NFKB1 and MDM4) were associated with favorable prognosis in this patients' cohort and were validated with cross-validation. The proportions of patients categorized as having low or high risk were 75% and 25%, respectively. The Kaplan–Meier estimates of the rates of distant recurrence at 10 years in the low- and high-risk groups according to gene expression signature were 62% (95% CI, 48.6–78.9%) and 85% (95% confidence interval, CI, 79.2–90.7%), respectively. When adjusting for tumor–node–metastasis (TNM) stage, the distant recurrence-free survival (DRFS)s in the low-risk groups were significantly longer than that in the high-risk group (p<.001) in each of TNM stages I plus II, and III. In a multivariate Cox regression model, the gene expression signature provided significant predictive power jointly with the TNM staging system.

CONCLUSION: A seven-gene signature could be used as a prognostic model to predict DRFS in patients with TNBC who received curative surgery followed by adjuvant chemotherapy.
Title: Next generation sequencing to find predictors for chemotherapy response in triple negative breast cancer

Esther H Lips, Magali Michaut, Marlous L Hoogstraat, Lennart Mulder, Nicolle Besselink, Marco J Koudijs, Emile E Voest, Rene Bernards, Petra M Nederlof, Jelle Wesseling, Sjoerd Rodenhuis, Marco J Koudijs, Emile E Voest, Rene Bernards, Petra M Nederlof, Jelle Wesseling, Sjoerd Rodenhuis, and Lodewyk FA Wessels. The Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands; The Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands; The Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands; UMC Utrecht, Utrecht, Netherlands; Center for Personalized Cancer Treatment, Netherlands; UMC Utrecht, Utrecht, Netherlands and The Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands.

Background: Neoadjuvant chemotherapy is the standard of care for locally advanced triple negative breast cancer (TNBC). A complete remission after chemotherapy is associated with a good prognosis. However, patients with a poor response often relapse and die of metastatic disease. Biomarkers are urgently needed to predict which patients will respond to standard chemotherapy. In the current study we deep-sequenced responding and non-responding tumors to find response prediction markers. In addition, the encountered mutations might provide clues for targeted treatment options.

Methods: Next generation sequencing (NGS) was performed for 2,000 genes involved in oncogenesis with an average coverage of 150 reads. DNA from 56 pre-treatment TNBC-biopsies was sequenced, as was non-tumor DNA from each patient for comparison. Biopsies were obtained from patients scheduled to receive neoadjuvant chemotherapy with doxorubicin/cyclophosphamide. The median follow up was 2.5 years. The presence of a BRCA1 germline mutation or BRCA1 promoter methylation was also known. We assessed three variables. First, we compared samples with a pathological complete remission (pCR) with those that did not achieve a pCR, to find mutations predicting chemotherapy response. Second, we performed the same comparison with relapse free survival as outcome measure. Third, we compared tumors with BRCA mutations or BRCA1 promoter methylation with tumors with a functional copy of the BRCA genes. The goal of this analysis was to detect mutations associated with BRCAness.

Results: The mutations observed were diverse and few recurrent mutations were detected. Most mutations were in TP53, TTN, and PIK3CA (57%, 22%, 9%). The mutation rates were similar between responders and non-responders (average mutation rate of 13 versus 16 mutations per tumor, p=0.27). The analysis of individual genes did not reveal significant differences between responders and non-responders. NOTCH4 mutations showed an association with relapse free survival, with three out of nine relapsing patients bearing a mutation and no mutations occurring in the relapse-free patients (p=0.008). Interestingly, PIK3CA mutations were only observed in patients with a functional BRCA gene (5/26 (19%) versus 0/30, p=0.017). Tumors with BRCA impairment may develop via different routes than tumors with functioning BRCA genes, and the PI3K pathway may play a role in the latter. This finding has also been observed in an independent dataset (Severson et al, 2014, submitted).

Conclusions: Although few recurrent mutations were found, two interesting leads for follow up studies were identified. First, three out of nine tumors with a relapse had a NOTCH4 mutation while NOTCH4 mutations were not occurring in the relapse free group. Second, PIK3CA mutations were associated with BRCA proficient tumors. After validation in larger series, triple negative tumors with PI3K or NOTCH4 mutations can be candidates for agents targeting these oncogenic pathways. In this manner, a more individualized treatment of triple negative breast cancer might become possible in the near future.
Identification of ERBB2 gene variants in HER2 positive disease associated with trastuzumab response in an adjuvant trastuzumab chemotherapy trial

Shelly Gunn1, Alexander Zien2, Markus Hartenfeller2, Francesca Diella2, Stephan Brock2, Martin Stein2, Sasha Badbanchi2, Anja Doerks2, David Jackson2, Lina Asmar3, Yunfei Wang3 and Steve Jones1. 1MolecularHealth, Woodlands, TX; 2MolecularHealth, Heidelberg, Germany and 3McKesson Specialty Health, Woodlands, TX.

Body

Background
Among 493 patients with early stage, lower risk (70% node negative), HER2 positive breast cancer, the 2-year DFS was 97% with adjuvant docetaxel and cyclophosphamide plus 1 year of trastuzumab (TCH) in a phase 2 study (Jones et al, Lancet Oncology 14: 1121, 2013). The objective of this work was to investigate the presence of ERBB2 specific DNA biomarkers in HER2 amplified tumors associated with relapse compared to those which did not recur during 3 years of followup.

Methods
The 26 coding exons of the ERBB2 gene were analyzed by next generation sequencing (NGS) on the HiSeq-2500 (Illumina) platform using DNA samples from the primary tumors of 13 of the 493 patients who progressed. Treatment refractory cases were analyzed in parallel with a clinically and pathologically matched group of 11 patients from the same trial with 2-year relapse free survival (RFS).

Results
ERBB2 gene variants were detected in all 11 relapse-free patients and 10/13 patients with relapse. Heterogeneity for sub-clonal ERBB2 variants at <1% tumor variant frequency (TVF) was pronounced. There were 126 total distinct ERBB2 SNVs across the 24 tumors. Only 4 occurrences of SNVs known to us to be activating (G309A, D769H, D769Y, V777L, V842I, R896C, S310Y, S310F, T798M, 611M, 678M) were found: 3 times V842I and a single case with R896C. Of the investigated tumors, 5 exhibited sub-clonal hyper-mutability with >30 ERBB2 SNVs. These sub-clonal ERBB2 SNVs were not detected in a comparison set of non-HER2 positive solid tumors. We did find variants that seem to be associated with later relapse, of which one is statistically significant (I655V; p=1.2%, Fisher exact test, no compensation for multiple testing), and further 5 have p<10%.

Conclusions
We find no evidence for known activating HER2 mutations to confer increased (nor decreased) risk of relapse after TCH therapy (p=30.0%). Remarkably, I655V is found significantly less often in the relapse group. This variant is known to increase BC risk by activating HER2 dimerization; an effect that may be muted by trastuzumab, in contrast to some other causes of BC. Moreover, I655V mutations with high TVF (>75%, hence suggesting homozygous germline presence) are exclusively found in the no-relapse group (p=3.1%). These biomarkers in combination with other molecular studies including immune function gene status may help define the subset of patients at risk for relapse during TCH therapy. The hyper-mutability genotype does not seem to correlate with later relapse, so it may be hypothesized that the low TVF mutations are passengers rather than drivers. Further studies are needed to verify and precisely define the role of these ERBB2 biomarkers in HER2 positive breast cancer.
Title: Immunoglobulin G fragment C receptor polymorphisms and clinical efficacy of preoperative chemotherapy plus trastuzumab and lapatinib in patients with HER2-positive operable breast cancer

Antonino Musolino, Valentina Guarneri, Nadia Naldi, Beatrice Bortesi, Daniela Boggiani, Paolo Sgargi, Daniele G Generali, Federico Piacentini, Maria V Dieci, Massimo Ambroggi, Katia Cagossi, Lorenzo Gianni, Samanta Sarti, Giancarlo Bisagni, Antonio Frassoldati, Pierfranco Conte, and Andrea Ardizzoni.

1University Hospital of Parma, Parma, Italy; 2Istituto Oncologico Veneto-IRCCS, University of Padova, Padova, Italy; 3AO Istituti Ospitalieri di Cremona, Italy; 4Modena University Hospital, Italy; 5Hospital of Piacenza, Italy; Ramazzini Hospital, Italy; 7Ospedale Infermi, Rimini, Italy; 8Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori, Italy; 9Arcispedale Santa Maria Nuova-IRCCS, Italy and 10Ferrara University Hospital, Italy.

Body: Introduction: In vitro studies have shown that lapatinib enhances the immune-mediated cytotoxicity (ADCC) of trastuzumab. FcγR polymorphisms have been associated with both ADCC and clinical activity of trastuzumab in patients with HER2-positive metastatic breast cancer. There are no data on the relationship between these polymorphisms and the combination of trastuzumab plus lapatinib in the early stage setting. We performed a pharmacogenomics analysis of CHER-LOB, a randomized phase II trial of preoperative chemotherapy plus trastuzumab (arm A), lapatinib (arm B), or both (arm C) in HER2-positive operable breast cancer.

Methods: FcγRIIa-H131R and FcγRIIIa-V158F polymorphisms were analyzed on DNA from peripheral blood samples. Pathologic complete response (pCR) of genotyped cases was evaluated by FcγR polymorphism and treatment arm.

Results: Genotyping was successfully performed in 73/121 (60%) patients. No deviation from the Hardy-Weinberg equilibrium was observed. Similarly to the overall results of the CHER-LOB study, in the subset of patients genotyped in this analysis, a significant improvement in pCR rate was observed in favor of the combination of lapatinib plus trastuzumab (arm C) compared to arm A (OR=3.66, P=0.037), and B (OR=3.03, P=0.049). Such improvement was restricted to carriers of FcγRIIIa V allele (C vs. A, OR=5.33, P=0.043; C vs. B, OR=6.50, P=0.012), while it was not observed in patients with FcγRIIIa F/F genotype (C vs. A, OR=2.14, P=0.642; C vs. B, OR=0.71, P=0.737). Disease free survival (DFS) was not different by treatment arm in all genotyped cases, but a trend toward significance for an interaction between FcγRIIIa V allele and better DFS with the combination of lapatinib plus trastuzumab was detected (P=0.058). No significant associations were observed by FcγRIIa polymorphism.

Conclusions: Host-related immune signatures may mediate lapatinib enhanced trastuzumab-dependent ADCC. FcγRIIIa genotypes may help predict different outcomes to lapatinib plus trastuzumab in HER2-Positive Early Breast Cancer.
Title: Brief exposure to trastuzumab prior to preoperative chemotherapy confirms predictors of response to treatment

Aditi Shirish Vadodkar¹, Vinay Varadan¹, Kristy Miskimen¹, VV Bossuyt², MM Abu-khalaf², I Krop³, E Winer³ and LN Harris¹. 'Case Western Reserve University, Cleveland, OH; ²Yale Cancer Center, New Haven, CT and ³Harvard Medical Center.

Body: Background: HER2-positive (HER2+) breast cancer is biologically heterogeneous; no consistent biomarkers of response to trastuzumab (T) have been identified. Recent data suggest intrinsic subtypes applied to HER2 cohorts are able to predict response to T-based therapy. We designed a multicenter trial (DFCI 03-311) to determine if exposure to T-alone prior to combination T-based chemotherapy (C) could produce predictors of pathologic complete response (pCR). We previously reported that change in AKT and IGF signatures by gene expression could predict pCR. New gene expression pathways (immune and HER2 subtypes) were evaluated.

Design: Fresh tumor core biopsies were taken at baseline and 2 weeks after a single dose of T (8mg/m2) from 80 HER2-overexpressing, stage II/III breast cancer patients enrolled on a clinical trial of T>T+C. A total of 122 samples (50 matched pairs and 22 single timepoints) produced useable gene expression data. Nucleic acids were extracted using Qiagen AllPrep and were analyzed with Illumina HT12v3. Data generated was normalized and median subtracted using Illumina's Genome Studio software; intrinsic subtyping was performed using median-subtracted PAM50 genes; ‘Estimation of STromal and Immune cells in Malignant Tumors using Expression data’ (ESTIMATE) algorithm was used to infer the fraction of stromal and immune cells in tumor samples.

Results: ESTIMATE Immune score predicts pCR after one dose of treatment of T compared with patients with stable disease or progression as best response ,(NOR;p=0.01) PAM50 subtyping of baseline samples showed increase in pCR in the HER2-enriched cluster (7/17; 41%) versus the Luminal B (2/14; 14%) and HER2 basal clusters (1/8; 12.5% ; P=0.04). ). Change in subtype between baseline and 2 weeks appeared less common in HER2-Luminal B (4/13; 31%) compared with HER2 enriched (9/16; 56%) or HER2 Basal (5/9; 55%). Of note, HER2-Basal had the highest frequency of NOR (4/13; 31%; P=0.06), despite having greater subtype change than HER2-luminal tumors.

Conclusions: Biomarkers of response to T-based regimens are greatly needed as no consistent predictors beyond HER2 are available. This data confirms (Carey et al, ASCO 2014) that the HER2 enriched subtype predicts pCR and (Harris et al, CCR 2007) that HER2 basal tumors are more likely to be non-responders. In this study, one dose of T is necessary to predict pCR using ESTIMATE immune signature. Validation of these findings a large cohort is needed.
Title: MammaPrint High1/High2 risk class as a biomarker of response to veliparib/carboplatin plus standard neoadjuvant therapy for breast cancer in the I-SPY 2 TRIAL

Body: Background: Further stratification of the 70-gene MammaPrint™ signature into ‘high’ and ‘ultra-high’ risk groups may help predict chemosensitivity. In I-SPY 2, patients were classified as MammaPrint High1 (MP1) or MammaPrint (ultra) High2 (MP2), with MP2 defined as MP_score <-0.154. MP1/MP2 classification was added to HR and Her2 to define the cancer subtypes used in the I-SPY 2 adaptive randomization engine. HER2- patients were randomized to receive standard chemotherapy or the oral PARP inhibitor veliparib in combination with carboplatin (V/C) and chemotherapy. V/C graduated in the triple-negative (TN) signature, where MP2 was not an eligible signature for graduation. Here, we assess the performance of MP1/MP2 class as a specific biomarker of response to V/C.

Methods: 115 HER2- patients (V/C: 71 and concurrent controls: 44) were considered in this analysis. We assess association between MP1/MP2 and response in the V/C and control arms alone using Fisher’s exact test, and relative performance between arms (biomarker x treatment interaction, likelihood ratio p < 0.05) using a logistic model. This analysis is also performed adjusting for HR status as a covariate. To assess MP1/MP2 in the context of the graduating signature, we added the MP2 patients to the graduating TN subset and evaluated the treatment effect in this ‘biomarker-positive’ group. Our study is exploratory with no claims for generalizability of the data. Statistical calculations are descriptive (e.g. p-values are measures of distance with no inferential content). This analysis does not adjust for multiplicities of other biomarkers in the trial but outside this study.

Results: In the V/C arm vs. concurrent controls, there were 66 MP1 (V/C: 32, Control: 34) and 49 MP2 patients (V/C: 39, Control: 10), 78% of which are TN. The distribution of pCR rates among MP1/MP2 dichotomized groups are summarized in Table 1.

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<th>V/C (n=71)</th>
<th>Control (n=44)</th>
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<tr>
<td></td>
<td>MP1 (n=32)</td>
<td>MP2 (n=39)</td>
</tr>
<tr>
<td>TN (n=59)</td>
<td>3 / 8</td>
<td>19 / 30</td>
</tr>
<tr>
<td>HR+HER2- (n=56)</td>
<td>1 / 24</td>
<td>4 / 9</td>
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</table>

The OR between MP1/MP2 risk groups for predicting pCR is 9.71 in the V/C arm (p=6.63E-05), in comparison to an OR of 0.97 in the control arm (p=1). There is a significant biomarker x treatment interaction (p=0.023), which remains upon adjusting for HR status (p= 0.028). Based on the I-SPY 2 Bayesian model, a Phase III trial with 300 MP2 patients has a 95% predictive probability of success. When the MP2 patients are added to the graduating TN subset, the OR associated with V/C is 4.36, which is comparable to that of the TN signature (OR: 4.29), while increasing the prevalence of biomarker-positive patients by ~10%.

Conclusion: In our exploratory analysis, MP2 suggests higher sensitivity to V/C combination therapy relative to controls. This observation has prompted an investigation into the biological mechanisms distinguishing the MP1/MP2 subtype that may account...
for this specificity.
Measurement of domain-specific HER2 (ERBB2) expression classifies benefit from Trastuzumab in breast cancer

Daniel E Carvajal-Hausdorf1, Kurt A Schalper1, Lajos Pusztai2, Amanda Psyrri3, Konstantine T Kalogeras4, Vassiliki Kotoula5, George Fountzilas4 and David L Rimm1. 1Yale University, New Haven, CT; 2Yale University, New Haven, CT; 3Attikon University Hospital, Athens, Greece; 4Hellenic Cooperative Oncology Group (HeCOG), Athens, Greece and 5Aristotle University of Thessaloniki School of Medicine, Thessaloniki, Greece.

Body: Background: The ASCO/CAP guidelines consider chromogen-based immunohistochemistry (IHC) as the primary assay to determine HER2 status in breast cancer. Studies have shown that antibodies targeting different protein domains (intracellular [ICD] or extracellular domain [ECD]) are equivalent using traditional methods. Here we define a new method for standardization of domain specific measurements, then determined their effect on trastuzumab benefit in the adjuvant setting.

Methods: We measured HER2 protein using quantitative immunofluorescence (QIF) in a standardization tissue microarray (TMA) with CLIA-lab defined HER2 status with 2 antibodies targeting the ICD (CB11 and A0485) and 2 against the ECD (SP3 and D8F12). Cut-points were generated using Joinpoint software. HER2 IHC and FISH results were used as reference to determine sensitivity and specificity. Finally, domain-specific HER2 levels were measured in 180 samples from a clinical trial of adjuvant chemotherapy followed by trastuzumab (HeCOG 10/05).

Results: HER2 ICD showed slightly higher sensitivity to predict HER2 gene amplification than the ECD, while the ECD was more specific and had higher positive predictive value. Fifteen percent of trastuzumab-treated patients from HeCOG 10/05 showed discordant results using ICD and ECD antibodies. ECD-high status was significantly associated with longer disease-free survival (DFS) (log-rank P=0.049, HR=0.31, 95% CI: 0.144-0.997), while ICD status was not. In patients with low ECD, there was no difference in DFS between ICD-low and ICD-high. However, when ICD was positive, high ECD was significantly associated with longer DFS (log-rank P=0.027, HR=0.23, 95% CI: 0.037-0.82) compared to low ECD. Since this trial was not randomized for trastuzumab, interaction could not be tested, but neither ICD nor ECD showed prognostic value in the 462 patients that were traditionally classified as HER2-negative and did not receive trastuzumab.

Conclusion: Determination of HER2 status in breast cancer tumor tissue samples using a standardized system and antibodies against the ECD or ICD suggests a biological difference in the tumors. High ECD was associated with benefit from trastuzumab, while elevated ICD alone was not. This observation could be useful in developing a new HER2 assay that can subclassify traditionally HER2-positive patients into groups for subsequent antibody (ECD) vs tyrosine kinase inhibitor (ICD) therapies.
Title: BRCAness is important to identify TNBC subtype resistant to taxanes

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Body: BACKGROUND: Triple negative breast cancer (TNBC) is heterogeneous and consists of tumors associated with basal like, BRCA related and cancer stem cell (CSC) phenotypes. Although anti-cancer agents are substantial for treating TNBC, existing ones do not work in some subpopulation in TNBC at all. However, it has not been reported that subdivision of TNBC is useful for choosing ant-cancer agents.

AIM: To examine whether subdividing TNBC is beneficial for tailored chemotherapy and to identify predictive factors for existing anti-cancer agents in TNBC.

METHODS: Sixty-six TNBC cases from a randomized phase II trial comparing TCx6 (TC6) with FEC followed by docetaxel (FEC-D) as neoadjuvant chemotherapy for hormone receptor-negative breast cancer (Kanagawa Breast Oncology Group 1101 Study). TNBC was subdivided by 1) IHC of CK 5/6 and EGFR into basal- and non-basal subtypes, and 2) MLPA of BRCA1 into BRCA1 and non-BRCA1 subtypes. The pCR rates were examined according to each regimen and subtype. 3) The association of grade 3 pCR was examined with Ki-67, p53, aldehyde dehydrogenase (ALDH) 1 and topoisomerase 2A (topoIIα) by IHC and TOP2A by FISH for each regimen.

RESULTS: 1) In basal subtype, the pCR rate was significantly higher for FEC-D (42.9%) compared with TC6 (13.6%) (p=0.033), but it was equivalent in non-basal subtype (FEC-D vs TC: 25.0% vs 36.4%, p=0.554). 2) In BRCA1 subtype, it was more significant (FEC-D vs TC: 53.8 % vs 13.3%, p=0.022). 3) An association between pCR and low ALDH1 expression was found in both FEC-D and TC6 (OR: 3.75 and 2.73). High topo IIα protein expression was associated with pCR in FEC-D (OR: 3.5).

DISCUSSION: TC6 was less effective than FEC-D in basal subtype and BRCA1 subtype, showing that taxanes cannot exert their anticancer role in tumors with BRCA1 dysfunction. Although basal subtype may contain more BRCA1-defective tumors than non-basal subtype, MLPA of BRCA1 was better to identify subtype resistant to taxanes than CK5/6 and EGFR. ALDH1 predicted treatment efficacy, and could therefore represent a marker of resistance to conventional chemotherapy.
Title: Alterations in HER2 status and outcome following neoadjuvant chemotherapy in HER2-positive breast cancer (BC)

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¹UCSF Medical Center, San Francisco, CA and ²Buck Institute for Research on Aging, Novato, CA.

Body: Background: Emerging data suggest that chemotherapy and anti-HER2 agents can change HER2 status in HER2+ BC and that this may have prognostic implications. The findings suggest a role for post-treatment (tx) retesting of HER2 status in surgical specimens. However, few studies have specifically examined this phenomenon, and direct comparisons of post-tx HER2 testing modalities are lacking. Furthermore, the biologic basis for HER2 alterations remains poorly understood. The aims of this study were to systematically evaluate the effects of neoadjuvant chemotherapy (NAT) including anti-HER2 agents on HER2 protein expression (P-EXP) and gene amplification (G-AMP), and to examine the potential prognostic impact of HER2 alterations on outcomes in HER2+ BC.

Design: We retrospectively identified 129 patients with HER2+ BC who received NAT at our institution. HR (ER and PR) and HER2 P-EXP were evaluated by immunohistochemistry (IHC) and HER2 G-AMP by fluorescence in situ hybridization (FISH) in pre- and post-tx samples. Pathologic tumor responses were categorized as no residual disease (RD; no invasive or in situ cancer), residual ductal carcinoma in situ (DCIS) only, and residual invasive carcinoma. Pathologic complete response (pCR) was defined as DCIS only or no RD. Association between groups was determined by Fisher exact test. For survival analysis, Kaplan Meier curves were constructed and significance in curve separation was assessed by log rank test.

Results: Mean follow-up was 52.6 months (range 6-162). Eighty-four (65%) cases had residual invasive cancer, 21 (16%) residual DCIS only and 24 (19%) no RD. Post-tx HER2 status was available in 70 cases with residual invasive cancer, and 27 (39%) of these showed reduced HER2 P-EXP (staining intensity 0, 1+, 2+) by IHC. Twenty-one (78%) of 27 cases with reduced HER2 P-EXP retained HER2 G-AMP (IHC-/FISH+), and 6 (22%) were negative for both HER2 P-EXP and G-AMP (IHC-/FISH-). The subset of IHC-/FISH- patients showed 100% 5-year recurrence free survival (RFS), whereas IHC-/FISH+ and IHC+ (intensity 3+) cases demonstrated similarly decreased 5-year RFS of 67% and 71%, respectively. There was a trend towards reduced HER2 expression in HR+ versus HR- cases (46% vs 26%, p=0.19). HR-HER2+ tumors were more likely to achieve pCR than HR+HER2+ cases (48% vs 26%; p=0.014, OR=0.375). In the HR- subset, RFS was significantly better in patients with no RD compared to those with residual invasive cancer (p=0.047); this relationship was not observed in the HR+ group (p=0.693).

Conclusion: A significant fraction of pre-tx HER2+ BC demonstrate reduced HER2 P-EXP following NAT. In a subset of these, post-tx decreased HER2 P-EXP is associated with retained HER2 G-AMP, suggestive of HER2 protein downregulation. In another subset, decreased HER2 P-EXP with associated lack of HER2 G-AMP indicates selection for HER2 non-amplified clones in HER2 heterogeneous tumors. Array comparative genomic hybridization studies are in progress to further elucidate these mechanisms. Lastly, our results suggest that post-tx evaluation of HER2 status by FISH in addition to IHC may have prognostic and/or predictive value in HER2+ BC. Further studies in larger prospective study populations are needed to confirm our findings.
Title: MammaPrint High1/High2 risk class as a biomarker of response to neratinib plus standard neoadjuvant therapy for breast cancer in the I-SPY 2 TRIAL

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Body: Background: Further stratification of the 70-gene MammaPrint™ signature into ‘high’ and ‘ultra-high’ risk groups may help predict chemo-sensitivity. In I-SPY 2, patients were classified as MammaPrint High1 (MP1) or MammaPrint (ultra) High2 (MP2), with MP2 defined as MP_score <-0.154. MP1/MP2 classification was added to HR and HER2 to define the cancer subtypes used in the I-SPY 2 adaptive randomization engine. Neratinib (N), one of the experimental agents evaluated in I-SPY 2, graduated in the HR-HER2+ signature. All patients received at least standard chemotherapy (paclitaxel followed by doxorubicin/cyclophosphamide; T->AC). HER2- patients were randomized to receive N+T- >AC vs. T->AC. For HER2+ patients, neratinib was administered in place of trastuzumab (N+T->AC vs. H+T->AC). Here, we assess the performance of MP1/MP2 class as a specific biomarker of neratinib response.

Methods: 115 patients in the neratinib arm and 76 concurrently randomized controls had Agilent 44K microarrays and pCR data available for analysis. We assess association between MP1/MP2 and response in the neratinib and control arms alone using Fisher’s exact test, and relative performance between arms (biomarker x treatment interaction, likelihood ratio p < 0.05) using a logistic model. This analysis is also performed adjusting for HR status as a covariate, and in receptor subsets. Our study is exploratory with no claims for generalizability of the data. Statistical calculations are descriptive (e.g. p-values are measures of distance with no inferential content). Our analyses do not adjust for multiplicities of other biomarkers in the trial but outside this study.

Results: There are 133 MP1 patients (neratinib: 74, Control: 59) and 58 MP2 patients (neratinib: 41, Control: 17), 84% (49) of which are Her2-. The distribution of pCR rates among MP1/MP2 dichotomized groups are summarized in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Neratinib (n=115)</th>
<th>Control (n=76)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MP1 (n=74)</td>
<td>0 / 17</td>
<td>7 / 39</td>
</tr>
<tr>
<td>MP2 (n=41)</td>
<td>15 / 33</td>
<td>5 / 16</td>
</tr>
<tr>
<td>HER2- (n=105)</td>
<td>22 / 57</td>
<td>4 / 8</td>
</tr>
<tr>
<td>HER2+ (n=86)</td>
<td>22 / 57</td>
<td>5 / 20</td>
</tr>
</tbody>
</table>

MP2, one of the 10 eligible signatures, did not meet the graduation threshold; and MP1/MP2 did not show a significant biomarker x treatment interaction (OR in neratinib relative to control arm = 1.25). The MP1/MP2 x treatment interaction remains non-significant after adjustment for HR and HER2 status (p=0.54). In HER2- patients receiving neratinib, 45% (15/33) of MP2 patients achieved a pCR, compared to 0% (0/17) of MP1 patients. In the HER2- controls, there is a 31% pCR rate in MP2 (5/16) vs. 18% in MP1 (7/39) patients (OR=2.14). This difference in performance between treatment arms appears significant (p=0.041). 90% of HER2+ patients are MP1, thus MP1/MP2 status x treatment interaction within the HER2+ subtype cannot be evaluated.
Conclusion: Within the I-SPY 2 population as a whole, MP1/MP2 stratification does not appear to be a specific biomarker of response to neratinib relative to the control arm. The number of HER2- patients is small and precludes any definitive conclusion, but these data motivate further investigation of the biological mechanisms distinguishing MP1 from MP2 to better understand chemotherapy and/or neratinib responsiveness.
Title: Patient-derived xenografts accurately predict patient response in breast cancer patients

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¹Champions Oncology, Inc, Hackensack, NJ; ²Icahn School of Medicine at Mount Sinai, New York, NY; ³Mount Sinai Hospital, New York, NY, United Kingdom and ⁴Imperial College, London, United Kingdom.

Body: PURPOSE/OBJECTIVES: A growing body of evidence demonstrates that patient-derived xenografts (PDXs) represent living tumor models that accurately reflect the biology of the primary patient tumor. More importantly, we have previously shown that PDX models show responses to therapeutic agents that are concordant with patient clinical response and can be used to direct personalized cancer treatments (Stebbing, 2014). Here we report the ability of PDX models to predict for patient response to drug treatment in a cohort of breast cancer patients.

MATERIALS/METHODS: Tumors were resected from patients with either primary or metastatic breast cancer and implanted into immunodeficient mice to establish PDX models. Successfully engrafted PDX models were expanded and randomized for drug sensitivity testing. Tumor growth inhibition and tumor regression were captured and results were correlated with a patient’s clinical response. In some cases, PDX results were used to personalize cancer treatment and some patients used PDX-directed treatments over multiple lines of therapy.

RESULTS: A total of 42 tumors from 40 patients were implanted resulting in 21 successfully engrafted PDX models (50% engraftment rate). Notably, engraftment rates were much higher for patients with triple negative breast cancer (TNBC) and resulted in 7 successful PDX models from 8 TNBC patients (87.5% engraftment rate). Drug sensitivity testing was offered to patients with established PDX models. Drugs and drug combinations tested included standard and nonstandard chemotherapy as well as biologics. At that time of this publication, 4 patients (3 TNBC and 1 HER2+) with completed drug sensitivity tests also had clinical data available resulting in 7 clinical correlations; 4 retrospective and 3 prospective. In all 7 cases, the PDX model accurately predicted patient clinical response demonstrating an accuracy of 100%. Five of the drug tests predicted drug sensitivity and 2 tests predicted resistance, indicating the potential of the PDX platform to predict for both sensitivity and resistance to therapy. The 3 prospective correlations resulted in concordant clinical benefit in 2 patients for duration greater than 6 months each.

CONCLUSIONS: These data support the use of the personalized PDX model as a platform for therapeutic decision making that can guide treatment for patients with breast cancer. A prospective clinical trial in TNBC is currently underway.
Title: Genetic heterogeneity for Her2 accounts for a significant percentage of breast cancers changing Her2 status following implementation of the 2013 CAP/ASCO HER2 reporting guidelines

Monica V Estrada¹, Jena M Giltinan¹, Ferrin C Wheeler¹, Ashwini Yenamandra¹, Vandana Abramson¹, Ingrid A Mayer¹, Julie Means¹, Brent Rexer¹, Ingrid M Meszoely¹ and Melinda E Sanders¹. ¹Vanderbilt University Medical Center, Nashville, TN.

Body: Background: Current evidence indicates that patients with Human Epidermal Growth Factor Receptor 2 (HER2)-positive Breast Cancer (BC) benefit from HER2-targeted therapies. Accurate determination of HER2 status is critical to ensure that the correct patients are offered targeted treatment while patients with HER2-negative tumors—who are unlikely to benefit from anti-HER2 therapy— are spared from the adverse effects of these costly drugs. Guidelines for performance and reporting of HER2 testing, first published in 2007 by American Society of Clinical Oncology (ASCO)/College of American Pathologists (CAP), were updated in October 2013. The new reporting criteria are based on a combination of HER2:CEP17 ratio and average HER2 copy number.

Methods: We retrospectively reviewed HER2 dual probe FISH test results sequentially performed by the Cytogenetics Laboratory at Vanderbilt University from January to May 2014 since implementation of the updated guidelines. The cases were then rescored using the 2007 guidelines. The clinicopathologic features of cases with a change in Her2 status after scoring with the 2013 guidelines were examined for statistical significance.

Results: If the 266 performed HER2 FISH tests had been scored using the 2007 guidelines the results would have been the following: amplified (n = 44, 16%), equivocal (n = 28, 10%) and not amplified (n = 194, 70%). However, using the 2013 guidelines the cases were actually reported as follows: amplified (n=57, 21%), equivocal (n=22, 8.3%) and not amplified (n=187, 68%). Additionally, 18 cases demonstrated genetic heterogeneity in >25% of cells. The updated guidelines resulted in a change in Her2 status in 12% of tests (32/266; p less than 0.0001); 13 changed from equivocal to amplified, 13 cases changed from not amplified to equivocal and 6 cases changed from not amplified to equivocal to not amplified. Cases with a change in Her2 status following implementation of the new guidelines were more likely to demonstrate genetic heterogeneity (28% [9/32] vs. 4% [9/234]; p less than 0.0001). Furthermore, hormone receptor (HR)-negative tumors scored as not amplified by the 2007 guidelines were more likely to undergo an upgrade in HER2 status under the 2013 guidelines than HR-positive tumors (26% [11/41] vs. 7% [11/153], p less than 0.0001).

Conclusions: The 2013 reporting criteria, based on a combination of HER2:CEP17 ratio and average HER2 copy number, increase the number of patients eligible for HER2-targeted therapies while decreasing equivocal results. Tumors that demonstrate genetic heterogeneity for Her2 or are HR-negative account for a significant percentage of these newly eligible cases. Correlation with clinical response is required to confirm the proposed improved analytical validity of the updated guidelines. Clinical trials to evaluate the benefit of anti-Her2 therapy in patients with genetic heterogeneity are in the planning phase.
Title: Comparison of pathologic response evaluation systems after neoadjuvant chemotherapy among different molecular subtypes of breast cancers

In Ah Park¹, Hee Jin Lee¹, In Hye Song¹ and Gyungyub Gong¹. ¹University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea.

Body: Background: Several ways to assess pathologic response of breast cancer after neoadjuvant chemotherapy (NAC) are currently available. However, clinical usefulness of assessment systems in each molecular subtype of breast cancer is unclear. Therefore, we compared four pathologic response assessment systems predicting patients' clinical outcome in specific subtypes of breast cancer.

Methods: In total, 598 tumors from 590 female breast cancer patients who received anthracycline and taxane-based NAC and subsequent surgery from 2010 to 2012 were analyzed. Molecular subtypes were defined by immunohistochemistry (HER2 and hormone receptor (HR): estrogen receptor and progesterone receptor). Miller-Payne grading, Residual Cancer Burden, Residual Disease in Breast and Nodes and ypTNM stage were evaluated. Results of each assessment system were analyzed for survival with Kaplan-Meier and Cox hazard model. Median follow-up period was 35.2 months (range 21.1-54.4 months).

Results: Pathologic complete response was achieved in 4.4% (12/275) of HR+/HER2-, 10.7% (8/75) of HR+/HER2+, 17.8% (16/90) of HR-/HER2+, and 29.7% (47/158) of triple negative (TN) tumors. Results of all four examined assessment systems were significantly correlated with disease-free and overall survival in all tumors. In HR+/HER2- and TN tumors, all systems were associated with disease-free and overall survival.

Comparison of pathologic response assessment systems after neoadjuvant chemotherapy for disease-free and overall survival

<table>
<thead>
<tr>
<th>Disease-free survival</th>
<th>HR+/HER2- (95% CI)</th>
<th>HR+/HER2+ (95% CI)</th>
<th>HR-/HER2+ (95% CI)</th>
<th>TN (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller Payne grade</td>
<td>0.629 (0.429-0.925)</td>
<td>0.310 (0.159-0.607)</td>
<td>0.647 (0.428-0.977)</td>
<td>0.451 (0.343-0.593)</td>
</tr>
<tr>
<td>P value</td>
<td>.018</td>
<td>&lt;0.001</td>
<td>.039</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RCB class</td>
<td>2.621 (1.398-4.914)</td>
<td>2.454 (0.917-6.564)</td>
<td>1.696 (0.929-3.094)</td>
<td>2.966 (1.982-4.441)</td>
</tr>
<tr>
<td>P value</td>
<td>.003</td>
<td>.074</td>
<td>.085</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RDBN level</td>
<td>2.892 (1.642-5.093)</td>
<td>2.587 (1.015-6.595)</td>
<td>2.189 (1.118-4.286)</td>
<td>3.065 (2.108-4.456)</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.001</td>
<td>.047</td>
<td>.022</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ypTNM Stage</td>
<td>2.975 (1.606-5.512)</td>
<td>2.062 (0.918-4.632)</td>
<td>2.004 (1.121-3.582)</td>
<td>2.950 (2.152-4.044)</td>
</tr>
<tr>
<td>P value</td>
<td>.011</td>
<td>.080</td>
<td>.019</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Overall survival</th>
<th>HR (95% CI)</th>
<th>HR+/HER2- (95% CI)</th>
<th>HR+/HER2+ (95% CI)</th>
<th>HR-/HER2+ (95% CI)</th>
<th>TN (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Miller Payne grade</td>
<td>0.416 (0.222-0.778)</td>
<td>0.399 (0.177-0.896)</td>
<td>0.633 (0.357-1.124)</td>
<td>0.397 (0.294-0.537)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.006</td>
<td>.026</td>
<td>.119</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>RCB class</td>
<td>8.611 (1.924-38.531)</td>
<td>2.971 (0.733-12.032)</td>
<td>1.900 (0.771-4.680)</td>
<td>2.976 (1.927-4.594)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.005</td>
<td>.127</td>
<td>.163</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>RDBN level</td>
<td>11.369 (2.618-49.375)</td>
<td>3.042 (0.801-11.550)</td>
<td>1.970 (0.805-4.821)</td>
<td>3.262 (2.145-4.961)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.001</td>
<td>.102</td>
<td>.138</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>ypTNM Stage</td>
<td>5.812 (1.666-20.270)</td>
<td>2.623 (0.851-8.080)</td>
<td>1.619 (0.739-3.546)</td>
<td>3.040 (2.150-4.300)</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>.006</td>
<td>.093</td>
<td>.228</td>
<td>&lt;0.001</td>
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</table>

RCB, residual cancer burden; RDBN; residual disease breast and nodes.

Especially in TN, Kaplan-Meier survival curves for disease-free survival were clearly separated by all assessment methods.
However, in HR+/HER2+ and HR-/HER2+ tumors, the association of patients’ survival with response assessment results was variable according to the examined systems.

**Conclusion:** By the available pathologic assessment systems after neoadjuvant chemotherapy, HR+/HER2- and TN breast cancers could be effectively classified into groups showing different prognosis. However, for the evaluation of pathologic response of HR+/HER2+ and HR-/HER2+ tumors, the development of effective assessment methods is warranted.
Title: Platelet predominant breast cancer is a new predictor for pathological complete response to neoadjuvant chemotherapy

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Body: Background and Objective: Platelets, the smallest anucleate hematopoietic cells, are increasingly recognized as the key regulator of tumor progression and metastasis in breast cancer. They contribute significantly to hematogenous metastasis, which increases tumor invasiveness, by protecting tumor cells from shear stress or immune surveillance and facilitating their extravasation to distant sites. Additionally, recent studies have demonstrated direct signaling between platelets and tumor cells during epithelial–mesenchymal transition (EMT) in the blood stream. However, the existence of platelets in primary breast cancer and its relationship with clinicopathological factors and pathological response to chemotherapy remain poorly understood. This study aimed to investigate (1) the presence of platelet infiltration in tumor tissue, (2) relationships between platelets and clinicopathological features, and (3) the association of platelets with pathological complete response (pCR) to neoadjuvant chemotherapy and EMT markers in primary breast cancer.

Methods: We retrospectively analyzed the pre-chemotherapeutic biopsy specimens from 100 breast cancer patients who underwent surgical resection after anthracycline/taxane-based neoadjuvant chemotherapy at Kanazawa University Hospital. Platelet subsets (glycoprotein Ib [CD42b]) and the expression of EMT markers (E-cadherin, vimentin, and β-catenin) were evaluated by immunohistochemistry and correlated with pCR after neoadjuvant chemotherapy. Platelet-predominant breast cancer (PPBC) was defined as the presence of ≥10% of anucleate cells positively stained for CD42b in direct contact with tumor cells. pCR was defined as the absence of residual invasive tumor cells in breast and lymph nodes (ypT0/is, ypN0).

Results: PPBC was observed in 48 patients (48%). The prevalence of PPBC was significantly higher in human epidermal growth factor receptor 2 (HER2)-negative patients (HER2 [+] vs. HER2 [-]; 65% vs. 90%, p = 0.008). There was no significant association between CD42b expression and stage, nuclear grade, histology, and estrogen receptor status. PPBC patients had a significantly decreased pCR rate (6/48, 12.5%) compared to non-PPBC patients (33/51, 64.7%; p < 0.001). Tumor cells surrounded by platelets exhibited EMT-like morphological changes, EMT marker expression, nuclear-staining of β-catenin, loss of E-cadherin expression, and expression of vimentin.

Conclusions: Our results suggested the presence of tumor-associated platelets in breast cancer as a new predictor of response to neoadjuvant chemotherapy. Platelets might facilitate EMT at the primary site by potentiating tumor cell adhesion. Platelet-tumor interaction might be a new therapeutic strategy for breast cancer. If our finding is validated in further investigations, it might serve as the basis for novel therapeutic approaches of combining conventional chemotherapy with antiplatelet therapy. Our data also identified new predictive parameters to stratify patients with an increased chance of response to anthracycline/taxane-based neoadjuvant chemotherapy.
**Title:** Association of estrogen receptor (ER) levels and prediction of antiproliferative effect of hormone therapy (HT) in lower ER-expressing tumors

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**Body: Intro**
Accurate measurement of ER in early stage invasive breast cancer (EBC) is important to identify patients likely to benefit from HT. While immunohistochemistry (IHC) is the most common method to quantify ER, other methods can also accurately measure ER, such as RT-PCR. ER is one of the genes included in the RT-PCR based 21-gene Recurrence Score assay (OncoType DX®, Genomic Health, Redwood City, CA) and is also reported separately as a single gene expression. Additionally, the association between ER expression by RT-PCR (ER-PCR) and tamoxifen benefit has been reported by Kim, et al (2011). A recent study reported that patients with ER levels <10% by IHC were largely negative by RT-PCR (Singh, et al; 2014) and that this has potential implications for which patients may be expected to benefit from HT. Robust measures of ER and the proliferative response may be useful in identifying patients likely to respond to HT.

**Aim**
The study aims are: (1) To correlate quantification of ER in EBC as assessed by Allred Score (AS) and ER as measured by RT-PCR in the 21-gene assay; (2) To describe changes in ER, Recurrence Score, and measures of proliferation after 2wks of an aromatase inhibitor (AI); (3) To perform exploratory analyses of factors associated with changes in proliferation.

**Methods**
55 postmenopausal EBC patients with lower ER (AS 2-7) were treated with 2wks of an AI followed by wide excision. All patients had a 21-gene assay on a pre-and post-treatment (Tx) sample. Proliferation was measured by both Ki67 by IHC (in 45 patients) and by the proliferation gene group score (PGS) in the RT-PCR based 21-gene assay (in all patients). Proliferation response was defined by a 20% relative decrease in Ki67 or a decrease in PGS. Changes in proliferation were correlated with AS, ER-PCR and Recurrence Score result.

**Results**
The Table shows the correlation of AS with ER-PCR measured in the pre-Tx (r=0.83) samples. 94% of AS (2-3) patients and 56% of AS (4-5) were ER(-) by RT-PCR There was a significant change (pre to post) in the average Ki67 level (18% to 11%; p<0.001) and PGS but not Recurrence Score result. 37/45 patients showed a 20% decrease in Ki67 while only 32/55 had a decrease in PGS. Changes in Ki67 levels were greatest in AS 6/7 patients with a 76% relative decrease vs 21% in AS 2/3 patients. The range of PGS change was 1% increase in AS 2/3, 1% decrease in AS 4/5 and 14% decrease in AS 6/7 patients. Univariate predictors of decrease in Ki67 were AS of 5/6/7 (vs 2/3/4), Recurrence Score result, ER-PCR (continuous or binary), and PR-PCR. The same variables were predictors of PGS change.

<table>
<thead>
<tr>
<th>Pre-Tx AS</th>
<th>ER-PCR Status (Pre-Tx)</th>
<th>Negative</th>
<th>Positive</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>AS 2-3</td>
<td></td>
<td>17 (94%)</td>
<td>1 (6%)</td>
<td>18</td>
</tr>
<tr>
<td>AS 4-5</td>
<td></td>
<td>9 (56%)</td>
<td>7 (44%)</td>
<td>6</td>
</tr>
<tr>
<td>AS 6-7</td>
<td></td>
<td>0 (0%)</td>
<td>21 (100%)</td>
<td>21</td>
</tr>
</tbody>
</table>
Conclusions

• Results confirm earlier reports showing substantial disagreement in ER measured by IHC vs RT-PCR in patients with lower ER-expressing tumors
• The clinical implications are that a substantial number of patients with low ER by IHC may have little to no benefit from HT
• The 21-gene assay may be useful in selecting patients likely to benefit from HT
• Further studies in larger cohorts are required to confirm these findings.
Title: Assessment of phosphorylated HER2 protein as predictive biomarker to stratify anti-HER2 treatment in HER2 non-amplified patients – A translational study in the GeparQuattro and GeparQuinto trials

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Body: Aims: There is evidence that the benefit of anti-HER2 treatment in combination with chemotherapy may not be limited to patients with HER2 amplification. This study tested if phosphorylated HER2 (pHER2) and co-activation of HER2 downstream targets are predictive of response to anti-HER2 treatment in HER2 non-amplified patients.

Methods: Patients initially classified as HER2 positive (IHC, FISH) by local testing from the GeparQuattro and GeparQuinto trials received neoadjuvant anti-HER2 treatment (trastuzumab or lapatinib) in combination with anthracycline-taxane-based chemotherapy. However, on a central pathology review 98 out of 1060 patients were considered HER2 non-amplified. In order to assess the potential benefit from anti-HER2 treatment in these patients the levels of pHER2 and downstream targets including pHER3, EGFR, AKT, PI3K, ERK, PTEN, S6RP and their phosphorylated forms were quantitatively assessed using reverse-phase protein arrays. Histopathological complete response (pCR; ypT0/is) and disease free and overall survival served as reference standard. Optimal cutpoints for each protein were calculated to achieve maximum separation between pCR and non-pCR using ROC analysis.

Results: 25 (26%) patients achieved pCR. The level of pHER2 was not significantly different between groups of histopathologic responders and non-responders. S6RP and pS6RP were the only proteins significantly associated with pCR (p=0.01 and 0.03, respectively) with a higher pretherapeutic expression in patients who subsequently achieved pCR. In contrast, low expression of S6RP and pS6RP were associated with longer disease free (p<0.01) and overall survival (p<0.05). Expression of S6RP and pS6RP did not correlate with that of other HER2 signaling proteins, whereas all remaining proteins were positively correlated with each other.

Conclusions: Expression of S6 ribosomal protein (S6RP), a downstream target of S6 kinase, and its phosphorylated form pS6RP are significantly associated with short and long-term outcome following anti-HER2 treatment in HER2 non-amplified breast cancer patients. In contrast, activation status of the HER2 pathway reflected by pHER2 and other downstream targets was not predictive of response, and showed no significant correlation with S6RP expression. These results suggest that S6RP and pS6RP may be novel predictive biomarkers for response to anti-HER2 treatment in non-amplified patients, possibly through a mechanism independent of global HER2 pathway activation.
Title: I-SPY 2 qualifying biomarker evaluation (QBE): The challenge and opportunity for interrogating predicted pathways in an adaptive design biomarker rich trial

Christina Yau1, Denise Wolf1, Ashish Sanil2, Laura van ’t Veer1, Emanuel F Petricoin3, Meredith Buxton1, Joe Gray4, Angela DeMichele5, Mike Hogarth6, Nola Hylton1, Jane Perlmutter7, Melissa Paoloni8, Fraser Symmans9, Doug Yee10, Don Berry9 and Laura Esserman1. 1University of California, San Francisco, CA; 2Berry Consultants, LLC; 3George Mason University; 4Oregon Health & Science University, Portland, OR; 5University of Pennsylvania, Philadelphia, PA; 6University of California, Davis, CA; 7Gemini Group; 8Quantum Leap Healthcare; 9University of Texas MD Anderson Cancer Center, Houston, TX and 10University of Minnesota, Minneapolis, MN.

Body: I-SPY 2, a multicenter phase 2 neoadjuvant trial in high-risk breast cancer, uses adaptive randomization within biomarker subtypes to evaluate novel agents added to standard chemotherapy. In addition to efficiently evaluating agent/signature pairs, I-SPY 2 is a biomarker rich trial, where samples are profiled for gene expression, protein levels, and mutation status. Biomarkers are classified as established, qualifying, or exploratory. Established biomarkers are those used clinically (HR/HER2 status) or FDA cleared (MammaPrint), and used for adaptive randomization to generate the 10 signatures from which a drug can graduate. Qualifying biomarkers (QB) represent evidence-based, biologic pathway markers (e.g. cell line predictors, known drug targets). QB analyses must be pre-specified and performed under CLIA. Exploratory markers are for discovery and may allow integration of data from different technologies.

The QBE goal is to (1) evaluate biomarkers related to an agent’s mechanism of action to identify promising candidates for testing/patient selection in future trials, and (2) create a resource to elucidate biological mechanisms of response. The wealth of biomarker data is both a boon and a challenge. Our small size limits the generalizability of our findings. There are multiple genes in each pathway measured on multiple platforms, creating the problem of multiplicity, which is compounded by the evaluation of multiple proposals. Biomarkers may correlate with HR/HER2/MP subtypes. The adaptive randomization may increase the prevalence of biomarker positive subsets and bias our findings. These challenges limit definitive conclusions, so our statistics are descriptive rather than inferential, and are intended to avoid adding to the false positive biomarker literature.

Methods: Three filters are applied: 1-The difference in biomarker performance in the experimental vs control arm (biomarker x treatment interaction) is evaluated using a logistic model under a pre-specified analysis plan 2-Biomarkers with a treatment interaction are dichotomized. The QB-High group is added to the graduating subtype to define a novel signature and the treatment effect in this group is evaluated 3-If the treatment effect is comparable to the graduating signature, and the prevalence is increased, the I-SPY 2 Bayesian model is modified to include the QB to assess the novel signature.

QBE to date: Veliparib in combination with carboplatin (V/C) and neratinib (N) are the first two agents to graduate from I-SPY 2. For V/C, we have completed initial evaluation for 5 biomarker proposals, including BRCA1/2 germline mutations and expression signatures associated with DNA repair deficiencies. For N, 6 biomarker proposals, including HER family protein signaling markers, have been assessed. Evaluation of the best candidates from these initial analyses in the I-SPY 2 Bayesian framework is ongoing. Mutational analyses are pending.

Conclusions: We have developed a rigorous approach for QB analysis. A small number of QB warrant further assessment. However, I-SPY 2 QB require validation, and should be considered preliminary efforts to effectively screen QB candidates for evaluation in ongoing and future trials.
Optical metabolic imaging predicts therapeutic response in breast cancer tumors and organoids

Alex J Walsh¹, Rebecca S Cook², Melinda E Sanders², Carlos L Arteaga² and Melissa C Skala¹. ¹Vanderbilt University, Nashville, TN and ²Vanderbilt University Medical Center, Nashville, TN.

The standard of care for breast cancer patients includes treatment with chemotherapy, antiestrogens, and targeted inhibitors. There are more than 100 drugs approved to treat breast cancers with more in the pipeline of discovery and approval; however, few biomarkers have been identified for individualized, patient-specific treatment planning. Currently, drug regimens are chosen based on the presence or absence of specific receptors, including progesterone receptor (PR), estrogen receptor (ER), and human epidermal growth factor receptor 2 (HER2) and patient response is assessed by changes in tumor size weeks after treatment. Unfortunately, many patients do not initially respond to therapy or develop a resistance, and face a greater risk of recurrence and death.

Herein we present a novel, light-based technology that can accurately predict individual tumor response to anti-cancer drugs based on drug-induced changes in metabolism. Cellular metabolism is a powerful indicator response to treatment, because the oncogenic drivers targeted by therapeutic agents often regulate cellular metabolism. In this study, we demonstrate the sensitivity of optical metabolic imaging to predict therapeutic response in xenografts in vivo, and in primary tumor derived organoids. Optical metabolic imaging utilizes multiphoton fluorescence intensity and fluorescence lifetime imaging to probe the concentrations and protein-binding dynamics of cellular NADH and FAD, two coenzymes of metabolism. A composite endpoint, the OMI index, provides a robust, dynamic readout of cellular metabolism.

First, we probed metabolic changes within ER+/HER2+ tumors treated with trastuzumab, an anti-HER2 antibody. After 48h of a single injection of trastuzumab (HER2 antibody), the OMI index of responsive tumors (ER+/HER2+) decreased (p<0.001), but remained unchanged in resistant tumors. To optimize clinical utility of this technology, we developed protocols to generate organoids from fresh biopsy samples. This allows high-throughput screening to directly test individual tumor response to a panel of drugs. We validated this approach in trastuzumab responsive and resistant tumors: the OMI index decreased in responsive ER+/HER2+ organoids treated with trastuzumab, paclitaxel, and XL147 (PI3K inhibitor) (p<0.05). Combination therapies resulted in the lowest OMI index values (p<0.001). Measurements on patient-derived organoids resulted in similar findings: reductions in ER+ tumors treated with tamoxifen and paclitaxel (p<0.05), and the greatest reductions in OMI index observed with combination treatments. The high-resolution images of OMI allow segmentation of the cells within each organoid for cellular-level analysis of heterogeneity. We identified heterogeneity profiles of resistant cell populations within the xenograft and human organoids and were able to track the emergence of resistant sub-populations of cells over the first 72 hours of drug treatment. Heterogeneity analysis is important for clinical utility of this technology to ensure the optimal drug regimen is selected. With these results, optical metabolic imaging shows potential for development into a high-throughput screen to test the efficacy of a panel of drugs to direct clinical therapy selection and expedite pre-clinical studies.
**Title:** Anaplastic lymphoma kinase (ALK) protein overexpression is not a feature of inflammatory breast cancer

Cecile Colpaert¹, Melike Marsan², Peter Vermeulen¹, Luc Dirix², Steven Van Laere² and Inflammatory Breast Cancer International Consortium³.

¹Oncology Centre, GZA Hospitals, Iridium Cancer Net, Antwerp, Belgium; ²Translational Cancer Research Unit, Oncology Centre, GZA Hospitals, Iridium Cancer Net, Antwerp, Belgium and ³Inflammatory Breast Cancer International Consortium.

**Body:**
Recent studies suggest that anaplastic lymphoma kinase (ALK) could serve as a therapeutic target for inflammatory breast cancer (IBC) and that strategies targeting ALK should be considered for evaluation in clinical trials. Reverse phase protein arrays revealed activation of the ALK receptor tyrosine kinase and biochemically-linked downstream signalling molecules in pre-clinical models of IBC. ALK genetic abnormalities have been reported in 80% (20/25) of IBC patients with a high prevalence of ALK alterations in basal-like IBC (Robertson F. et al., 2013). However, ALK protein expression has not been studied in human IBC samples.

Immunohistochemical detection of the ALK protein is increasingly being used as a screening tool to test samples for ALK rearrangements. The biological premise is that the genetic abnormality of the ALK gene leads to overexpression of the ALK protein and therefore overactivity of the ALK tyrosine kinase; since this kinase is the target of crizotinib, it makes sense to assess the drug target directly.

Formalin fixed paraffin embedded tissue samples from pre-treatment surgical biopsies of 79 consecutive IBC patients were immunohistochemically (IHC) tested for ALK protein over-expression using a validated IHC test (NCL-ALK, clone 5A4) with a sensitivity of 93% and a specificity of 100% when the Vysis ALK-FISH break apart test is used as a gold standard. Stained tissue sections were evaluated using the IHC histoscore proposed by Thunnissen E. et al., 2012, assessing both the proportion of tumor cells showing cytoplasmic staining and the staining intensity. Cytoplasmic staining of appendiceal ganglion cells was used as an on-slide external positive control; weak to moderate cytoplasmic staining of macrophages was used as an internal positive control.

In 75 of the 79 tissue samples none of the tumor cells showed any ALK immunoreactivity. One tumor showed moderate cytoplasmic staining in a few cells (<1%, score 2+); ALK (2p23) FISH showed no rearrangement. This tumor was hormone receptor negative and HER2 negative. Three tumors showed minimal cytoplasmic staining in a few cells (<1%, score 1+). These results demonstrate that ALK protein overexpression is not a feature of IBC. Although genetic abnormalities of ALK without protein overexpression are not excluded, our results show that ALK IHC cannot be used to identify IBC patients eligible for enrollment in clinical trials evaluating ALK targeted therapeutics.
Title: Prognostic impact of PgR and Ki-67 expression in ER-positive and HER2-negative breast cancer patients

Yasuyuki Nishiyama¹, Reiki Nishimura¹, Tomofumi Osako¹, Yasuhiro Okumura¹ and Nobuyuki Arima². ¹Kumamoto City Hospital, Kumamoto, Japan and ²Kumamoto City Hospital, Kumamoto, Japan.

Body: Background:
The 13th St Gallen International Breast Cancer Conference (2013) proposed that conventional and new clinico-pathological factors provided a surrogate subtype classification. Moderate or strong expression of PgR has been proposed as an additional restriction in the surrogate definition of Luminal disease. Ki-67 level is also important for this distinction. We divided ER-positive and HER2-negative breast cancer into 4 groups according to the PgR and Ki-67 expressions. The aim of this study was to evaluate the clinical and prognostic significance of these markers among 4 groups.

Patients and Methods:
We analyzed recurrence-free survival (RFS) in 2100 invasive cancer patients who underwent surgery at our institution between September 2001 and December 2013 retrospectively. Patients with T4, neoadjuvant chemotherapy and adjuvant trastuzumab therapy were excluded. The age of the patients ranged from 25 to 75 years. The subtypes were defined as LA (PgR≥20% and Ki-67<20%; n=677), LB-1 (PgR≥20% and Ki-67≥20%; n=524), LB-2 (PgR<20% and Ki-67≥20%; n=198), LB-3 (PgR<20% and Ki-67<20%; n=187), LH (HER2 positive; n=198), HER2 (non-luminal; n=105), and triple negative (TN, ER- and PgR- and HER2-; n=211). RFS was compared among subtypes using Kaplan-Meier method and logrank test. The median follow-up period was 62.9 months.

Results:
In terms of RFS, patients with LA had significantly better prognosis than other subtypes (p<0.001). Patients with LB-2 had a significantly poorer prognosis than LA, LB-1, LB-3 and HER2 (non-luminal), and the PFS of LB-2 was equivalent to LH (HER2 positive) and TN. Among ER-positive and HER2-negative patients (LA, LB-1, LB-2 and LB-3), LB-2 patients had significantly worse prognosis than the other subtypes in node negative cases (p<0.001). On the other hand, in node positive cases, there was no difference in PFS between LB-2 and LB-3 (p=0.298). Moreover, LB-2 patients showed a significantly worse PFS in the endocrine therapy group (p<0.001). In the chemo-endocrine therapy group, LB-2 and LB-3 had a significantly poorer prognosis than LA; however, there was no difference between LB-2 and LB-3 (p=0.371).

Conclusion:
In luminal type breast cancer, patients with LB-2 (PgR<20% and Ki-67≥20%) had the worst prognosis. On the other hand, in the chemo-endocrine therapy group, LB-2 and LB-3 (PgR<20% and Ki-67<20%) had similar and poorer prognosis. These findings suggested that lower expression of PgR (<20%) correlated with lower PFS and lower sensitivity to endocrine therapy, and lower Ki-67 index (<20%) reflected lower sensitivity to chemotherapy. The expression of PgR and Ki-67 might provide a clinical significance in the luminal type.
Title: Upfront identification of chemoresistance in locally advanced breast cancer using 99m Tc tetrofosmin scan: A low cost technology

V Seenu¹, Rakesh Kumar¹ and Siddharth Dattagupta¹. ¹All India Institute of Medical Sciences, New Delhi, Delhi, India.

Body: With the availability of newer chemo therapeutic drugs for breast cancer with excellent efficacy; it has become essential to identify breast cancers that are chemoresistant to doxorubicin based chemotherapy early in course of treatment so that a different regimen can be used to improve response rates & survival. PET scan/ contrast MRI are used routinely used to assess response to neoadjuvant chemotherapy (NACT) in locally advanced breast cancer (LABC). However, both these modalities are expensive & not easily available in most centres in India. 99m Tc Tetrofosmin is taken up by metabolically active breast cancer cells, is inexpensive & easily available at most centres. This study was undertaken to evaluate the ability of Tc99 tetrofosmin scan to identify upfront chemo resistance in LABC.

Aims: Evaluate the role of Tc 99m-Tetrofosmin scan in distinguishing chemo sensitive from chemo resistant LABC patients. Methods: N=65 pts. Inclusion: LABC as per TNM classification. Exclusion: Pts not fit to receive chemotherapy or refusing enrollment. Place of study: Breast Cancer Clinic, Department of Surgical Disciplines. 99m Tc Tetrofosmin Scintimammography performed in all the patients. NACT was CAF regimen Cyclophosphamide (600mg/m2), Doxorubicin (50mg/m2) and 5-Fluorouracil (600mg/m2) 3 cycles @ 3 weekly intervals. Response evaluated after 3rd cycle. Response evaluation: Scintimammo: CR: No detectable abnormal uptake on the post-chemotherapy scan. PR: Some uptake on the post-chemotherapy scan; NR: No change in uptake when compared with pre-chemotherapy scan. Histopathology: CR: Lesion entirely replaced by fibrosis or when only few isolated malignant cells persist. PR: Viable invasive carcinoma persisting in more than 25% of the area of the lesion. Chemosensitivity: A ROC curve was plotted for the T/B ratio & pathological response. Area under the curve was found to be 84% with a C.I. between 73%-95%. Using this curve, a cut off value of 2.55 was chosen to characterize the chemosensitivity of the tumors.

<table>
<thead>
<tr>
<th>Scintimammography</th>
<th>pathological response</th>
<th>CR</th>
<th>PR(Tumor yes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no uptake</td>
<td>11</td>
<td>3</td>
<td>12</td>
</tr>
<tr>
<td>uptake</td>
<td>1</td>
<td>20</td>
<td>30</td>
</tr>
</tbody>
</table>

Sensitivity-92%, Specificity-87%, Positive predictive value-79%, Negative predictive value-95%, LR+7, LR-0.09, LR---73.3, accuracy-89% p value <0.000

shows T/B ratios When the T/B ratio was >2.55, the tumor can be considered to be chemosensitive to Doxorubicin based NACT. On the contrary, when the T/B ratio was <2.55, it is unlikely that the tumor regresses completely following Doxorubicin based NACT. Ability of Scintimammography to assess response to NACT was done comparing post-NACT scintimammograms with the final histopathology.

T/B ratio & path response

<table>
<thead>
<tr>
<th>Scintimammography</th>
<th>CR (tumor no)</th>
<th>PR(Tumor yes)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T/B Ratio2.55&gt;</td>
<td>18</td>
<td>12</td>
<td>&lt;0.003</td>
</tr>
<tr>
<td>T/B ratio 2.55&lt;&lt;&lt;</td>
<td>1</td>
<td>30</td>
<td>&lt;0.003</td>
</tr>
</tbody>
</table>

LR+:3.3, LR-:0.07 sen:95% spe: 72%

Conclusion: Tc99 tetrofosmin can accurately identify chemosensitive breast tumors and predict response to NACT in LABC. Its low, cost, high accuracy and wider availability in developing nations makes it an attractive option in management of LABC.
Title: Detection of PIK3CA mutations in cell-free plasma DNA from women with early breast cancer

Kent F Hoskins¹, Amer Sidani¹, Tiffany Jones¹, Stefan Green¹, Rajyasree Emmadi¹ and Anjen Chenn¹. ¹University of Illinois at Chicago, Chicago, IL.

Body: Background
Activating mutations in the PIK3CA gene represent the most common molecular aberration in luminal breast cancers. Three hot-spot mutations (E542K, E545K, H1047R) account for approximately 70% of the PIK3CA mutations identified. The presence of a mutation is likely to become an important predictive biomarker to select patients for treatment with PIK3CA inhibitors. Tumor-derived DNA can be detected in the systemic circulation in the form of cell-free plasma DNA (cfpDNA), and the ability to interrogate tumor-derived DNA from an easily acquired biospecimen like cfpDNA has practical advantages over analysis of tissue specimens. Furthermore, cfpDNA can be monitored serially and could provide a dynamic measure of tumor progression and response to therapy. Droplet digital PCR (ddPCR) is a high-throughput technique that provides highly precise and sensitive detection of rare alleles in samples with ultra-low abundance of target DNA.

Methods
A nested case-control study was conducted with plasma samples collected as part of a study to identify blood-based biomarkers. Blood was collected from women scheduled for a breast biopsy at one of seven participating mammography units. All blood samples were collected prior to the biopsy procedure and were processed using a standard protocol. Cases are women diagnosed with invasive ductal carcinoma, stage I-III, that is positive for the estrogen receptor (ER+) and negative for HER2/neu (0 or 1+ by IHC). Controls are women with a benign, non-proliferative lesion, matched by collection site, year of collection and age. cfpDNA was isolated using a commercial kit (Macherey-Nagel, Bethlehem, PA). DNA was extracted from FFPE biopsy specimens by standard techniques. Central pathologic review confirmed at least 10% tumor cellularity in biopsy specimens from cases. A ddPCR assay was developed on a BioRad QX100 Droplet Digital PCR instrument using standard TaqManR assays with probe pairs designed to detect the 3 PIK3CA hotspot mutations and corresponding wild type (wt) alleles. The assay was validated using DNA from four cell lines, each positive for one of the hotspot mutations or wt at all 3 loci (Horizon Diagnostics, Cambridge, UK). ddPCR was performed on cfpDNA and DNA extracted from FFPE biopsy specimens in cases and controls. Next generation DNA sequencing (NGS) of exons 9 and 20 of PIK3CA was performed on biopsy specimens from cases using an Ion Torrent Personal Genome Sequencer.

Results
Validation of the ddPCR assay with serial dilutions of cell line DNA demonstrated the assay is highly sensitive with mutant alleles detected at an allele frequency down to 25% when input DNA template is as low as 2 ng. With DNA template input of 20 ng (typical recovery of cfpDNA), mutations can be detected down to an allele frequency of 0.78%. We will present data on the rate of hotspot mutation detection with the ddPCR assay performed on cfpDNA and FFPE samples in cases (n=80) and controls (n=80), and we will report performance characteristics of the assay. Mutation status of tumors determined by NGS will serve as the gold standard.

Conclusion
We have developed a ddPCR assay to detect PIK3CA hotspot mutations in cfpDNA, and will report the performance characteristics of the assay in 80 cases of untreated, ER+ early breast cancer.
Title: A genetic deletion in the uridine diphosphate glucuronosyltransferase 2B17 affects the pharmacokinetics of exemestane

Harriet Johansson¹, Valentina Aristarco¹, Jennifer Gjerde², Sara Gandini¹, Aliana Guerrieri-Gonzaga¹, Matteo Lazzeroni¹, Serena Mora¹, Debora Macis¹, Davide Serrano¹, Antonio Toesca¹, Luca Bottiglieri¹, Gunnar Meligren², Giuseppe Viale¹, Andrea DeCensi³ and Bernardo Bonanni¹. ¹Istituto Europeo di Oncologia, Milan, Italy; ²Haukeland University Hospital, Bergen, Norway and ³E.O. Ospedali Galliera, Genoa, Italy.

Body: Background Exemestane (EXE) is an aromatase inactivator used in the prevention and treatment of breast cancer (BC). The majority of EXE and its active metabolite 17-dihydroEXE are excreted as glucuronide conjugates by uridine diphosphate glucuronosyltransferase (UGT). The UGT2B17 enzyme is the most expressed in human liver. A deletion spanning the entire UGT2B17 gene (*2) decreased the EXE glucuronidation process by 14-fold in human liver microsomes (HLM) from UGT2B17(*2/*2) genotype subjects compared to wild-type UGT2B17(*1/*1) HLMs.

Aim The aim of this study was to investigate whether the UGT2B17 deletion is associated with increased serum levels of EXE and 17-dihydroEXE and to assess if this deletion predicts the anti-proliferative effect of EXE in BC tissue as measured by Ki67 changes 6 weeks apart.

Methods In a phase II pre-surgical trial, 50 postmenopausal women with histologically-confirmed ER positive BC; stage T1-2, N0-1, M0 were assigned to EXE 25 mg/d for 6 weeks before surgery. Morning fasting blood was collected at baseline (B) and after 6 weeks and stored at -80° C until assayed. Time of last drug intake was collected at blood draw. DNA was extracted from whole blood (Qiagen, Italy). We used Taqman copy number variation assay (Life Technologies, Monza, Italy) for the UGT2B17 genotyping. EXE and 17-dihydroEXE concentrations were determined by mass spectrometry (MS) using Waters® Xevo™ TQ MS in electrospray positive ionization mode (Waters, Manchester, UK), optimized by multiple reaction monitoring mode. We used Zorbax Eclipse Plus C18 columns and mobile phase MeOH/H2O with 0.1% formic acid. EXE pure substance was provided by Pfizer Inc., 17β-hydroxy EXE and EXE-19-d3 purchased from Toronto Research Chemicals (Toronto, Ontario, Canada). Wilcoxon Signed Rank Test was used to assess differences in serum concentrations of EXE, its metabolites, and post-pretreatment tissue Ki67 changes according to UGT2B17 genotypes.

Results Median age and BMI were 62 years and 26 kg/m2, respectively. The UGT2B17 genotype (n=50) was 24 homozygote wt (*1/*1), 20 heterozygote (*1/*2) and 6 homozygote variant (*2/*2). Minor allele frequency = 0.32. Hardy-Weinberg Equilibrium (P=0.57) was respected.

Median and IQRs of EXE, 17-dihydroEXE and Ki67 change from B by UGT2B17 genotypes

<table>
<thead>
<tr>
<th>Genotype</th>
<th>EXE (nM)</th>
<th>17-dihydroEXE (nM)</th>
<th>Ki67 change</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>UGT2B17 *1/*1 (n=22)</td>
<td>5.3 (2.2, 11.4)</td>
<td>1.85 (1.4, 3.06)</td>
<td>-9 (-16, -5)</td>
<td>28 (24, 32)</td>
</tr>
<tr>
<td>UGT2B17 *1/*2, *2/*2 (n=25)</td>
<td>9.3 (2.6, 17.6)*</td>
<td>2.5 (2.0, 3.6)**</td>
<td>-11 (-19, -3)♦</td>
<td>26 (23, 30)♦♦</td>
</tr>
</tbody>
</table>

P-values between genotype groups:*P=.28; **P=.04; ♦P=.82; ♦♦P=.41

Conclusions Our study demonstrates a significant association of the UGT2B17 deletion with increased serum concentrations of
17-dihydroEXE, irrespective of time since last drug intake. Neither 17-dihydroEXE, nor genotype explained the significant anti-proliferative effect of EXE on BC tissue. Larger studies should determine the clinical implications of this gene on EXE efficacy.
**Title:** ALK and BRAF genes are associated with early failure of breast cancer (BC) who received primary neoadjuvant chemotherapy for patients with locally advanced BCs

Yeon Hee Park\(^1\), Moon Ki Choi\(^1\), In-Gu Do\(^1\), Eun Yoon Choi\(^1\), Won Ho Kil\(^1\), Seok Won Kim\(^1\), Jeong Eon Lee\(^1\), Seok Jin Nam\(^1\), Jin Seok Ahn\(^1\) and Young-Hyuck Im\(^1\). \(^1\)Samsung Medical Center.

**Body:** **BACKGROUND:** Neoadjuvant chemotherapy (NAC) has been used widely in patients with locally advanced breast cancer (LABC). NAC has the added advantage of increasing breast conservation rates with similar disease-free and overall survival compared with adjuvant chemotherapy. A subset of patients receiving NAC experiences early failure during the course of therapy or within a short period after breast surgery. There are no established predictors for early therapy failure in LABC patients who received NAC. This study was performed to identify candidate actionable mutation to explain early failure and refractoriness to chemotherapy in BC patient groups that may not benefit from NAC.

**METHODS:** Seventy eight patients among 397 Patients with LABC (cT2-4N0-3) who were available for preoperative FFPE tumor block for next generation sequencing (NGS) included in this analysis excluding 21 patients whose FFPE were not qualified for Ampliseq. Early failure was defined as the development of an inoperable state caused by locoregional or systemic progression during NAC or relapse after curative surgery within 1 year after the initiation of NAC. Patients who developed recurrence after 1 year from the start of NAC or exhibited no failure during the follow-up period were defined as control in this study. Thus, our cohort was composed of pCR (pathologic CR to NAC), early failure, and control. The clinicopathological characteristics and disease courses of the patients whose disease progressed within 1 year of receiving neoadjuvant chemotherapy were analyzed to compare with the other patients. Using the Ion Torrent Ampliseq Cancer Panel v2 after DNA isolation from FFPE samples, we sequenced 2,855 loci from 50 cancer-related genes to identify genetic mutations in 78 BC patients who received NAC for patients with locally advanced BCs and available preoperative tumor tissue.

**RESULTS:** Thirty-eight of the 397 patients (9.6%) exhibited progression within 1 year after receiving neoadjuvant chemotherapy. Among 78 patients who were available tumor tissue in this analysis, the number of patients with early failure, pCR, and control was 22, 19, and 27, respectively. The sequencing analysis revealed TP53 mutations were observed in 95% or more patients, evenly irrespective of patients’ group. Missense mutations in ALK and BRAF were found to be predominantly much higher in patients with early failure than in other groups (p<0.01). To the contrary to this, missense mutations in JAK3 and MPL were only found in patients with pCR than in other groups (p<0.01).

**CONCLUSION:** ALK and BRAF genes are associated with early failure in this analysis. Our results support that targeted sequencing using cancer panel may function to identify actionable targets which are associated with responsiveness to NAC for patients with LABC. Further research to figure out the functioning role of these genes for each group is warranted.
Title: Concordance/discordance rates of HER2, ER, PR, and Ki67 in matched pair samples of primary (PBC) and metastatic breast cancer (MBC) tissues when comparing IHC with MammaTyper® RT-PCR kit

Markus Wallwiener¹, Andreas Hartkopf², Thomas Deutsch¹, Lakis Sotiris⁴, Florin-Andrei Taran², Andreas Trumpp⁴, Ralph Wirtz³ and Andreas Schneeweiss⁴. ¹University of Heidelberg, Heidelberg, BW, Germany; ²University of Tuebingen, Tuebingen, BW, Germany; ³STRATIFYER Molecular Pathology GmbH, Cologne, Germany and ⁴National Center for Tumor Diseases, Heidelberg, Germany.

Body: Background
Clinical decisions in the primary and advanced situation of breast cancer are based on immunohistochemical staining (IHC) and semiquantitative assessment of ER, PgR, HER2 and Ki67. However, IHC carries an up to 20% risk of erroneous results. Moreover, metaanalysis revealed variable discordance rates between primary and metastatic sites (P/M) which may have implications for patient management (Aurilio, Eu J Cancer 2013). Herein, we undertook a pilot study aiming to investigate whether the reported discordance between primary lesion and paired distant metastatic sites is due to technical limitations of the IHC technique and could be improved by mRNA analysis using the MammaTyper® in vitro diagnostic assay.

Materials and Methods
One 10µm-thick sections from clinical routine FFPE tissues of 28 tumor samples were reexamined by RNA quantitation of ESR1, PGR, HER2 and Ki67 using the RNXtract® kit for RNA extraction and MammaTyper® kit for objective assessment of receptor status. RNA levels were normalized using a synthetic in-vitro transcript for normalization according to the 40-DDCT method. Receptor status was reported based on predefined cut-offs according to the instruction for use. mRNA and IHC results were compared between paired primary and metastatic sites.

Results
Concordance per tumor sample between the two methods was 100% for HER2, 81.9% for ESR1 and 81.5% for PR. Among discrepant samples (N=8), 6 were primary and among these, half (N=3) were due to negative ER by IHC. With the exception of HER2, where no differences were observed for either method, the P/M concordance was superior by MammaTyper® kit (ESR1: 92.85 vs. 76.82, PR: 71.43 vs. 69.23). Importantly, several discordant cases by IHC were ER negative in the primary site, while being ER positive in the metastatic lesion, while being consistently ESR1 positive in both sites, when determined by the more sensitive PCR method. The subtype discordant cases were situated in the lung (2), the liver (1) and the bone marrow (1).

Conclusion
Our data indicate that receptor status shifts less frequently when determined by PCR methods compared to IHC. While some shifts between primary site and metastases may arise from true progression-related biologic alterations (Prat et al Nat Med 2009), others seem to be related to technical limitations of semiquantitative and subjective IHC, with several cases being conspicuous for ESR1. This finding is of great clinical significance and is consistent with previous reports implicating technical confounders of IHC for large part of the observed P/M deviations (Pusztai, The Oncologist 2010). The implied superiority of RT-qPCR by MammaTyper® vs. routine IHC in detecting true P/M tumor receptor status reversals and the possible clinical impact is clearly worth pursuing in larger datasets.
Title: Serum tumor markers levels may have little significance in evaluating neoadjuvant treatment response

Yu Jie Wang¹, Xiao Yan Huang¹, Miao Mo¹, Xiao Qing Jia¹, Jian Wei Li¹, Zhi Min Shao¹ and Guang Yu Liu¹. ¹Fudan University Shanghai Cancer Center, Shanghai, China.

Body: Background: Serum tumor markers such as carcinoembryonic Antigen(CEA), cancer antigen 15-3(CA15-3)and cancer antigen 12-5 (CA12-5) are widely used in clinical practice for surveillance of localregional, regional recurrence and distant metastasis. Testing for serum tumor markers is widely accepted as a fast, noninvasive, reproducible, and quantitative laboratory test. In this study, we aimed to determine the potential prognostic value of serum tumor markers in predicting pathological complete response (pCR) during neoadjuvant chemotherapy.

Patients and methods: We prospectively measured the pro-, mid- and post- neoadjuvant treatment serum tumor markers concentration in locally advanced breast cancer (stage II-III) patients who accepted pre-surgical chemotherapy or chemotherapy in combination with targeted therapy at Fudan University Shanghai Cancer Center between September 2011 and January 2014 and investigated the association of serum tumor markers levels with therapeutic effectiveness. Immunohistochemistry(ICH) of core needle biopsy was assessed before neoadjuvant treatment to determine hormone receptors, human epidermal growth factor receptor 2(Her-2) and proliferation index Ki67 value. In our study, therapeutic response was evaluated by pCR, defined as disappearance of all invasive cancer cells from excised tissue (including primary lesion and axillary lymph nodes) after completion of chemotherapy. General Linear Model and ROC curve were used to analyze the statistics.

Results: A total of 348 patients were recruited in our study after excluding patients with incomplete clinical information, 813 blood specimens from 271 patients were taken into final analysis. 97 patients were observed to acquire pCR status after completing treatment, accounting for approximately 35.8% of study individuals. 106 patients were determined to be Her-2 positive, among whom the pCR rate was 40% (42 patients). General Linear Model analysis showed that the concentration of CA153 increased after neoadjuvant chemotherapy in both pCR and non-pCR groups, and that there were significant differences between two groups (P=0.008). The areas under the ROC curve(AUC) of pre-, mid- and post-treatment CA153 concentration demonstrated low-level predictive value (P=0.594, 0.644, 0.621, respectively). No significant differences in CEA nor CA125 serum levels were observed between pCR group and non-pCR group (P=0.228 and 0.157, respectively). No efficient AUC (Area Under the Curve) of CEA or CA125 concentration could be observed to predict response towards neoadjuvant treatment (both less than 0.7), nor were difference observed between the two groups at different time points. We then analyzed the Her-2 positive subset. There were significant differences between pCR and non-pCR groups (P=0.039) in CEA concentration, but no significance in CA15-3 and CA12-5 (p=0.092 and 0.89, respectively). None of the ROC curve showed underlying prognostic values, for AUCs of these three makers were less than 0.5.

Conclusion: Serum levels of tumor markers CA15-3, CA12-5 and CEA serum levels may have little clinical significance in predicting response towards neoadjuvant treatment.
Title: Bcl2 and p53 immunohistochemical expression in 501 early luminal B breast cancers with long term follow up: Impact on disease free survival

Alessandra Fabi, Irene Terrenato, Francesca Sperati, Anna Di Benedetto, Sabrina Vari, Elisa Melucci, Cristiana Ercolani, Paola Malaguti, Simonetta Buglioni, Paola Papaldo, Cecilia Nisticò, Gianluigi Ferretti, Patrizia Vici, Laura Pizzuti, Letizia P Veracchio, Claudio Botti, Francesco Cognetti and Marcella Mottolese. 

Body: Over the last few years the ever-expanding investigation on cancer have provided accumulating evidence on the molecular characteristics of breast cancer (BC). Specifically, among the estrogen receptor (ER)-positive types of BC, the Luminal B (LB) subtype represents one of the most complex subtype, both from the diagnostic and the therapeutic point of view. Although in the clinical setting LB is typically regarded as an ER+, hormone-sensitive disease, more research is needed to improve the treatment. In this study we analyzed p53 and Bcl2 expression, together with conventional bio-pathological parameters, in a large set of LB BC patients with long term follow up in order to determine their potential impact on tumor progression (DFS) according to the treatment regimen.

Five-hundred and one BC patients (pts) who underwent surgery at our Institute between 2000 and 2009 (median follow-up 73 months [4-156]) were classified as LB according to Saint Gallen classification (2013) [(estrogen receptor +(ER ≥1%) and/or progesterone receptor+( PgR ≥20%), high Ki67 (≥15%) and/or HER2+ (2+Amplified –ISH test or 3+)]. All pts underwent systemic treatments, namely 132 anthracycline based therapy, 157 anthracycline plus taxanes, 103 regimens not including anthracyclines and/or taxanes and 109 hormonal therapy (HT). Forty-two pts underwent trastuzumab therapy. p53 and Bcl2 expression was routinely determined by immunohistochemistry at the time of surgical treatment along with other conventional biological factors, before any adjuvant therapy was planned. Kaplan-Meier method was applied to determine the impact of clinical and biological factors on disease-free survival (DFS) and the log-rank test was used to assess differences between subgroups. p53 was defined positive when a nuclear immunostaining was observed in ≥30% of tumor cells whereas Bcl2 when ≥50% of tumor cells presented a distinct cytoplasmic positivity.

Of the 501 LB pts included in the study (median age 54 yrs [21-85]), 183 (37%) were in the pre-menopausal status, 257 (51%) were N+, and 28% relapsed. In this series, 451 tumors (90%) were invasive ductal carcinomas, 47 (10%) invasive lobular carcinomas, 95% and 65% were ER and PgR positive respectively and 26% overexpressed HER2. p53 was found positive in 25% and Bcl2 in 68% of the cases. When we considered p53(+/−) Bcl2(−/+) combination, we observed that the 109 pts presenting a p53(+) Bcl2(−) tumor phenotype and receiving only hormonal therapy (HT), showed a higher probability of relapse in comparison to the others (p<0.0001). When these pts were stratified by menopausal status, the pre-menopausal group showed the poorest outcome (p=0.041).

Among the 370 HER2(−) LB pts, 177 were N-. In the latter group we did not find any differences between treatment with chemotherapy+HT and HT in terms of DFS regardless of bio-pathological parameters including p53 and Bcl-2. These data indicate that p53+/Bcl2- tumor phenotype may influence the outcome of Luminal B patients mainly in pre-menopausal women treated with HT. In LB HER2(−) N(−) BC, HT seems to be a valid option in the adjuvant setting, regardless of clinical and bio-pathological parameters.
2014 San Antonio Breast Cancer Symposium

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Average Grade: 5.50

Title: Pharmacogenetic dosing of epirubicin in FEC chemotherapy

John R Mackey¹, Edith Pituskin¹, Ann Vlahadamis¹, Katia Tonkin¹, Karen King¹, Sanraj Basi¹, Maria Ho¹, Judith Meza-Junco¹, Anil Joy¹, Dick Au¹, Sambasivarao Damaraju¹ and Michael B Sawyer¹. ¹Cross Cancer Institute, Edmonton, AB, Canada.

Body: Background:
Epirubicin dosing affects important clinical outcomes in breast cancer, with higher dose regimens improving efficacy but producing more myelosuppression. Epirubicin is metabolized by uridine glucuronosyltransferase 2B7 (UGT2B7). We previously reported relationships between UGT2B7’s promoter polymorphisms and epirubicin clearance and clinical outcomes in the (neo)adjuvant breast cancer setting; we identified a trend for increased grade 3/4 neutropenia but better efficacy outcomes in patients having at least one deficient allele (i.e. CT or CC) vs. patients who were wild type homozygotes. Patients homozygous for the deficient allele (CC) were at statistically significant increased risk for leucopenia compared to patients who were wild type homozygotes or heterozygotes. In this study we hypothesized patients with CT and TT genotypes would tolerate a higher epirubicin dose compared to CC genotype patients.

Methods:
Female breast cancer patients with histologically confirmed non-metastatic invasive breast cancer scheduled to receive at least three cycles of FE100C in the (neo)adjuvant setting were enrolled into the study. Peripheral blood was analyzed for UGT2B7 genotype. Patients received standard dose IV FE100C during the first 21 day cycle. Based on genotype, epirubicin dosing was escalated in the 2nd and 3rd cycles.

**Epirubicin Dose Escalation Scheme**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Cycle 1</th>
<th>Cycle 2</th>
<th>Cycle 3</th>
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</thead>
<tbody>
<tr>
<td>CC</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>CT</td>
<td>100</td>
<td>115</td>
<td>130</td>
</tr>
<tr>
<td>TT</td>
<td>100</td>
<td>120</td>
<td>140</td>
</tr>
</tbody>
</table>

Results:
To date 32 patients are evaluable for pharmacogenetic guided epirubicin dosing (8 CC genotypes, 14 CT genotypes and 10 TT genotypes). All 32 patients received epirubicin100 mg/m² in cycle one and a single patient in each of the CC and CT genotypes experienced grade 3 febrile neutropenia and were not dose escalated. All other patients with CT and TT genotypes were dose escalated in cycle 2 and all but two patients in the CT and TT genotypes were dose escalated in cycle 3. The incidence of febrile neutropenia was not dose dependent as all three genotypes had similar incidence in each cycle whereas leucopenia was genotype and dose dependent. The incidence of leukopenia increased in patients with CT and TT genotypes as their dose was increased and cycle 3 leukopenia rates were similar to patients with the CC genotype receiving standard dose epirubicin.

Conclusions:
Pharmacogenetic guided epirubicin dosing is well tolerated. This study is ongoing and updated data will be presented.
Title: Prognostic impact of discordance in hormone receptor status after the neoadjuvant chemotherapy in primary breast cancer

Toshiaki Kurihara1, Hanako Ueno1, Masaru Takemae1, Aiko Nagayama1, Maiko Takahashi1, Tetsu Hayashida1, Hiromitsu Jinno1 and Yuko Kitagawa1. 1Keio University School of Medicine, Shinanomachi Shinjuku, Tokyo, Japan.

Body: Background: Hormone receptor (Estrogen receptor (ER), Progesterone receptor (PgR)) is an important biological marker for predicting prognosis and making effective treatment decisions. Discordance in these biomarkers between the primary tumor and recurrent lesions is reported frequently. However, it is not well known whether these biomarkers are affected by neoadjuvant chemotherapy and their impacts on outcomes still remain to be elucidated. The aim of the present study is to evaluate the changes in HR status after neoadjuvant chemotherapy in patients with operable breast cancer and their relationship with response to treatment and prognosis.

Patients and Methods: Of 162 patients with stage II/III breast cancer patients receiving neoadjuvant chemotherapy from January 2005 to September 2012 at Keio University Hospital, 140 patients with non-pCR were analyzed. Patients were treated with sequential anthracycline and taxane. ER and PgR were assessed in both CNB performed prior to neoadjuvant chemotherapy and surgical samples. HR status was assessed by immunohistochemistry (IHC). ER/PgR status was determined using the Allred score and defined as positive when score was 3 and more. HR status was considered positive in cases of ER and/or PgR positivity. Pathological response criteria were classified as grade 0, 1, 2, or 3: grade 0 includes almost no change in cancer cells; grade 1 includes slight or marked changes in less than two thirds of area; grade 2 includes marked changes in more than two thirds of area; grade 3 includes necrosis or disappearance of all tumor cells.

Results: ER, PgR and HR positive rates before neoadjuvant chemotherapy were 72.9%, 67.1% and 76.4%, respectively. Changes in ER, PgR and HR status between CNB and surgical samples were 12.1% (4.3% gain; 7.8% loss), 17.1% (2.1% gain; 15.0% loss) and 9.3% (2.9% gain; 6.4% loss), respectively. In ER-discordant group, grade 2 rate of pathological response was significantly higher than ER-concordant group (61.1% vs. 30%, p=0.033), whereas there were no significant differences of pathological response between discordant and concordant group in PgR status. In the disease free survival (DFS), there were no significant difference between concordance and discordance group for ER, PgR and HR (p=0.216, 0.859, 0.233) after a median follow-up of 40.4 months. But patients with a loss in ER and/or HR status had a trend to a shorter DFS compared with the concordant ER and/or HR-positive group (p=0.169 and 0.154)

Conclusions: After neoadjuvant chemotherapy, discordance of biomarkers was seen in 5-20%. The pathological response was significantly associated with the change in ER status. A loss in ER and/or HR status may affect a prognosis of breast cancer after neoadjuvant chemotherapy, but still need further investigation.
**Title:** Thyroid function is associated with the response to neoadjuvant chemotherapy in breast cancer patients: Results from the NEOZOTAC trial on behalf of the Dutch Breast Cancer Research Group (BOOG 2010-01)

S de Groot\(^1\), A Charehbili\(^1\), L GM Janssen\(^1\), E M Dijkgraaf\(^1\), V THBM Smit\(^1\), L W Kessels\(^2\), A van Bochove\(^3\), H WM van Laarhoven\(^4\), E Meershoek-Klein Kranenburg\(^1\), A E van Leeuwen-Stok\(^4\), G J Liefers\(^1\), C JH van de Velde\(^1\), J WR Nortier\(^1\), J JM van der Hoeven\(^1\), H Pijl\(^1\) and J R Kroep\(^1\). \(^1\)Leiden University Medical Center, Leiden, Netherlands; \(^2\)Deventer Hospital, Deventer, Netherlands; \(^3\)Zaans Medical Center, Zaandam, Netherlands; \(^4\)Academic Medical Center, Amsterdam, Noord-Holland, Netherlands and \(^5\)Dutch Breast Cancer Research Group (BOOG), Amsterdam, Noord-Holland, Netherlands.

**Body:**

**Background:** Thyroid hormones, regulators of metabolism and development in healthy tissue, stimulate tumor growth in vitro and are associated with breast cancer risk. We investigated the effect of chemotherapy on thyroid function and the extent to which it can predict the pathological response in patients with HER2 negative stage II/III breast cancer taking part in the NEOZOTAC phase III trial, randomizing between 6 cycles of neoadjuvant TAC chemotherapy with or without additional zoledronic acid. Moreover, we examined the impact of thyroid function on chemotherapy toxicity.

**Methods:** Serum samples of 38 of the 105 patients who participated in the side study of the NEOZOTAC trial were available for analyses. Serum free thyroxin (fT4) and thyroid stimulating hormone (TSH) levels were measured at baseline and compared with fT4 and TSH levels before the 2\(^{nd}\) and 6\(^{th}\) chemotherapy cycle. FT4 and TSH levels were also compared between subjects with and without pathological complete response (pCR). The relation between toxicity, per side effect of any CTC grade, and the variation in fT4 and TSH levels during chemotherapy was tested.

**Results:** Serum samples at baseline, before the 2\(^{nd}\) chemotherapy cycle and at end of treatment were available for 31, 30 and 21 patients, respectively. In the total population, the mean baseline fT4 level was 16.0 pmol/L and the mean TSH level 1.11 mU/L. There were no differences between subjects solely treated with TAC chemotherapy and subjects treated with zoledronic acid as an adjunct to TAC with respect to the mean fT4 and TSH at each time point. Baseline TSH levels tended to be higher in patients who achieved pCR (\(p=0.035\) univariate analysis and \(p=0.074\) multivariate analysis) (Table 1). During 6 cycles of chemotherapy, fT4 levels decreased (\(p<0.000\)) and TSH levels increased significantly (\(p=0.019\)). Interestingly, the decrease of fT4 was significantly greater in patients without nausea, vomiting or sensory neuropathy, than in patients with those side effects (\(p=0.037\), \(p=0.043\) and \(p=0.050\) respectively).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Univariate analysis</th>
<th>Multivariate analysis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>N stage: N0 vs. N+</td>
<td>0.33</td>
<td>0.03-3.64</td>
</tr>
<tr>
<td>T stage: &lt;5cm vs. &gt;5cm</td>
<td>0.33</td>
<td>0.03-3.63</td>
</tr>
<tr>
<td>ER receptor: Pos vs. Neg</td>
<td>2.56</td>
<td>0.20-33.1</td>
</tr>
<tr>
<td>fT4</td>
<td>0.78</td>
<td>0.43-1.42</td>
</tr>
<tr>
<td>TSH</td>
<td>3.24</td>
<td>1.09-9.70</td>
</tr>
</tbody>
</table>

Table 1. Univariate and multivariate logistic regression models of baseline characteristics and TSH and fT4 predictive of pCR.

**Conclusion:** TSH levels at baseline were higher in breast cancer patients with pCR. Chemotherapy blunts thyroid function, and a large decline of fT4 was associated with less side effects. These data suggest that thyroid hormones may interact with chemotherapy to modulate treatment (side-) effects in patients with breast cancer.
Circulating vitamin D concentrations and breast cancer risk: A pooled analysis of 17 cohorts

Kala Visvanathan1, Alison Mondul2, Anne Zeleniuch-Jacquotte3, Toqir K Mukhtar4, Stephanie A Smith-Warner4, Regina G Ziegler2 and On Behalf of Investigators in the Vitamin D Pooling Project of Breast and Colorectal Cancer. 1Johns Hopkins, Baltimore, MD; 2National Cancer Institute, Washington, DC; 3New York University and 4Harvard.

Body: Optimizing vitamin D status through the use of supplementation and/or judicious sun exposure has been proposed as a BC risk reduction strategy. This simple and non-invasive approach to BC prevention is extremely appealing given that vitamin D concentrations in circulation are low in many individuals around the world. However, an Institute of Medicine (IOM) report found the evidence on vitamin D and breast cancer to be inconsistent. To clarify the relationship between vitamin D and breast cancer, data was pooled on approximately 25,000 women from 17 prospective cohorts worldwide. The association between pre-diagnostic circulating 25(OH)D levels, the accepted measure of vitamin D status, and breast cancer incidence was examined. For five cohorts, vitamin D status was assessed at a central laboratory (Heartland Assays, Inc.) using a direct, competitive chemiluminescence immunoassay that measures 25(OH)D2 and 25(OH)D3 equivalently. In 12 cohorts with previously measured 25(OH)D levels, a stratified sample of 29 controls was re-assayed at Heartland Assays and used to calibrate existing levels to a central assay using robust linear regression analyses. We standardized 25(OH) D levels for season using a periodic sine/cosine function. Conditional logistic regression analyses were performed in each study and were then pooled to generate pooled odds ratios by study-specific quantiles, consortium wide-quantiles, absolute cut points based on IOM guidance. Our preliminary analyses included 10,353 cases of incident invasive breast cancer (5305 estrogen receptor (ER) positive cases and 1311 (ER) negative cases and 12,313 matched controls. Median calibrated 25(OH)D levels in controls varied from 33 to 70 nmol/L across the cohorts. The consortium-wide median 25(OH)D among controls was 22% higher in summer as compared to winter months. Across all studies, median age at blood draw was 41 to 70 years; and median elapsed time from blood draw to diagnosis ranged from 2 to 13 years. The pooled odds ratio of breast cancer, comparing the highest to lowest study-specific 25(OH)D quintile, was 0.99 (95% confidence interval 0.90-1.08) after adjusting for body mass index, physical activity, menopausal status, menopausal hormone therapy use, parity/age at first birth, and family history of breast cancer. Results were not significantly different in analyses stratified by age of diagnosis (<50, 50-60, 60+ y).or by ER status. When calibrated circulating 25(OH)D levels were categorized based on the IOM definitions of "deficiency", "inadequacy", "adequacy", and "beyond adequacy", risk was similar across the categories. Further analyses are ongoing to examine especially low and high 25(OH)D concentrations, whether the vitamin D association varies according to tumor characteristics, the importance of elapsed time between blood draw and diagnosis. These will be completed before the meeting.

In conclusion, preliminary results from the largest pooled analysis of prospective studies to date show no association between 25(OH)D levels and breast cancer risk and therefore suggest that increasing Vitamin D levels may not be an effective risk reduction strategy for breast cancer.
Body: Background: Obesity in relation to breast cancer risk reveals different impact on women, warranting studies on circulating lipids and risk for breast cancer. Apolipoprotein A (ApoA), and apolipoprotein B (ApoB) are recognized actors in cardiovascular disease as the major protein components of High-Density-Lipoprotein (HDL) and Low-Density-Lipoprotein (LDL), respectively. LDL and HDL particles transport cholesterol from the liver to peripheral tissues and back. Blood levels of circulating lipoproteins and obesity are positively correlated. However the epidemiological evidence for the lipoprotein-cancer linkage has been mixed.

Aim: The aim of this study was to investigate pre-diagnostic levels of ApoA, ApoB, LDL and HDL in relation to breast cancer risk.

Methods and Material: The prospective cohort, Malmö Diet and Cancer Study was initiated in 1991 and enrolled 17035 women. These were followed-up until 31st of December 2011 and 1024 women were diagnosed with breast cancer. Blood samples were collected at baseline. Lipid quartiles (ApoA, ApoB, HDL, LDL) were constructed based on the entire female cohort, excluding women with a prevalent breast cancer. Associations between circulating lipoproteins and patient characteristics; age at baseline, body mass index (BMI) and socioeconomic index were described with ANOVA. A multivariate Cox Proportional Hazards analyses was applied to study breast cancer risk in relation to levels of circulating lipids and a trend analysis was performed.

Results: Age at baseline was evenly distributed among quartiles of ApoA (mean 56.4 SD 8.0- 58.0 SD 7.6) and HDL (mean 57.7 SD 6.1 – 57.4 SD 5.8) For ApoB (mean 53.7 SD 7.2 - 60.3 SD 7.3 and LDL (mean 54.9 SD 5.8 – 59.5 SD 5.2), age increased with levels of lipids. BMI was negatively associated with ApoA; women in the lowest quartile had the highest BMI (kg/m2) (mean BMI in ApoA-quartiles 1-4 (26.5, 25.7, 25.0, 24.4). The same pattern was seen for quartiles of HDL whilst a reversed relationship was seen for ApoB and LDL. Socioeconomic status was evenly distributed between all measured lipid quartiles. The multivariate Cox analyses revealed a statistically significant risk reduction for women with increasing levels of ApoB. Compared with the first quartile, the risk for incident breast cancer in quartiles 2-4 was 1.01 (95%CI, 0.85-1.20); 0.82 (95%CI, 0.68-0.99); 0.73 (95%CI, 0.60-0.89), P-trend<0.0003. A similar trend was seen for LDL, however only statistically significantly in the unadjusted analyses (P-trend <0.016). Levels of ApoA and HDL were not significantly associated with breast cancer risk.

Conclusions: This study confirms previous studies indicating an inverse association between ApoB and breast cancer risk. Studies addressing total cholesterol have similarly shown risk-reducing effects on breast cancer risk with increasing levels of cholesterol. However, BMI does not seem to explain this association. Previous breast cancer studies on circulating lipids including all separate components, ApoA, ApoB, HDL, and LDL are sparse, and the association is still insufficiently elucidated. It is possible that ApoB and LDL as risk factors for cardiovascular events have diverse effects in breast cancer. The interaction between circulating levels of lipids and breast cancer needs to be further investigated.
Title: Endocrine sensitivity is decisive for patient outcome in small node-negative breast cancers (BC) (pT1a,b) – Results from the Munich Cancer Registry

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Body: PURPOSE In clinical routine, adjuvant systemic therapy (ADST) in small node-negative (N0) breast cancers is controversial, in particular in HER2-positive disease. We aimed to define the patient subgroups with tumors <1 cm which would benefit from ADST based on their risk of BC-recurrence and survival using population-based cancer registry data.

METHODS From 2002-2009 (median follow-up 6 years), 9,707 primary breast cancer patients with N0 tumors <2 cm (pTis, pT1N0M0) were reported to the Munich Cancer Registry. Patients with pTis tumors (n= 1870) served as internal control. Time to progression, observed (OS) and relative survival rates (Kaplan-Meier estimates) are presented. Cox regression analysis was used to assess the influence of tumor size, age, HR-, and HER2-status.

RESULTS 10-year-OS in HR-positive tumors was 91.9% in pT1a (n=537), 90.6% in pT1b (n=1958), and 86.8% in pT1c (n=4513). In HR-negative tumors, rates were 91.7% (n=78), 86.8% (n=134), and 86.8% (n=427), respectively. In HER2-positive, it was 81.2% for pT1a (n=116), 88.1% for pT1b (n=171), and 86.7% for pT1c (n=427), in HER2-negative tumors it was 93.1% (n=431), 90.6% (n=1751), and 86.0% (n=3942), respectively.

In the multivariate model, age, tumor size, and HR-status showed a significant impact on OS, while HER2-status was not an independent prognostic factor.

CONCLUSION Prognosis of pN0 tumors <1 cm is excellent, especially if they are HR-positive, even in HER2-positive cases. Weighing potential benefits vs. side-effects, there seems to be no need for chemotherapy in tumors <0.5 cm. In pT1b tumors, chemotherapy may be considered, if tumors are triple negative or HER2-positive and HR-negative. In pT1c guideline-based adjuvant therapy using all therapeutic options seems to be warranted.
Title: Effects of statin use on volumetric mammographic density: Results from the Karolinska mammography project for risk prediction of breast cancer (KARMA) study

Ida Skarping¹, Judith Brand², Per Hall² and Signe Borgquist¹. ¹Lund University, Lund, Sweden and ²Karolinska Institutet, Stockholm, Sweden.

Body: Introduction:
High mammographic density is an established risk factor for breast cancer, whereas epidemiological data on statins and breast cancer risk have been inconclusive. The aim of this study was to address the role of statins in breast cancer risk by studying their effect on mammographic density in a large screening-based cohort.

Methods:
The KARolinska MAmmography project for the risk prediction of breast cancer (KARMA) study includes 70,876 women who performed either a screening or clinical mammography from January 2011 to December 2013. In all, 41,102 women responded to a web-based questionnaire, and their raw digital mammograms were stored and their volumetric mammographic density was estimated using the Volpara system. Information on statin use was obtained through the Swedish National Prescription Register. Analysis of covariance was used to study the effect of current statin use on mammographic density, adjusting for a large set of potential confounders. Analyses were stratified by statin lipophilicity and exposure duration. The potential effect modification by hormone replacement therapy (HRT) was analyzed.

Results:
Statin use was recorded in approximately 3,300 women (8.1%) of the study population of 41,102, the majority of which was prescribed a lipophilic statin (93.4% of statin users). After multivariable adjustment, volumetric percent density was lower in statin users than in non-users (P<0.001). Further, statin users had a larger non-dense volume than non-users (P<0.001), but no difference in absolute dense volume was detected. No differential effects were observed according to lipophilicity of the statin or drug duration. Interaction analyses revealed effect modification by hormone replacement therapy (HRT) (P-interaction=0.03) with statin use being associated with a larger dense volume among ever HRT users.

<table>
<thead>
<tr>
<th>Participant characteristics</th>
<th>Total</th>
<th>Statin use</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td>N = 37,765</td>
<td>N = 3,337</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>55.0</td>
<td>54.2</td>
<td>63.8</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.4</td>
<td>25.2</td>
<td>27.1</td>
</tr>
<tr>
<td>Menopausal status, % (N)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>40.2 (16,506)</td>
<td>43.1 (16,272)</td>
<td>7.0 (234)</td>
</tr>
<tr>
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<td>3.3 (1,349)</td>
<td>3.5 (1,304)</td>
<td>1.4 (45)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>56.6 (23,247)</td>
<td>53.5 (20,189)</td>
<td>91.6 (3,058)</td>
</tr>
</tbody>
</table>

Volumetric mammographic density measures by current statin use.
<table>
<thead>
<tr>
<th>Volumetric percent density (%)</th>
<th>No</th>
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<td>Yes</td>
<td>7.73</td>
<td>7.74</td>
<td>7.66</td>
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<tr>
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<td>P value</td>
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<td>0.001</td>
<td>&lt; 0.001</td>
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<tr>
<td>Dense volume (cm³)</td>
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<td>57.5</td>
<td>57.0</td>
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<tr>
<td></td>
<td>Yes</td>
<td>57.6</td>
<td>57.6</td>
<td>58.0</td>
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<tr>
<td></td>
<td>Hydrophilic</td>
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<tr>
<td></td>
<td>P value</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Dense volume (cm³)</td>
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<td>57.1</td>
<td>57.0</td>
</tr>
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<td>57.6</td>
<td>57.5</td>
</tr>
<tr>
<td></td>
<td>Hydrophilic</td>
<td>58.2</td>
<td>55.0</td>
<td>54.8</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>&lt; 0.001</td>
<td>0.20</td>
<td>0.66</td>
</tr>
</tbody>
</table>

Model 1: adjusted for age Model 2: Model 1 + BMI Model 3: Model 2 + menopausal status, HRT use, parity, age at menarche, education level, smoking, alcohol consumption and benign breast disease Model 4: Model 3 + low-dose aspirin and metformin use

Conclusions:
Statin use was associated with a lower mammographic percent density, although no evidence was found for an effect of statins on absolute dense volume. The observed interaction between statin and HRT use requires further investigation.
Title: Oncotype Dx use and its relationship with chemotherapy administration in the general population

Mariana Chavez-MacGregor¹, Jiangong Niu¹, Benjamin Smith¹, Hui Zhao¹, Thomas A Buchholz¹ and Sharon H Giordano¹.
¹University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Oncotype Dx is a 21-gene assay performed in paraffin-embedded tumor tissue. This test is used to assist in estimating the likelihood of recurrence and the benefit from chemotherapy in patients with hormone receptor (HR)-positive, node-negative breast cancer. One of he purported benefits of this assay is that by identifying low risk patients, Oncotype Dx use will be associated with a decrease in chemotherapy use among those patients less likely to benefit from it. In this study we sought to describe the utilization patterns of Oncotype Dx and its relationship with the use of adjuvant chemotherapy in a large population-based study.

Methods: We identified 112,522 patients younger than 65 years old diagnosed with early stage breast cancer between 2004-2012 in the MarketScan database. Standard algorithms were used to identify our cohort and HR-positivity was defined according to prescription information. A total of 34,245 patients older that 66 and diagnosed with early stage breast cancer between 2006-2009 were identified in the SEER-Medicare database. Descriptive statistics were used as well as logistic regression models to estimate the impact of Oncotype Dx testing on adjuvant chemotherapy administration.

Results: In the cohort of young patients (<65 years old), 13.9% (n=15,643) underwent Oncotype Dx testing. Among those with HR-positive disease, the percentage use was 71.8%. The use of Oncotype Dx increased according to age and year of diagnosis (p<.001), going from 0.11% in 2005 to 21.9% in 2011. In the multivariable model, Oncotype Dx testing was associated with a reduction in the use of adjuvant chemotherapy (OR 0.78; 95% CI 0.75-0.81).

Among the older patients (≥66 years old), 11.35% (n=3,017) underwent Oncotype Dx testing. Utilization was associated with younger age, histological grade, educational level, marital status, geographic location and comorbidities (p<0.001). Oncotype Dx use increased according to year of diagnosis going from 3.7% in 2006 to 14.11% in 2009 (p<0.001). Among the patients with Oncotype Dx test, 85.5% had node-negative disease. In the multivariable model Oncotype Dx testing was not associated with a statistically significant reduction in the use of chemotherapy (OR 0.95; 95% CI 0.86-1.06).

Conclusions: The use of Oncotype Dx in clinical practice is common, with an increase use in more recent years. Utilization was determined by patient-related characteristics. Among younger patients Oncotype Dx testing was associated with a reduction in the use of adjuvant chemotherapy, but this decrease was not observed among those ≥66 years old. It is possible that this discordance is related to comorbidities and the fact that older patients are less likely to be considered candidates for chemotherapy.
Title: Survival benefit increases after breast conserving therapy compared to mastectomy when axillary node status is positive in early stage breast cancer: A registry based follow-up study of Norwegian women with screening and interval detected breast cancer primary operated between 1998 and 2009

Olaf Johan Hartmann-Johnsen, Ellen Schlichting, Rolf Kaaresen and Jan Franz Nygaard. 1Oslo Universitetssykehus HF, Norway and 2Cancer Registry of Norway, Norway.

Body: BACKGROUND: Recent register studies on early stage breast cancer have shown better survival when women underwent breast-conserving treatment (BCT) compared to mastectomy (MTX). It is unclear if this is due to selection effects or to a systemic benefit of BCT compared to MTX. If there is a systemic benefit this could theoretically be more prominent in some subgroups of breast cancer. The aim of this study is to evaluate if there are any subgroups with early stage breast cancer that benefits more of BCT compared to MTX.

METHOD: A cohort of 6,629 women aged 50-69 with primary operable breast cancer detected in the screening programme (on screening or between screening, interval cancer) were selected from January 1998 until December 2009 and followed up until end of 2010. Life tables for overall survival (OS) and breast cancer specific survival (BCSS) were calculated in surgery groups and stages. Corresponding Kaplan-Meier plots were stratified in T1N1M0 and Ductal Carcinoma. OS and BCSS were compared using Cox proportional hazard for estimating hazard ratio between BCT and MTX in crude and adjusted analysis.

RESULTS: 5-years BCSS in T1N0M0 was 99% for both surgical cohorts (BCT and MTX). 5-years BCSS in T1N1M0 was 98% for women undergoing BCT and 91% for women undergoing MTX. Respectively, 10-years BCSS in T1N1M0 was 96% for women undergoing BCT and 83% for women undergoing MTX.

In adjusted analysis, HR for breast cancer death for women with stage T1N1M0 undergoing MTX compared to BCT was 3.12 (95% CI: 1.71-5.72). Respectively, 3.67 (95% CI: 1.20-11.22) stratified on grade III and 4.89 (95% CI: 1.25-19.01) stratified on hormone receptor negative disease.

CONCLUSION: The benefit of BCT compared to MTX seems to increase with increasing severity of the breast cancer disease, positive nodal status (N1), grade III and hormone receptor negative disease. These finding indicate a systemic benefit of BCT compared to MTX.
Title: Familial risk of breast density in extended Utah pedigrees

Karen Curtin1, Leigh Neumayer2, Matthew B Morgan3, Matthew A Stein3, Nicola J Camp1, Geraldine P Mineau1, Kerry G Rowe4 and Saundra S Buys1. 1University of Utah School of Medicine & Huntsman Cancer Institute, Salt Lake City, UT; 2University of Utah Surgery Department & Huntsman Cancer Institute, Salt Lake City, UT; 3University of Utah, Salt Lake City, UT and 4Intermountain Healthcare Clinical Oncology Program, Salt Lake City, UT.

Body: Background: Mammographic breast density (MBD) and family history are consistently associated with breast cancer risk, and breast density may account for a proportion of susceptibility to this disease. MBD has been shown to correlate in small cohort studies of twins and siblings. However, MBD has not been studied on a large scale in multi-generation families. We investigated the familial relative risk of MBD in the Utah population, as clustering of breast density in extended relatives may provide evidence for the role of genetics in breast density and inform screening recommendations.

Methods: Using the Utah Population Database (UPDB), an extensive genealogical database linked to medical records) we identified 189,812 women ages 35-85 with pedigree information, who underwent digital mammography between 2005-2012, with no history of breast/ovarian cancer and no indication of tamoxifen/aromatase inhibitor use. Individuals with unusually frequent screening (>1/yr) or with inconsistent MBD assessments were not included. Subjects were categorized according to Breast Imaging-Reporting and Data System (BI-RADS®) composition classification at index mammogram available in radiology records of Intermountain Healthcare and University of Utah Healthcare systems, representing the majority of mammograms in Utah, as: (I) 0-25% fibroglandular densities (mostly fat); (II) 26-50% fibroglandular (scattered densities); (III) 51-75% fibroglandular (heterogeneously dense); or, (IV) >75% fibroglandular densities (extremely dense). Familial recurrence risks of MBD classification and breast cancer were estimated using Cox regression models in relatives (mothers, daughters, and sisters or 1st-degree; aunts, nieces, grandmothers, and granddaughters or 2nd-degree; first- and second-cousins) of probands classified as BI-RADS I (N=18,170) or BI-RADS IV (N=11,787), compared to those in the most common classifications, BI-RADS II (N=79,825) and III (N=80,030) combined. Women in the comparison group were randomly selected and matched 5:1 to probands on birth year.

Results: Relatives of women with a history of extremely dense breasts (BI-RADS IV) were at increased relative risk (RR) of extremely dense breasts compared to women in BI-RADS II/III: 1st-degree, RR=2.3 (95%CI 2.0-2.7, P<10^-15); 2nd-degree, RR=1.8 (95%CI 1.5-2.2, P<10^-9); first cousins, RR=1.2 (95%CI 1.1-1.4, P<10^-3); and second cousins, RR=1.1 (95%CI 1.06-1.2, P<10^-5). Conversely, relatives of women in BI-RADS I (mostly fat) were at decreased risk of BI-RADS IV: 1st-degree, RR=0.5 (95%CI 0.4-0.5, P<10^-15); 2nd-degree, RR=0.7 (95%CI 0.6-0.8, P<10^-9); first cousins, RR=0.85 (95%CI 0.8-0.9, P<10^-4); and second cousins, RR=0.9 (95%CI 0.9-<1.0, P<10^-8). First-degree relatives of women in BI-RADS IV were at slightly greater risk of breast cancer compared to women in BI-RADS II/III (RR=1.2, 95%CI 1.1-1.4; P=0.004), while women in category I showed no increased cancer risk.

Conclusions: BI-RADS composition categories available from radiology records in the UPDB appear to be useful in assessing familial risk of MBD at the population level. Our results may inform screening guidelines in more distant as well as close relatives of women with a history of extremely dense breasts, whose families may be more susceptible to breast cancer.
Body: Background: In population-based datasets breast cancers are often classified as screen-detected or not screen-detected (or symptoms-detected) without further information, resulting in substantial heterogeneity in the latter group. In fact, this group includes symptoms-detected and fortuitously detected cancers, as well as 'interval breast cancers' (i.e. detected between screenings), all of which have different morphological characteristics than screen-detected cancers.

Objective: To compare the demographic features, tumour characteristics and survival of women with not-screen detected breast cancer according to whether or not they reported previous mammography screening.

Methods: The study population comprised 1696 women aged 50-69 years old with non-screen detected invasive breast cancer recorded at the Geneva cancer registry between 1990 and 2007. According to the information on the variable "previous mammography screening" the women were classified as those never having had a screening test and those having had one. We compared tumour characteristics and prognostic factors through chi square test, and calculated 5-year breast cancer specific survival and multivariate Cox proportional hazard models to assess independent determinants of mortality.

Results: Among the non-screen detected breast cancers there were 904 women who never had a screening mammography and 792 who declared to have had one. Women previously screened were more often 55-59 years old, diagnosed after 1996, of high social class, treated in the private sector and had a positive family history. These women had smaller tumours (mean 21.6 mm vs. 27.2, p<0.001), more often lobular cancers (18.4% vs 10.4%, p<0.001), less often poorly or undifferentiated cancers (22.8% vs 34.2%, p<0.001), and less often stage III or IV cancers (13.4% vs. 24.3%, p<0.001). Previously screened women more often received breast conserving surgery with radiotherapy (70.7% vs 49.1%, p<0.001), and endocrine therapy (73.0% vs 55.8%, p<0.001).

Five year breast cancer-specific survival was 83% among never screened women (95% Confidence Intervals [95%CI] 81-86) and 91% among those previously screened (95% CI: 89-93; log rank test=20.62, p<0.001). In the Cox multivariate model adjusted for all confounders and for prognosis and treatment variables, the difference in breast cancer mortality risk between women with and without a previous mammography screening disappeared (Hazard Ratio for previously screened women 1.06, 95%CI: 0.73-1.54).

Conclusions: Within the category of non-screen detected breast cancers co-exist at least two distinct groups of patients, with different tumours and, potentially, treatments and prognoses. Comparing women with screen-detected breast cancer against this heterogeneous group of women could produce biased results. Recording information about screening use before the breast cancer diagnosis would help to correctly classify these women.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-07-09
Average Grade: 3.60

Title: Trends and determinants of breast cancer survival among unscreened women: A population-based study

Elisabetta Rapiti¹, Thomas Agoritsas¹, Massimo Usel¹, Robin Schaffar¹, Hyma Schubert¹ and Christine Bouchardy¹. ¹Geneva Cancer Registry, Geneva, Switzerland.

Body: Background: Breast cancer mortality has been declining in many western countries, including Switzerland, since the late 1980s. This has largely been credited to mammography screening and improved treatment. However, mortality trends are also decreasing among unscreened women, though most breast cancer deaths still occur in that population.

Objective: The objective of this study was to analyse trends in, and factors affecting the survival of, women whose breast cancer was not detected by screening in the Geneva female population from 1990 to 2007. In Geneva an organized screening programme started in 2001 while opportunistic screening has existed since the beginning of the 1990s.

Methods: The study population comprised 1696 women aged 50-69 years old with invasive breast cancer that was not detected by screening and that was recorded at the population-based Geneva cancer registry. We studied tumour characteristics and prognostic factors across 6 time periods through chi square and trend tests. To assess whether breast cancer specific survival had improved over time, we calculated 5-year specific survival and performed multivariate Cox proportional hazard models to assess independent determinants of mortality.

Results: Median age of the women at diagnosis was 59 years. During the 18 year study period there was a decrease in the proportion of diagnoses among women of low social class (25.8% in 1990-92 vs 17.3% in 2005-07, p=0.001). No change in the distribution of stage at diagnosis or hormone receptor status was observed, while between the first and last period there was an increase in cancers with lobular morphology (6.8% vs. 19.0%, p<0.001), and a decrease in poorly or undifferentiated cancers (36.7% vs 28.7%, p<0.001). The use of breast conserving surgery with radiotherapy (BCS), chemotherapy and endocrine therapy increased significantly along the period (41.2% to 58.8%, p<0.001; 41.6% to 52.8, p=0.030; and 34% to 69.9%, p<0.001, respectively).

Five year breast cancer-specific survival was 82% in 1990-92 (95% Confidence Intervals [95%CI] 77-87) and 89% in 2005-07 (95% CI: 83-92; log rank test=8.68, p=0.122). In the Cox multivariate model there was a trend towards improved survival, but it was only statistically significant when comparing the period 2005-07 to the first period (Hazard Ratio 0.47, 95%CI: 0.22-0.98). Increasing age, stage, and grade, hormone receptor–negative disease, and not receiving BCS or endocrine therapy were all independently associated with a worse breast cancer–specific survival.

Conclusions: We observed an improvement in survival only in recent years among women whose breast cancers were not detected by screening; this appears to be associated with improved treatment. This suggests that the breast cancer mortality reduction observed in Switzerland since the late 1980s is not likely attributable to changes in treatment before 2005, but rather to the generalization of screening.
Title: Rising incidence of primary breast lymphoma: SEER 1973-2011

Alexandra Thomas1, Mary C Schroeder1, Bradley D McDowell1 and Brian K Link1. 1University of Iowa, Iowa City, IA.

Body: Background: Primary breast lymphomas (PBL) are rare and most commonly of B-cell origin: diffuse large B-cell or extranodal marginal zone lymphomas. Recent reports have described breast-implant associated anaplastic large T-cell lymphoma. Other lymphomas arising from the breast have also been described less frequently, with plastic surgery. We hypothesized that the overall incidence of primary breast lymphomas may also be increasing. This study reports the incidence of PBL as first malignancy as recorded in Surveillance, Epidemiology and End Results Program (SEER) from 1973-2011. Mortality and first local therapy are also reported.

Methods: Using the SEER 9 Research Data, we conducted a retrospective cohort analysis of women diagnosed with microscopically confirmed, first primary malignant lymphoma arising in the breast from 1973 through 2011. Cases were excluded if they were reported to the registry by a nursing home or diagnosed by autopsy or death certificate. PBLs were categorized by the SEER lymphoid malignancy recode variable. We evaluated trends in age-adjusted incidence rates, first local therapy, and age-adjusted all-cause and cause-specific mortality across time.

Results: The overall incidence of PBL has increased more than 3 fold, from 0.07/100,000 in 1973-82 to 0.23/100,000 in 2003-11 (p< 0.001) (Table 1).

Table 1: Primary Breast Lymphoma- Incidence (age-adjusted rate, per 100,000 women)

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0.1</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
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<td>Age &lt;50</td>
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<td>0.0</td>
<td>0.1</td>
<td>0.05</td>
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<td>Age 50+</td>
<td>0.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.7</td>
<td>&lt;0.001</td>
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<td>Lymphoma</td>
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<td></td>
<td></td>
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<tr>
<td>Hodgkins</td>
<td>0.0</td>
<td>0.002</td>
<td>0.003</td>
<td>0.002</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>NHL-B-cell</td>
<td>0.04</td>
<td>0.1</td>
<td>0.2</td>
<td>0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DLBCL</td>
<td>0.03</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.001</td>
</tr>
<tr>
<td>Follicular</td>
<td>0.005</td>
<td>0.02</td>
<td>0.03</td>
<td>0.04</td>
<td>0.062</td>
</tr>
<tr>
<td>Marginal Zone</td>
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<td>0.0</td>
<td>0.03</td>
<td>0.1</td>
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<tr>
<td>NHL-T-cell</td>
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<td>0.001</td>
<td>0.004</td>
<td>0.01</td>
<td>&lt;0.001</td>
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</table>

By age, the greatest incidence increase was for women 50 and older (0.02/100,000 in 1973-82 to 0.68/100,000 in 2003-11, p<0.001). Both B-cell marginal zone lymphoma and T-cell lymphoma have had steep increases in incidence from 1983-1992 to 2003-2011 (0/100,000 to 0.06/100,000, p<0.001 and 0.001/100,000 to 0.008/100,000, p<0.001, respectively). The incidence of DLBCL and follicular lymphomas arising in the breast rose from 1973-1992 and subsequently stabilized. Age-adjusted, all-cause mortality has dropped significantly since 1992, as has cause-specific mortality (Table 2).

Table 2: Primary Breast Lymphoma-Mortality and Initial Local Therapy

<table>
<thead>
<tr>
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<tr>
<td>All-cause</td>
<td>51%</td>
<td>54%</td>
<td>33%</td>
<td>22%</td>
<td>&lt;0.001</td>
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<tr>
<td>Cause-specific</td>
<td>43%</td>
<td>43%</td>
<td>25%</td>
<td>15%</td>
<td>&lt;0.001</td>
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</table>
For the entire cohort, no local therapy and radiation therapy, as initial local treatment, has increased over time. Surgery alone has decreased substantially.

Conclusions: Primary breast lymphoma remains a rare diagnosis; however the incidence has risen markedly over the last four decades, with B-cell marginal zone and T-cell lymphomas increasing significantly in the most recent time period. Further research is required to better understand the factors contributing to these trends. Survival gains over time suggest either that lymphoma treatments have improved or that the excess cases are treatment responsive or non-survival-threatening.
Title: Trends in clinicopathologic features and systemic therapy use in breast cancer patients: Findings from the National Cancer Database

Annees B Chagpar¹, Sarah Mougalian¹, Donald Lannin¹, Maysa Abu-Khalaf¹, Brigid Killelea¹, Tara Sanft¹ and Lajos Pusztai¹. ¹Yale University, New Haven, CT.

Body: Introduction: Breast cancer remains the most common malignancy affecting women in the United States. We sought to identify temporal trends in the clinicopathologic features of breast cancer over the last 13 years, along with changes in systemic therapy usage to treat this disease.

Methods: The National Cancer Database is a national resource maintained by the CoC and the American Cancer Society that captures 70% of the newly diagnosed breast cancers in the US. We utilized the beta-PUF file of the NCDB, which included 1,669,679 patients with invasive breast cancer patients from 1998 to 2010, and analyzed trends in clinicopathologic features as well as use of systemic chemo- and hormonal therapy.

Results: Over the 13 years of the study, a significant trend was noted to older age, lower grade and fewer positive lymph nodes (all p<0.001). Yet, the proportion of patients receiving systemic chemotherapy and hormonal therapy also increased over this time period (p<0.001). These trends remained when the time period was bisected into an early (prior to 2004) and later (after 2004) time period (shown in the table below). Receipt of systemic therapy was positively correlated with younger age, larger tumor size, higher grade and more positive lymph nodes over this time period (all p<0.001). There was a small increase in tumor size (<0.1 mm) in the latter period, but other factors were more favorable. Nonetheless, there has been a significantly higher utilization of systemic chemotherapy (OR: 1.107; 95% CI: 1.017-1.098, p<0.001) and hormonal therapy (OR: 1.588; 95% CI: 1.578-1.598, p<0.001) in more recent years, even when controlling for changes in clinicopathologic features on multivariate analysis.

<table>
<thead>
<tr>
<th></th>
<th>Prior to 2004</th>
<th>After 2004</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age at diagnosis (years)</td>
<td>60.1</td>
<td>60.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean tumor size (mm)</td>
<td>20.1</td>
<td>20.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean number of positive nodes</td>
<td>1.3</td>
<td>1.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proportion grade 3 (%)</td>
<td>36.8</td>
<td>34.6</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proportion receiving chemotherapy (%)</td>
<td>41.1</td>
<td>42.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Proportion receiving hormonal therapy (%)</td>
<td>41.7</td>
<td>53.5</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Conclusions: These data demonstrate that while early detection may be increasing the number of low grade node-negative tumors in older patients, our use of systemic therapy has become more liberal. Increasing use of hormonal therapy may be due to more aggressive guidelines and a lower threshold for ER-positivity; increasing use of chemotherapy may be the result of more efficacious drugs with lower toxicity being available to treat limited disease. Nonetheless, implications in terms of cost vs. benefit of our more aggressive approach may warrant further evaluation.
Title: Outcomes of women with small, early-stage breast cancer in Manitoba from 1995-2011

Aly-Khan Lalani1, Katherine Fradette3, Rashid Ahmed3, Debjani Grenier2,3 and Marshall Pitz2,3. 1University of Manitoba, Winnipeg, MB, Canada; 2University of Manitoba, Winnipeg, MB, Canada and 3CancerCare Manitoba, Winnipeg, MB, Canada.

Body: OBJECTIVES:
The objectives of this study were to describe the distribution, management, and outcomes of women with early-stage breast cancer in Manitoba during the period from 1995-2011. Specifically, we looked at tumours 1cm or smaller in size (T1mic/T1a/T1b) across the spectrum of molecular phenotypes: estrogen-receptor (ER) status, progesterone-receptor (PR) status, and human epidermal growth factor receptor 2 (HER2) status. This is the first study to evaluate these objectives in Manitoba, and will contribute significantly to the limited data of long-term outcomes for smaller-sized breast tumours.

METHODS:
Using the Manitoba Cancer Registry, we created a retrospective cohort of all patients with primary breast cancer of 1.0 cm or less, between 1995 and 2011. Women with previous or synchronous contralateral breast cancers were excluded. Data included patient demographics, diagnostic procedure, tumour size, nodal disease, treatment modalities (surgery, radiotherapy, hormone therapy, and chemotherapy), ER status, PR status, and HER2 status. Linkage to the Manitoba Health Drug Program Information Network (DPIN) database allowed capture of all hormonal therapies. Patient outcomes were evaluated, including risk of recurrence, overall survival and relative survival. Risk of recurrence was illustrated using cumulative incidence of recurrence, accounting for the competing risk of death. Kaplan-Meier curves were used to illustrate overall survival. The relative survival estimate was used to estimate the probability of surviving from breast cancer while controlling for differences in mortality due to other causes.

RESULTS:
Our study captured 2,341 women. Mean age at diagnosis was 62. ER+, PR+, and HER2+ status were 71% (11% unknown), 61.6% (11% unknown), and 8.6% (31% unknown), respectively. Clinically, 78% were Stage I disease, and by tumour size overall: T1mic 11.5%, T1a 18.9%, and T1b 69.7%. Ninety-eight percent had primary surgery, 58% had primary radiation, 48% received hormone therapy, and 16% received chemotherapy. At last follow-up, 21% of all patients were deceased. Relative survival estimates revealed that the survival of the overall cohort was not different than the general population, when comparing based on age groupings for Manitoban females. Recurrence occurred in 6.6% (156) of all cases. ER+, PR+, and HER2+ status were 63% (4% unknown), 58% (4% unknown), and 48% (5% unknown), respectively. By staging, 65% were Stage I disease and by tumour size 60% were T1b. Overall, 64% of recurrences were node-negative. For the HER2+, node-negative subpopulation at diagnosis: 21% of the T1mic group recurred, 41% of T1a recurred, and 19% of T1b recurred. In all recurrences, 39% of patients were deceased at last follow-up.

CONCLUSIONS:
Small, early-stage breast cancers are common and a significant proportion of patients recur. HER2-positivity appears to be an important risk factor for recurrence and may independently warrant treatment with trastuzumab, regardless of primary tumour size.
Increasing proportion of de novo compared with recurrent HER2-positive metastatic breast cancer: Early results from the systemic therapies for HER2-positive metastatic breast cancer registry study

Debu Tripathy¹, Adam Brufsky², Melody Cobleigh³, Mohammad Jahanzeb⁴, Peter Kaufman⁵, Ginny Mason⁶, Musa Mayer⁷, Joyce Oâ–Shaughnessy⁸, Hope Rugo⁹, Sandra M Swain¹⁰, Denise A Yardley¹¹, Mary Beattie¹², Bongin Yoo¹² and Sara Hurvitz¹³. ¹Keck School of Medicine, USC/Norris Comprehensive Cancer; ²University of Pittsburgh Cancer Institute, Pittsburgh, PA; ³Rush University Medical Center, Chicago, IL; ⁴University of Miami Sylvester Comprehensive Cancer Center Deerfield Campus; ⁵Norris Cotton Cancer Center, Dartmouth-Hitchcock Medical Center; ⁶Inflammatory Breast Cancer Research Foundation, West Lafayette, IN; ⁷AdvancedBC.org; ⁸Charles A. Sammons Cancer Center, Texas Oncology, The US Oncology Network; ⁹University of California, Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; ¹⁰Washington Cancer Institute, Medstar Washington Hospital Center, Washington, DC; ¹¹Sarah Cannon Research Institute and Tennessee Oncology, PLLC; ¹²Genentech, San Francisco, CA and ¹³University of California, Jonsson Comprehensive Cancer Center, Los Angeles, CA.

Body: Introduction
The Systemic Therapies for HER2-Positive Metastatic Breast Cancer Registry (SystHERs) is a US-based prospective observational cohort study currently enrolling patients with HER2-positive metastatic breast cancer (MBC). It began in 2012 and aims to provide real-world insight into the disease course, treatment patterns and associated clinical outcomes, and patient-reported experiences of disease. Here we describe the baseline characteristics of this population to date, including the proportion of patients with de novo and recurrent HER2-positive MBC.

Methods
SystHERs aims to enroll ~1000 patients with HER2-positive MBC within 6 months of first metastatic diagnosis. At enrollment, investigators report whether patients have recurrent or de novo MBC, the latter defined as distant metastases at the time of initial MBC diagnosis.

Results
As of February 17, 2014, data are available from 306 of 319 enrolled patients. Forty-six percent (142/306) had de novo HER2-positive MBC. For the 54% (164/306) with recurrent HER2-positive MBC, median disease-free interval (DFI, defined here as time from early-stage breast cancer diagnosis to diagnosis of MBC) was 43.6 (range 0.5–270.2) months. For these patients with recurrent disease, 54% (89/164) received (neo)adjuvant HER2-targeted therapy. Baseline patient and tumor characteristics are shown below.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overall (n=306)</th>
<th>De novo (n=142)</th>
<th>Recurrent (n=164)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median (range)</td>
<td>57 (27-86)</td>
<td>54 (27-83)</td>
<td>59 (30-86)</td>
</tr>
<tr>
<td>Race, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>233 (76)</td>
<td>105 (74)</td>
<td>128 (78)</td>
</tr>
<tr>
<td>Black</td>
<td>54 (18)</td>
<td>28 (20)</td>
<td>26 (16)</td>
</tr>
<tr>
<td>Other</td>
<td>8 (3)</td>
<td>3 (2)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Not available</td>
<td>11 (4)</td>
<td>6 (4)</td>
<td>5 (3)</td>
</tr>
<tr>
<td>Ethnicity, n(%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Not Hispanic</td>
<td>257 (84)</td>
<td>120 (85)</td>
<td>137 (84)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>31 (10)</td>
<td>17 (12)</td>
<td>14 (9)</td>
</tr>
<tr>
<td>Not available</td>
<td>18 (6)</td>
<td>5 (4)</td>
<td>13 (8)</td>
</tr>
<tr>
<td>Pre-menopausal, n (%)</td>
<td>61 (20)</td>
<td>42 (30)</td>
<td>19 (12)</td>
</tr>
<tr>
<td>Hormone receptor status, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER and/or PR positive</td>
<td>209 (68)</td>
<td>90 (63)</td>
<td>119 (73)</td>
</tr>
<tr>
<td>-----------------------</td>
<td>----------</td>
<td>--------</td>
<td>----------</td>
</tr>
<tr>
<td>ER and PR negative</td>
<td>92 (30)</td>
<td>50 (35)</td>
<td>42 (26)</td>
</tr>
<tr>
<td>Unknown</td>
<td>5 (2)</td>
<td>2 (1)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Median number of metastatic sites, (range)</td>
<td>2.0 (1-7)</td>
<td>2.0 (1-6)</td>
<td>2.0 (1-7)</td>
</tr>
<tr>
<td>Sites of metastasis, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone</td>
<td>141 (46)</td>
<td>76 (54)</td>
<td>65 (40)</td>
</tr>
<tr>
<td>Liver</td>
<td>120 (39)</td>
<td>63 (44)</td>
<td>57 (35)</td>
</tr>
<tr>
<td>Nodes</td>
<td>89 (29)</td>
<td>49 (35)</td>
<td>40 (24)</td>
</tr>
<tr>
<td>Lung</td>
<td>87 (28)</td>
<td>38 (27)</td>
<td>49 (30)</td>
</tr>
<tr>
<td>Chest wall</td>
<td>43 (14)</td>
<td>12 (9)</td>
<td>31 (19)</td>
</tr>
<tr>
<td>CNS</td>
<td>30 (10)</td>
<td>9 (6)</td>
<td>21 (13)</td>
</tr>
</tbody>
</table>

Conclusions
The proportion of patients with de novo MBC appears to have increased over time to 46% in SystHERs, compared with 33% in RegistHER, a registry study that enrolled 1023 patients with HER2-positive MBC from 2003–2006 (Yardley et al, 2014), and predated the broad use of HER2-targeted therapy in the (neo)adjuvant setting. The DFI for patients with recurrent HER2-positive MBC also appears to be longer (median DFI 43.6 months in SystHERs vs. 32.6 months in registHER). We hypothesize that the proportion of patients with de novo MBC within the metastatic population is higher due to the use of advanced screening techniques which allow better detection of metastases, and due to a reduction in recurrences related to the availability of HER2-targeted adjuvant therapy. Changes in the population characteristics of patients with HER2-positive MBC may impact treatment strategies and have trial design implications. Updated data from approximately 500 patients are expected by the time of presentation.
Recent changes in breast cancer incidence and mortality trends in Mexico: A population-based study

Enrique Soto-Perez-de-Celis1, Alejandro Mohar2 and Yanin Chavarri-Guerra1. 1Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán, Mexico City, DF, Mexico and 2Instituto Nacional de Cancerología, Mexico City, DF, Mexico.

Body: Background: Breast cancer (BC) is the most common malignancy in Mexican women since 2006, with a high number of cases reported in the more developed northern states. However, the analysis of national BC incidence in Mexico has not been previously reported. We sought to describe BC trends in Mexico using recent population-based data and to analyze geographical differences in BC incidence and mortality rates.

Methods: This population-based retrospective cohort study included all incident BC cases registered in the National Epidemiological Surveillance System and all BC deaths registered by the National Institute of Statistics and Geography in Mexico from 2001 to 2011. Yearly populations were obtained from national census data. The age-standardized (AS) incidence rate of BC was calculated using the female population over 15 years of age and standardized to the World Standard Population. AS incidence rates were calculated for 3 geographic regions of the country (North, Center and South). Joinpoint regression analysis was performed to examine trends in BC incidence and mortality. We estimated annual percentage change (APC) using weighted least squares log-linear regression.

Results: From 2001 to 2011, 69,651 new cases of BC were registered in Mexico. The AS incidence of BC significantly increased, rising from 14.2/100,000 person-years (PY) (Standard Error [SE] 0.23) in 2001, to 25.2/100,000 PY (SE 0.25) in 2011, with an APC of 5.9% (95% CI 4.1-7.7, p<0.05). Regional AS incidence rates were significantly increased in the Center and in the South, with a non-significant increase in the North (Table 1). For the same period, 48,817 deaths attributed to BC were registered. AS mortality rate also had a significant increase, rising from 14/100,000 PY (SE 0.23) in 2001 to 14.6/100,000 PY (SE 0.2) in 2011, with an APC of 0.4% (95% CI 0.1-0.7, p<0.05). Regional AS mortality rates were significantly increased in all three regions (Table 1).

Table 1. Regional BC incidence and mortality rates

<table>
<thead>
<tr>
<th>Region</th>
<th>2001 AS rate per 100,000 PY (SE)</th>
<th>2011 AS rate per 100,000 PY (SE)</th>
<th>APC% (95% CI)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>South</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence</td>
<td>6.1 (0.42)</td>
<td>16.57 (0.53)</td>
<td>10.5 (6.1-15.2)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mortality</td>
<td>7.8 (0.46)</td>
<td>9.56 (0.43)</td>
<td>2 (1-3.2)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Center</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence</td>
<td>12.9 (0.27)</td>
<td>26.9 (0.33)</td>
<td>7.6 (5.7-9.6)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Mortality</td>
<td>14 (0.29)</td>
<td>14.4 (0.25)</td>
<td>0.3 (0-0.6)</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>North</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence</td>
<td>24.2 (0.64)</td>
<td>26.8 (0.54)</td>
<td>1 (-1.6-3.7)</td>
<td>NS</td>
</tr>
<tr>
<td>Mortality</td>
<td>17.6 (0.57)</td>
<td>18.8 (0.48)</td>
<td>0.7 (0-1.3)</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Conclusions: BC trends in Mexico show a continuous increase in incidence and mortality from 2001 to 2011, which could reflect population growth, ageing, lifestyle modifications and changes in access to diagnosis and treatment. These changes could be an expression of epidemiological transition in developing countries such as Mexico. A significant growth in both incidence and mortality was found in both the Center and the South. In the North, incidence rates remained unchanged while mortality rates had a significant rise, which was comparable to that of the other regions. One possible unexplored explanation for this observation could be the recent wave of drug-related violence and high criminality rates in the north of the country, which may perhaps cause underreporting of cases and disrupt availability of medical attention and access to healthcare in an otherwise developed and
wealthy region.
Title: Does annual mammogram screening incur lower healthcare costs for breast cancer women after diagnosis?

Su-Hsin Chang¹, Lauren T Steward², Bettina F Drake¹, Sarah Lyons¹, Susan Kraenzle³ and Melody S Goodman¹. ¹Washington University School of Medicine, St Louis, MO; ²Washington University School of Medicine, St Louis, MO and ³Joanne Knight Breast Health Center, The Alvin J. Siteman Cancer Center at Barnes-Jewish Hospital and Washington University School of Medicine, St Louis, MO.

Body: Background: Breast cancer (BC) is the most commonly diagnosed cancer in women. The American Cancer Society (ACS) guidelines recommend annual screening mammography for women of average risk beginning at age 40.

Objective: Based on the ACS recommendation, we sought to determine whether women aged 40 years and older who had annual screening mammogram before BC diagnosis incurred lower healthcare costs after BC diagnosis compared to those who did not follow the guidelines.

Methods: We used data from a large private health insurance claims database (Thomson Reuters Marketscan Research Databases), 2006-2010. Our sample included women aged at least 40 years with a BC diagnosis between January 2009 and May 2010 and excluded women with cancer other than BC. The study period was chosen to allow for (1) the observation of their screening behavior (3 years) before BC diagnosis and (2) the determination of their metastatic status. A woman was determined to have metastasis upon diagnosis if within the period of 1 month before and 7 months after BC diagnosis, she had at least two metastasis codes and chemotherapy (algorithm adapted from Schootman et al. 2009). The outcome variable was total healthcare costs after BC diagnosis. Costs in MarketScan are defined as adjudicated reimbursement amounts from insurer claims data. Women who had annual mammogram screening (ACS guidelines followers) were defined as women who had a screening mammogram every calendar year from January 2006 until their BC diagnosis. Multivariable regression analyses of log-transformed total costs were conducted, adjusting for age, months between BC diagnosis and December 2010, employee classification (e.g., salary union), employment status, geographic location (e.g., northeast, south, etc.), metastatic status, and whether they received wire localization, lumpectomy, partial mastectomy, radical mastectomy, breast reconstruction, chemotherapy, and radiotherapy.

Results: 7,892 women with BC were identified. Among them, 27% had screening mammography every calendar year before their BC diagnosis. In the multivariable analysis, women who had annual mammogram screening were associated with a 4.6% higher cost (p=0.03). Women with metastatic diseases at diagnosis increased healthcare costs by 143.8% (p<.0001). Health care costs increased by 0.9% each month after diagnosis (p<.0001). Wire localization incurred 40.4% higher costs (p<.0001). Lumpectomy increased healthcare costs by 45% (p<.0001). Partial mastectomy was associated with 42.3% more costs (p<.0001). BC patients undergoing radical mastectomy had 34.6% more costs (p<.0001). Breast reconstruction increased costs by 62.6%. Patients who received chemotherapy had 189.8% higher healthcare costs compared to those who did not receive chemotherapy (p<.0001). Radiotherapy was associated with 95.9% more costs (p<.0001).

Conclusion: This study provides evidence suggesting that even amongst an insured population, it appears that less than 30% of breast cancer patients had annual mammogram screening before diagnosis and that these patients were associated with higher healthcare costs in the early stage after their BC diagnosis. Future studies need to be conducted to further examine total healthcare costs throughout breast cancer survivors' life span.
Rates of adherence and persistence to adjuvant endocrine therapy among women enrolled in Medicare Part D in a four-state region of Appalachia

Gretchen G Kimmick¹, Fabian Camacho² and Roger T Anderson². ¹Duke University Medical Center, Durham, NC and ²Pennsylvania State College of Medicine.

**Body:**

**Background:** Disparities exist in breast cancer outcomes by age, geographic location, and socioeconomic status, but there is little data regarding contributing factors to disparities within Appalachia. Underuse of adjuvant endocrine therapy for breast cancer can contribute to disparities. We studied older women within four states of Appalachia and explored adherence and persistence rates for adjuvant endocrine therapy.

**Methods:** The study group consisted of women with stage I-III breast cancer diagnosed 2008-2009 in North Carolina, Pennsylvania, Kentucky, and Georgia, who were continuously enrolled in Part D Medicare and filled a prescription for tamoxifen, anastrozole, letrozole, or exemestane within 1 year of diagnosis date. Adherence rate is defined by medication possession ratio (MPR=sum of days supply for all claims during the calendar year after first prescription). Persistence rate was defined as absence of a 90 day or greater gap in prescription coverage since the first prescription fill. Univariate analyses by Kruskall-Wallis nonparametric test were performed. Variables included age, year of diagnosis, Charlson comorbidity score, Medicaid/Medicare versus Medicare insurance status, rural versus urban residence, county-level economic status (Appalachia Regional Commission: Distressed, At risk, Transitional, or Competitive), state, stage (1, 2A, 2B, or 3), and breast conserving surgery (BCS) versus mastectomy.

**Results:** We identified 1,229 eligible cases. Mean age was 74.7 years (range 37-98); 97.5% were white; 46.7% had rural residence, 9.0% lived in distressed counties, 28.2% were dual-Medicaid/Medicare insured, mean Charlson comorbidity score was 1.51, and tumor was stage 1 in 56.7%, 2 in 34.1%, and 3 in 9.2%. Mean calendar MPR was 80% (SD 0.30). Persistence rate at 6-months was 87% (SD 0.34) and at 12-months was 67% (SD 0.47). MPR varied significantly between diagnosis years (p < .01), county-level economic status levels (p=0.03), geographical state (p < .01), and Singh Index Area Deprivation tertiles (p=0.01). MPR was highest among those in the Competitive ARC class at median levels of 86%, versus 76% in Distressed, 77% in At Risk, and 80% in Transitional. MPR was highest in PA (84%), compared to OH (79%), NC(77%), and KY (73%). Greatest area deprivation tertile also coincided with lower mean and median MPR adherence.

Persistence at 12 months varied significantly between diagnosis years (p < .01), geographical state (p = .01), and Singh Index Area Deprivation tertiles (p=0.01). Persistence rates for PA (71%) were higher than OH (66%), NC (52%), and KY (51%). Persistence rates for the highest area deprivation tertile were lower (62%) than rates in the lowest tertiles (71%, and 69%).

**Conclusions:** Among this group of women within four states in Appalachia with Medicare Part D who filled a prescription for endocrine therapy, overall adherence and persistence rates were lower than expected. Higher adherence and persistence rates were seen in areas with lower deprivation and economic distress.
Title: The NABON breast cancer audit; quality improvement in three years' time

Annelotte C van Bommel1, Marie-Jeanne T Baas-Vrancken Peeters2, Margriet van der Heiden - van der Loo3, Thijs van Dalen4, Emiel J Rutgers2, Michel W Wouters5, Marc B Lobbes6, Ruud M Pijnappel6, Marc A Mureau7, Pieter J Westenend8, Bart de Vries5, Carolien H Smorenburg5, Agnes Jager7, John H Maduro9, Henk Struikmans10, Carol Richel11, Marga Schrieks11, Maike Schepens12, Sabine Siesling13 and Vivianne C Tjan-Heijnen5.

1Leiden University Medical Center, Leiden, Netherlands; 2Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; 3Comprehensive Cancer Centre the Netherlands (IKNL), Utrecht, Netherlands; 4Diakonessenhuis Utrecht, Utrecht, Netherlands; 5Maastricht University Medical Centre, Maastricht, Netherlands; 6University Medical Centre, Utrecht, Netherlands; 7Erasmus MC Cancer Institute, Rotterdam, Netherlands; 8Laboratory for Pathology, Dordrecht, Netherlands; 9University Medical Centre, Groningen, Netherlands; 10Medical Centre Haaglanden, Hague, Netherlands; 11Dutch Breast Cancer Association, Utrecht, Netherlands; 12Health Insurer Netherlands, Netherlands and 13Comprehensive Cancer Centre the Netherlands (IKNL), Utrecht, the Netherlands / University of Twente, Enschede, Netherlands.

Body: Background
The lifetime risk of developing breast cancer is 1 in 8 for women in the Netherlands. Breast cancer care in the Netherlands is of high quality, resulting in low 5-year local recurrence rates of 1.5% after breast conserving therapy and 2.7% after mastectomy. Although good endpoints of breast cancer treatment are obtained, it is expected that further improvement of care can be achieved since unexplained variation in a number of treatment aspects was found between hospitals in the Netherlands. Clinical audits provide an important tool for quality assessment.

Worldwide, only a few nationwide clinical audits for breast cancer care are currently running. In the Netherlands, a nationwide multidisciplinary clinical audit started three years ago.

Material and methods
The multidisciplinary national NABON Breast Cancer Audit (NBCA) started collecting data of all Dutch hospitals in 2011, facilitated by Comprehensive Cancer Centre the Netherlands (IKNL) and Dutch Institute for Clinical Auditing (DICA). The NBCA has several purposes: nation-wide evaluation of quality parameters, evaluation of guideline adherence, and providing weekly updated feedback to participating institutions.

Results
All Dutch hospitals (n=92) participate by providing data regarding delivered breast cancer care resulting in a database of more than 42,000 breast cancer patients (5,745 DCIS and 36,396 invasive carcinomas) in three years time. Eighty-nine percent of invasive breast cancer patients were treated with primary surgery of which 62% (n=19,885) with breast conserving surgery. Within three years time, several quality assessments improved such as guideline compliance for pre- and postoperative multidisciplinary team meetings, percentage of patients starting surgery within five weeks (see table). The percentage of patients that were treated with preoperative systemic treatment (12%; 95% CI: 0 – 47%) and patients receiving an immediate reconstruction after ablative surgery (19%; 95% CI 0 – 73%) still remained low with a large variation between hospitals. At the conference, results will be substantiated by funnelplots. Other quality indicators will be presented as well.

Table 1. Quality indicator results and their improvement over years.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>2011</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preoperative MDT</td>
<td>81%</td>
<td>96%</td>
</tr>
<tr>
<td>Postoperative MDT</td>
<td>90%</td>
<td>98%</td>
</tr>
<tr>
<td>Time to operation ≤5 weeks (immediate reconstruction after mastectomy excluded)</td>
<td>80%</td>
<td>85%</td>
</tr>
<tr>
<td>Tumor positive margins invasive breast cancer (without PST)</td>
<td>6.1%</td>
<td>5.0%</td>
</tr>
<tr>
<td>Tumor positive margins DCIS</td>
<td>25%</td>
<td>20%</td>
</tr>
<tr>
<td>Procedure</td>
<td>Percentage 1</td>
<td>Percentage 2</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>PST for invasive breast cancer</td>
<td>10%</td>
<td>12%</td>
</tr>
<tr>
<td>Immediate reconstruction after ablative surgery for invasive breast cancer</td>
<td>15%</td>
<td>19%</td>
</tr>
<tr>
<td>Immediate reconstruction after ablative surgery for DCIS</td>
<td>39%</td>
<td>44%</td>
</tr>
</tbody>
</table>

MDT = Multi-disciplinary team meeting; PST = Primary Systemic Treatment; DCIS = Ductal Carcinoma In Situ

Conclusions
The continuous cycle of registration and providing feedback by clinical auditing provides a powerful tool for quality monitoring and improving breast cancer care. Improvements of monodisciplinary surgical and pathological aspects of care have been reached in a relatively short time period. However, for more complicated multidisciplinary issues like the use of primary systemic treatment and immediate reconstruction, detailed analyses of the variation between hospitals is needed to further improve these aspects of breast cancer care.
Body: Introduction: In the United States, nearly 200,000 cancer-related deaths each year are attributed to poor diet and physical inactivity. We sought to determine whether perceived personal breast cancer risk was associated with adherence to healthy lifestyle habits in a nationally representative sample.

Methods: The National Health Interview Survey (NHIS) is a population-based survey conducted each year by the CDC, designed to represent the non-institutionalized civilian population in the United States. We utilized data from the 2010 NHIS adult and cancer supplements to compare fruit/vegetable intake, alcohol use and exercise habits among women who perceived themselves to be at high risk of developing breast cancer versus those women who perceived themselves to be at average or low risk.

Results: In 2010, 12,055 women without a history of cancer were surveyed, representing 94,990,140 people in the population. Perceived risk of breast cancer was not associated with improved adherence to nutrition and physical activity guidelines (see Table). Less than 5% of all U.S. women meet the recommended fruit/vegetable intake regardless of perceived risk, and only one-third of all women achieve recommended physical activity. Approximately 95% of women adhere to guidelines regarding alcohol intake. On multivariate analysis, perceived risk did not affect health habits when controlling for age, ethnicity, education, insurance status, income, region and body mass index (BMI).

Conclusion: Thousands of breast cancer diagnoses and deaths could be prevented each year through healthy diet and exercise. Yet, a striking majority of U.S. women do not adhere to cancer prevention recommendations, including those women who perceive themselves to be at high risk of breast cancer. These findings suggest that patient education and awareness of personal breast cancer risk may be essential, but not sufficient, for motivating meaningful behavior change.

<table>
<thead>
<tr>
<th></th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Perceived High Risk-%</td>
<td>Perceived Low/Avg Risk-%</td>
</tr>
<tr>
<td>Fruit/Veg Intake (&gt;5 svg daily)</td>
<td>4.90%</td>
<td>4.42%</td>
</tr>
<tr>
<td>Alcohol Intake (&lt;=7 svg/wk)</td>
<td>94.22%</td>
<td>95.13%</td>
</tr>
<tr>
<td>Physical Activity (75-150 min vig-mod act/wk)</td>
<td>30.11%</td>
<td>27.74%</td>
</tr>
</tbody>
</table>
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-07-20
Average Grade: 2.75

Title: Survival comparative analysis of patients with invasive breast cancer treated by a military medical center and matched patients of the US general population

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1Windber Research Institute, Windber, PA; 2Walter Reed National Military Medical Center, Bethesda, MD; 3MDR Global, LLC, Windber, PA and 4Thomas Jefferson University, Philadelphia, PA.

Body: BACKGROUND
U.S. military beneficiaries differ from the U.S. general population with regards to access to health care as care is provided at no or much lower cost in the military health system. Other differences also exist. Many of these differences are known factors affecting invasive breast cancer outcomes. Thus it is desirable to conduct a comparative analysis of breast cancer patient outcomes between these two populations to find out whether there is any outcome difference, and if yes what the contributing factors are.

METHODS
We compared overall survival (OS), disease-specific survival (DSS), and 5-year OS and DSS rates in breast cancers between 399 patients from the Clinical Breast Care Project at the Walter Reed National Military Medical Center (CBCP-WR) and 1,000 sets of 1596 matched patients from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute. All patients were diagnosed between 2001 and 2010. Each CBCP-WR patient was randomly matched to four SEER patients on six demographic and clinicopathologic variables (age at diagnosis in 5-year groups, race, diagnosis year, estrogen receptor (ER), progesterone receptor, and AJCC stage).

RESULTS
The CBCP-WR cohort had better survival than the SEER population. At the whole cohort level, the mean hazard ratios (HRs) from 1,000 matched comparisons for OS and DSS were 0.774 and 0.708, with mean log-rank P-values of 0.124 and 0.125. The numbers of 175 and 141 comparisons showing a log-rank P-value <0.05 out of the 1,000 tests were significantly higher than what would be expected from a random distribution of these P-values (P<0.00001, exact binomial test). By stratifying the cohorts we identified that this survival disparity was mainly contributed by patients with a diagnosis age ≥50 years (for DSS, mean HR=0.550, mean P=0.049, and 642 of 1,000 tests showed a P<0.05; for OS, mean HR=0.713, mean P=0.081, and 377 of 1,000 tests showed a P<0.05), but not by patients with a diagnosis age <50 years. The absolute differences in 5-year DSS rates were 4.4% (94.6% in CBCP-WR vs. 90.2% in SEER; mean P=0.010) for all matched patients and 4.8% (95.2% vs. 90.4%; mean P=0.015) for patients diagnosed at an age ≥50 years. Again there was no significant difference for patients diagnosed at an age <50 years. When stratified by race, ER, stage or grade, most of the patient subpopulations showed favorable 5-year OS and DSS rates in the CBCP-WR cohorts.

CONCLUSION
Overall, these results suggested that breast cancer patients, especially older patients seen in the CBCP-WR, carried more favorable outcomes than those from the general population. The findings warrant further analyses of the contributing factors, such as health care access, treatments, population characteristics, additional pathologic characteristics, and socioeconomic statuses, to this outcome disparity. The views expressed in this article are those of the author and do not reflect the official policy of the Department of Defense, or U.S. Government.
Title: Breast cancer subtype and survival in a population-based cohort of patients from California

Christina A Clark¹, Scarlett Lin Gomez¹, Li Tao¹, Lisa Moy¹, Juan Yang¹, Lisa Wang², Mary S Beattie², Jennifer Eng-Wong², Melissa Brammer² and Laura Chu². ¹Cancer Prevention Institute of California, Fremont, CA and ²Genentech, San Francisco, CA.

Body: Background: Breast Cancer (BC) is a heterogeneous disease comprising distinct subtypes defined at present by tumor molecular markers. Over the past decades, more effective treatments targeted to specific markers have been developed and tested in clinical trials. While clinical trials have demonstrated the impact of these targeted treatments on recurrence and mortality rates, little is known about survival patterns associated with specific subtypes in the general population. We examined overall survival outcomes according to tumor subtype and stage in a diverse, population-based cohort of BC patients (pts) in California.

Methods: Through the California (CA) Cancer Registry, we identified all female CA residents diagnosed with primary invasive BC between 1/1/2005 and 12/31/2011. We classified these cancers as early breast cancer (EBC, stages I-III) vs. de novo metastatic (MBC, stage IV). We further grouped these cancers into 4 subtypes based on HER2 and hormone receptor (HR) status. For a subset of women with available survival data, we calculated the proportion surviving at 3 years (yrs), and median overall survival using the Kaplan-Meier method.

Results: 118,817 EBC pts (61.4% HR+/HER2-, 9.9% HR+/HER2+, 13.3% unclassified, 4.9% HR-/HER2+, and 10.6% triple-negative (TN)) and 6,268 MBC pts (43.7% HR+/HER2-, 13.0% HR+/HER2+, 23.3% unclassified, 8.9% HR-/HER2+, and 10.7% TN) were identified. Table 1 presents survival time in EBC vs. MBC, and for each of the receptor subtypes of BC. For EBC, 3-yr survival rate was highest (95.1%) for HR+/HER2- pts and shortest (84.3%) for TN. For the HER2- and HER2+ overall groups (regardless of HR status), 3-yr survival was similar (93.3% vs. 92.5%, respectively). The longest survival for de novo MBC was observed for the HR+/HER2+ subtype (median OS: 45.3 months (mos)), compared to 38.7 mos for the HR+/HER2- subtype, 23.1 mos for the HR-/HER2+ subtype and 12.7 mos for the TN subtype. For the overall HER2+ and HER2- subtypes, HER2+ MBC had slightly better survival (3-yr rate: 47.6% and median OS: 33.6 mos) than HER2- MBC pts (3-yr rate: 44.8% and median OS: 30.9 mos).

Conclusions: This study demonstrates the relevance of subtype on the OS of BC pts in a large population of CA women. Although HER2+ status is a negative prognostic factor, survival was similar between HER2+ and HER2- pts, likely due to available treatments targeting HER2. TNBC pts had the shortest survival, especially when they presented with metastatic disease. There remains an urgent unmet need for more effective treatments for TNBC.

Table 1. Survival by stage (EBC vs de novo MBC) and BC subtype

<table>
<thead>
<tr>
<th>Subtype</th>
<th>EBC</th>
<th>de novo MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3-yr Survival (95% CI)</td>
<td></td>
</tr>
<tr>
<td>HR+/HER2-</td>
<td>38118</td>
<td>95.1 (94.8-95.3)</td>
</tr>
<tr>
<td>HR+/HER2+</td>
<td>6920</td>
<td>94.6 (94.1-95.2)</td>
</tr>
<tr>
<td>Unclassified</td>
<td>10813</td>
<td>91.2 (90.6-91.7)</td>
</tr>
<tr>
<td>HR-/HER2+</td>
<td>3589</td>
<td>88.4 (87.3-89.4)</td>
</tr>
<tr>
<td>TN</td>
<td>7083</td>
<td>84.3 (83.4-85.1)</td>
</tr>
<tr>
<td>HER2-neg*</td>
<td>45550</td>
<td>93.3 (93.1-93.6)</td>
</tr>
<tr>
<td>HER2-pos*</td>
<td>10635</td>
<td>92.5 (92.0-93.0)</td>
</tr>
</tbody>
</table>

* Pts with available HER2 data, but missing HR data were included.
Title: Predictors of neoadjuvant chemotherapy use in women with breast cancer: A review of 169,329 patients from the American College of Surgeons' National Cancer Database

Lynn J Howie¹,², Rachel Greenup¹, Kevin Houck¹, Julie A Sosa¹,², E Shelley Hwang¹ and Jeffrey M Peppercorn¹. ¹Duke Cancer Institute, Durham, NC and ²Duke Clinical Research Institute, Durham, NC.

Body: Background: Randomized controlled trials have demonstrated that neoadjuvant chemotherapy (NAC) offers equivalent long-term outcomes when compared to adjuvant chemotherapy while improving rates of breast conservation therapy (BCT). We sought to evaluate the national use of NAC and the patient, tumor, and provider characteristics associated with its use among women with stage I-III breast cancer. We hypothesize that younger women with larger tumors and HER-2+ or triple negative disease are more likely to receive NAC than their older counterparts with ER+ disease, and that use of NAC varies by region and practice setting.

Methods: Using the American College of Surgeons National Cancer Database, which captures data on approximately 70% of new cancer diagnoses in the U.S., we identified women with diagnosed with stage I-III invasive cancer between 2008-2013. Women treated with both surgery and chemotherapy were included in the study. Demographic and clinical factors including race, ethnicity, income, insurance type, region of treatment facility, treatment facility type, tumor size, hormone receptor status, HER-2 status and Charlson Comorbidity score were analyzed to determine predictors associated with receipt of NAC. Utilization of preoperative chemotherapy and rates of breast conserving therapy (BCT) were evaluated as outcomes.

Results: 169,329 women with stage I-III breast cancer underwent treatment with chemotherapy and surgery during the study period. Of these, 81.0% were White, 14.4% were Black, 4.6% were classified as Other Race. 28.7% were 18-49, 46.8% were 50-64 and 24.5% were >65 years. 64.4% had private insurance while 35.6% had public insurance (Medicaid, Medicare and VA), and 71.4% were treated at Community-Based Clinics while 28.6% were treated at Academic Medical Centers.

Patients with larger tumors (p<0.0001) and triple negative disease were significantly more likely to be treated with NAC than those with ER+ or HER2+ disease (p<0.0001). Among women who received NAC, the median age was 54 as compared to 57 in those receiving adjuvant chemotherapy (p<0.0001). Treatment facility type impacted rates of NAC use, with academic centers being more likely than community-based practices to give chemotherapy preoperatively (12.1% vs. 9.8%, p<0.0001) and urban vs. rural settings (10.4% vs 8.2%, p<0.0001). Rates of NAC utilization differed regionally with the highest rate being 14.0% and the lowest rate 7.9% (p<0.0001).

The overall % of BCT following NAC was 36.1% compared to 59.0% for those receiving adjuvant chemotherapy. The proportion of BCT following NAC differed significantly by subtype, 54.6% for ER+, 54.5% for Her2 +, and 59.7% for triple negative breast cancer (TNBC) (p <0.0001).

Conclusions: In the treatment of stage I-III breast cancer across the US, variations in the utilization of neoadjuvant chemotherapy exist across the country suggesting clinical uncertainty about its use. Further research about the use of NAC therapy and the relationship to clinical outcomes can identify patient subsets who might obtain greatest clinical benefit from preoperative systemic therapy.
Title: Patient characteristics, clinical and economic outcomes of women with first-line therapy for HR+/HER2- metastatic breast cancer in a large US managed care health plan: Chemotherapy first vs. no chemotherapy first

Tanya Burton¹, Stacey DaCosta Byfield¹, Ying Fan¹, Yiyu Fang¹, Feng Cao¹, Gregory L Smith², Giovanni Zanotti², Timothy J Bell², Julia J Perkins², Ruslan Horblyuk² and April Teitlebaum³. ¹Optum, Eden Prairie, MN; ²Pfizer, New York, NY and ³Private Practice, San Diego, CA.

Body: BACKGROUND: NCCN guidelines clearly identify when chemotherapy may be an appropriate therapeutic approach for metastatic breast cancer (mBC). This study compared patient characteristics, mortality rates, and health care costs by initial chemotherapy (CT) use among women with HR+/HER2- mBC.

METHODS: A retrospective cohort study design was used to analyze administrative claims data linked to clinical information for commercial health plan enrollees with evidence of mBC between 1/2008 and 4/2013. Clinical status at diagnosis was obtained from physician reports, including date of diagnosis, hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status. Women with known HR+/HER2- subtype and diagnosed initially with Stage IV or Stages I-III with evidence of progression later to metastatic disease were evaluated for at least 6 months after their Stage IV diagnosis or first metastatic claim, or until death if sooner. Clinical characteristics were compared descriptively between women who initiated therapy with/without chemotherapy (CT ¹st vs. no CT ¹st, respectively) using t-test for continuous and chi-square test for categorical variables. Mortality was compared using the incidence rate ratio (IRR) and 95% confidence interval (CI) from a negative binomial distribution. Total average per-member-per-month (PMPM) health care costs were compared using a generalized linear model. Both the mortality rates and costs were adjusted for age, geographic region, stage at diagnosis, initial metastatic site(s), and initial use of CT.

RESULTS: Of 349 women with HR+/HER2- mBC, 204 (58%) had CT ¹st and 145 (42%) had no CT ¹st. Median follow-up was 17 months, and median length of the first-line of therapy (¹st LOT) was 4 months (including censored LOTs). The mean age of women with CT ¹st was slightly lower than those without CT ¹st (52 vs. 55 years, p<0.01), although the proportion ≥ 50 years old did not differ between cohorts (35% vs. 28%, p=0.14). Cohorts did not differ by geographic region or initial metastatic site(s) to brain, liver, or lung. Compared to women without CT ¹st, a lower proportion of women with CT ¹st were diagnosed de novo Stage IV (34% vs. 48%, p=0.01). Among women with CT ¹st, 24 (12%) also received hormonal therapy (HT) during their ¹st LOT. All women with no CT ¹st (100%) initiated treatment with HT, of which the most common were 52 (36%) with tamoxifen, 41 (28%) with anastrozole, and 39 (27%) with letrozole. After adjustment for baseline characteristics, no cohort differences were found in mortality (IRR: 0.93, 95% CI: 0.50-1.72), however adjusted total average PMPM costs were significantly higher in women with CT ¹st than those without ($11,666 vs. $6,639, p<0.001).

CONCLUSION: In this study of commercially insured women with HR+/HER2- mBC, use of CT ¹st (>50%) was higher than expected. While there were minor cohort differences in patient characteristics, CT ¹st does not appear to be associated with a survival benefit, but was associated with significantly higher costs when compared to no CT ¹st. Additional research is needed to determine subset(s) of mBC women with CT ¹st likely to benefit from initial HT.
Title: Clinical characteristics and treatment utilization by tumor subtype among metastatic breast cancer patients in a large US managed care health plan

Tanya Burton¹, Stacey DaCosta Byfield¹, Ying Fan¹, Yiyu Fang¹, Feng Cao¹, Gregory L Smith², Giovanni Zanotti², Timothy J Bell², Julia J Perkins², Ruslan Horblyuk² and April Teitlebaum³. ¹Optum, Eden Prairie, MN; ²Pfizer, New York, NY and ³Private Practice, San Diego, CA.

Body: BACKGROUND: Breast cancer is the second most common cause of cancer death among US women. Molecular profiling of breast cancer tumors is important for assessing prognosis and optimizing treatment. This study compared clinical characteristics and treatment utilization by tumor subtype among women with metastatic breast cancer (mBC).

METHODS: A retrospective cohort study design was used to analyze administrative claims data linked to clinical information for commercial health plan enrollees with evidence of mBC between 1/2008 and 4/2013. Clinical status at diagnosis was obtained from physician reports, including date of diagnosis, hormone receptor (HR) and human epidermal growth factor receptor 2 (HER2) status. Women with known HR/HER2 subtypes and diagnosed initially with Stage IV or Stages I-III with evidence of progression later to metastatic disease were evaluated for at least 6 months after their Stage IV diagnosis or first metastatic claim, or until death if sooner. Lines of therapy (LOTs) were identified based on the timing of claims for NCCN-recommended chemotherapy (CT), hormonal therapy (HT), and biologics (BIO). Clinical characteristics and treatments were compared descriptively across the HR/HER2 subtypes using t-test for continuous and chi-square test for categorical variables.

RESULTS: Table 1 presents study results by tumor subtype. There were 657 women identified (72% HR+ and 28% HR-). Median age was 53 years, and median follow-up was 16 months. Overall, 93% initiated therapy; of which, 48% started a 2nd LOT, 4% died, 28% discontinued or had a ≥90-day gap in therapy, and 20% had their 1st LOT censored due to disenrollment or end of study (EOS) period. More than half received CT during the 1st LOT, regardless of tumor subtype. Less than half of HR+ women initiated HT, and among HER2+ patients, most initiated a BIO agent.

<table>
<thead>
<tr>
<th></th>
<th>Total (N=657)</th>
<th>HR+/HER2- (N=365)</th>
<th>HR-/HER2- (N=118)</th>
<th>HR+/HER2+ (N=111)</th>
<th>HR-/HER2+ (N=63)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diagnosed at Stage IV*</td>
<td>265(40)</td>
<td>145(40)</td>
<td>37(31)</td>
<td>50(45)</td>
<td>33(52)</td>
</tr>
<tr>
<td>Initial site of metastasis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Bone</td>
<td>283(43)</td>
<td>167(46)</td>
<td>49(42)</td>
<td>46(41)</td>
<td>21(33)</td>
</tr>
<tr>
<td>- Brain*</td>
<td>105(16)</td>
<td>45(12)</td>
<td>32(27)</td>
<td>17(15)</td>
<td>11(17)</td>
</tr>
<tr>
<td>- Liver*</td>
<td>171(26)</td>
<td>76(21)</td>
<td>40(34)</td>
<td>35(32)</td>
<td>20(32)</td>
</tr>
<tr>
<td>- Lung*</td>
<td>127(19)</td>
<td>64(18)</td>
<td>36(31)</td>
<td>11(10)</td>
<td>16(25)</td>
</tr>
<tr>
<td>- Other (incl. distant lymph nodes)</td>
<td>494(75)</td>
<td>277(76)</td>
<td>97(82)</td>
<td>76(68)</td>
<td>44(70)</td>
</tr>
<tr>
<td>Initial NCCN-recommended therapy (1st LOT)</td>
<td>614(93)</td>
<td>349(96)</td>
<td>98(83)</td>
<td>109(98)</td>
<td>58(92)</td>
</tr>
<tr>
<td>- CT*</td>
<td>422(69)</td>
<td>204(58)</td>
<td>92(94)</td>
<td>78(72)</td>
<td>48(83)</td>
</tr>
<tr>
<td>- BIO*</td>
<td>124(20)</td>
<td>1(&lt;1)</td>
<td>4(4)</td>
<td>68(62)</td>
<td>51(88)</td>
</tr>
<tr>
<td>- HT*</td>
<td>210(34)</td>
<td>169(48)</td>
<td>9(9)</td>
<td>31(28)</td>
<td>1(2)</td>
</tr>
<tr>
<td>Reason for 1st LOT end</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Initiated a 2nd LOT*</td>
<td>293(48)</td>
<td>159(46)</td>
<td>45(46)</td>
<td>71(65)</td>
<td>18(31)</td>
</tr>
<tr>
<td>- Died</td>
<td>26(4)</td>
<td>10(3)</td>
<td>8(8)</td>
<td>5(5)</td>
<td>3(5)</td>
</tr>
<tr>
<td>- Discontinuation/gap*</td>
<td>174(28)</td>
<td>105(30)</td>
<td>36(37)</td>
<td>15(14)</td>
<td>18(31)</td>
</tr>
</tbody>
</table>
CONCLUSION: In this study of commercially insured mBC women, initial therapy choices were generally consistent with NCCN guidelines for three of the four subtypes, while the largest subtype of HR+/HER2- women had a higher than expected utilization of CT. Future analyses of study data will investigate appropriateness of these initial treatments and their impact on clinical and economic outcomes.
2 year survival analysis of triple negative breast cancer from SEER data

Moira Rushton¹, Tinghua Zhang² and Xinni Song³. ¹University of Ottawa; ²Ottawa Hospital Research Institute, Ottawa, ON, Canada and ³Ottawa Hospital Cancer Program, Ottawa, ON, Canada.

Body: Background
Triple negative breast cancer (TNBC) is a heterogeneous disease characterized by the lack of receptor expression (ER, PR and Her 2/neu negative). Amongst breast cancer types TNBC has a less favourable prognosis. There is a higher incidence of TNBC in African-American women than Caucasian women. What has not been clearly elucidated is whether survival outcomes are different among women with TNBC from different ethnic background.

Objective
The objective of our study was to use population data to determine if significant differences exist in overall survival (OS) of TNBC patients across various ethnicities, including but not limited to—white, black, Hispanic and Asian.

Methods
Retrospective cohort study of patients with TNBC from 1973-2011 Surveillance, Epidemiology, and End-Results (SEER) database to examine differences in OS across ethnicities. For each case data was collected on age, race, disease stage, treatment, insurance status, time to death and cause of death. Descriptive statistics and survival analysis was carried out on the data. Multivariate analysis was carried out to take into account age, stage, treatments received.

Results
12894 cases of TNBC across all ethnicities were reported in the SEER database. At two years follow-up, 720 patients (5.7%) had died of breast cancer. 9696 (78.77%) had early stage (stage 0 – II) disease, 1885 (15.31%) had locally advanced/stage III disease while 728 (5.9%) had stage IV disease. 12071 (95.53%) were insured, 11533 (91.27%) had surgery, and 5454 (43.17%) had radiation therapy. 7746 (61.53%) patients were white, 2429 (19.29%) black, 1548 (12.30%) were Hispanic and 490 (3.89%) were Asian. In multivariate analysis, increasing age, stage III or IV disease, lack of insurance, surgery or radiation all had significant hazard ratios. There was no significant survival difference found between any ethnicity compared with white patients when controlled for age, stage, insurance, surgery and radiation.

Discussion
After two-year follow-up of large cohort of TNBC patients no significant difference could be found between any ethnicity and the white population with this disease. While there is a large population of black and Hispanic patients in this study there are small numbers of other races. The small relatively small event rate could be masking potential differences given the majority if patients were early stage and are still alive. Longer follow-up is needed before conclusions can be made about differences between ethnic groups. if certain populations do worse will inform the medical oncology community of an area to focus greater research into how to optimize therapies for that patient group.
Characteristics of de novo metastatic breast cancer in California, 2005-2011

Christina A Clarke1, Laura Chu2, Li Tao1, Lisa Wang1, Lisa Moy1, Melissa Brammer2, Chunyan Song2, Marjorie Green2 and Scarlett Lin Gomez1. 1Cancer Prevention Institute of California, Fremont, CA and 2Genentech, San Francisco, CA.

BACKGROUND: Breast cancer (BC) that is metastatic at initial diagnosis (i.e. de novo metastatic or stage IV) has not been well described, especially in the general population.

OBJECTIVE: To describe demographics, tumor characteristics and survival in a population-based cohort of patients with de novo metastatic BC (MBC).

METHODS: We studied all 6268 de novo MBC cases diagnosed in California women between 1/1/2005 and 12/31/2011, as reported to the California Cancer Registry. Molecular subtypes were classified according to HER2 and hormone receptor (HR, based on estrogen and progesterone receptor) status. Median overall survival (OS) was calculated by Kaplan-Meier methods. Cox proportional hazards regression was used to assess independent predictors of OS.

RESULTS: 5% of all newly diagnosed BC were metastatic, representing 6% of all newly diagnosed HR+/HER2+, 8% of all HR-/HER2+, 4% of all HR+/HER2- and 6% of all triple negative BC (TNBC). Compared to patients with early BC, MBC patients were of similar age (mean age at diagnosis, (interquartile range): 61,(51-71) vs. 60, (50-70) years)). They were slightly more likely to be black (10% vs. 6%) or Hispanic (19% vs. 17%) but substantially more likely to be unmarried (56% vs. 40%), to live in neighborhoods of the lowest socioeconomic quintiles (39% vs. 29%), and to have public (e.g., Medicaid) or no insurance (39% vs. 21%). Most MBC patients presented with large tumors; however, 13% of patients had tumor sizes 2 cm or less, compared with 60% of patients with early BC (of TNBC: 15% MBC vs. 44% early BC were ≤2 cm). A minority of patients with de novo MBC received breast surgery (39%), with 24% receiving full or partial mastectomy, 9% lumpectomy, 3% bilateral mastectomy and 3% other/unknown surgery . 64% of de novo MBC patients received chemotherapy and 33% received radiation.

Median survival after MBC diagnosis was 27 months (mos), but varied substantially by patient characteristics including age (<40: 40 mos, 85+: 8 mos), race/ethnicity (Asian: 34 mos, black: 16 mos), and neighborhood socioeconomic quintile (lowest: 20 mos, highest 34 mos) and molecular subtype (HR+/HER2+: 45 mos, TNBC: 12 mos). In a multivariate Cox model including all available variables, TNBC was the most important predictor of death (Hazard Ratio 2.8, 95% CI: 2.4-3.3 vs. HR+/HER2+). Other significant and important predictors included HR-/HER2+ subtype (Hazard Ratio 1.6, 95% CI: 1.3-18 vs. HR+/HER2+), being unmarried, living in low socioeconomic status neighborhoods, and high tumor grade status.

CONCLUSIONS: In this large diverse population, de novo MBC was more likely to be diagnosed for certain breast cancer subtypes, and among minority and underserved women (black or Hispanic race, low socioeconomic neighborhood, no or public health insurance) that may have contributed to the detection of their tumor only after it had metastasized. A high proportion of patients with MBC are not treated surgically, but most receive chemotherapy. Median survival remains poor, with worse survival strongly associated with tumor biology (triple negative and HR-/HER2+ molecular subtypes) and patient characteristics indicative of low socioeconomic status.
Title: Patterns of chemotherapy (CT) use by molecularly-defined subtype among a population-based cohort of breast cancer (BC) patients (pts) from California

Christina A Clarke¹, Laura Chu², Lisa Wang², Chunyan Song², Marjorie Green², Lisa Moy¹, Juan Yang¹, Li Tao¹ and Scarlett Lin Gomez¹. ¹Cancer Prevention Institute of California, Fremont, CA and ²Genentech, San Francisco, CA.

Body: Background: Guidelines describe recommended BC treatments (tx) according to tumor subtype; however, population-based data regarding tx utilization are limited.

Objective: To describe tx utilization by tumor HER2, hormone receptor (HR) status, and stage among representative pts from California.

Methods: We examined patterns of CT (including biologic tx) reported as received for all 131,450 female BC pts diagnosed with primary invasive BC in California between 1/1/2005 and 12/31/2011 in the California Cancer Registry, including n=19,918 HER2+ [65% HR+, 33% HR-]; n=91,919 HER2- [84% HR+, 15% HR-]; and n=19,613 HER2 unknown. Neoadjuvant & adjuvant CT data was only available for a subset of 35,040 pts diagnosed 2010-2011 (4,609 HER+; 27, 604 HER2-). Descriptive statistics were used for comparisons.

Results: CT was reported as highest among pts with metastatic breast cancer (MBC) and HER2+ status (Table 1). The proportion of pts with early BC (EBC) receiving CT increased from 58% in 2005 to 69% in 2010, but was generally steady for MBC pts (74.2% in 2005 to 79.6% in 2011). 20.7% of HER2+ pts received neoadjuvant CT vs 8.6% for HER2- pts. Similarly, adjuvant CT was more common in HER2+ (55.5%) than HER2- (28.5%) pts. As expected the proportion of pts receiving CT was lower in HR+ vs HR- pts for EBC and MBC [eg. all stages HER2+ (62% vs 71%); HER2- (33% vs 68%)].

Conclusion: In this population-based cohort, ~20-40% of MBC pts may not have received any CT or biologics. CT was more commonly received by HER2+ patients compared to HER2- patients for both EBC and MBC.

Table 1. Patterns of chemotherapy (including biologics) use among California female BC pts by stage and HER2 status, 2005-2011

<table>
<thead>
<tr>
<th></th>
<th>EBC</th>
<th>MBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HER2+</td>
<td>HER2-</td>
</tr>
<tr>
<td>Any chemotherapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>5898 (33.4%)</td>
<td>52467 (61.0%)</td>
</tr>
<tr>
<td>Yes</td>
<td>11463 (64.8%)</td>
<td>32403 (37.7%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>322 (1.8%)</td>
<td>1128 (1.3%)</td>
</tr>
<tr>
<td>Any neoadjuvant therapy (2010-2011 data only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>3546 (76.9%)</td>
<td>24794 (89.8%)</td>
</tr>
<tr>
<td>Yes</td>
<td>953 (20.7%)</td>
<td>2381 (8.6%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>110 (2.4%)</td>
<td>429 (1.6%)</td>
</tr>
<tr>
<td>Any adjuvant therapy (2010-2011 data only)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>1943 (42.2%)</td>
<td>19296 (69.9%)</td>
</tr>
<tr>
<td>Yes</td>
<td>2556 (55.5%)</td>
<td>7879 (28.5%)</td>
</tr>
<tr>
<td>Unknown</td>
<td>110 (2.4%)</td>
<td>429 (1.6%)</td>
</tr>
</tbody>
</table>

* not applicable

Joseph Ragaz¹, Hubert Wong¹, Hong Qian¹, Joel Fox², Kenneth Wilson² and Andrew Coldman². ¹School of Population and Public Health, University of British Columbia, Vancouver, BC, Canada and ²British Columbia Cancer Agency, University of BC, Vancouver & Victoria, BC, Canada.

Body: INTRODUCTION:
The net benefit of ScreenMam has been questioned in recent literature. We report here the 1975-2010 population-based rates of DCIS, BrINV, and of BrMOR in two regions of Canada, British Columbia [BC] vs the Atlantic Provinces of Canada [Atl.P], with unequal adherence (high in BC, low in Atl.P) to ScreenMam and therapy [Th⁺] guidelines for BrCa [Ref. 1].

METHODS: Annual age-specific rates of DCIS, BrINV, and BrMOR were obtained for 17 age groups (ages 0-4 to 85+) in BC and Atl.P, and averaged over each 5-year periods [1975-1979 up to 2005-2009]. Four birth cohorts were defined within each region: women who in the 1975-79 were aged 30-34 [COHORT 1]; 35-39 [COHORT 2]; 40-44 [COHORT 3] and 45-49 [COHORT 4]. The rates [cases /100,000 person-years] of DCIS, BrINV and BrMOR in 5 year intervals between 1975 and 2009 were followed within each birth cohort and compared between BC and Atl.P in periods 1975-1984 and 2000-2009 [see Table]. Also, age-standardized rates [ASRs] for BrINV incidence and BrMOR were estimated for the entire population, and for ages 50-65 and 65+. The data were obtained from the Public Health Agency of Canada based on the Canadian Cancer Registry database at Statistics Canada.

RESULTS
Table 1. Cases / 100,000 person-years, years 1975-84 vs 2000-09. BrCa Incidence [DCIS, BrINV] and mortality in BC vs Atl.P. within four birth-cohorts.

<table>
<thead>
<tr>
<th>COHORTS*: 1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>IN-SITU</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1975-84</td>
<td>2 vs 1 [+1]**</td>
<td>4 vs 4 [0]</td>
<td>8 vs 5 [3]</td>
</tr>
<tr>
<td>INVASIVE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MORTALITY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1975-84</td>
<td>7 vs 9 [-2]</td>
<td>13 vs 14 [-1]</td>
<td>28 vs 29 [-1]</td>
</tr>
</tbody>
</table>

**[x] = Difference Cases / 100,000 person-years, BC vs Atl.P: + or –

In BC, the ASR for BrINV incidence increased by 20% between 1985 and 1989, and decreased by 20% between 1990-2007. In the Atl.P, the BrINV rates increased by 40% between 1980 and 1998 [i.e.increase to higher extent / over longer time period] and declined by 20% between 1999-2007. Compared to 1975, corresponding ASRs for mortality show a 47% decline in BC vs 30% for Atl.P [for ages 50-65: 52% vs 46%; for ages 65+: 37% vs 5%, respectively.].

CONCLUSIONS:
1. DCIS: Higher rates in BC than in Atl.P in the older cohorts [3 and 4] during 1975-84 likely reflect earlier clinical use of ScreenMam in BC. Substantially higher rates maintained during 2000-09 likely reflect persistent greater usage in BC.
2. BrINV. In every birth cohort, substantially higher rates in BC than in Atl.P during 1975-1984 but substantially lower rates in 2000-2009 are likely due to detection & Th earlier in life in BC.

3. BrMOR. Rates were similar between the two regions within each cohort during 1975-84 but substantially lower in BC for older cohorts [3 and 4] during 2000-09, likely due to earlier detection and Th in BC, concordant with more substantial ASR reduction of BrMOR observed in BC.

SUMMARY. In view of more ScreenMam in BC, these data suggest that the higher DCIS, but lower rates of BrINV, and more consistent mortality decline in BC than in Atl.P, could all be related - a likely reflection of earlier detection and earlier therapy. The associations of these interactions will be discussed.

*Th: surgery, radiation, Tamoxifen for DCIS; the same + chemotherapy for early BrINV.

Title: Association between adjuvant chemotherapy and risk of acute kidney injury in elderly women diagnosed with early stage breast cancer

Shuling Li\(^1\), Jiannong Liu\(^1\), Beth A Virnig\(^2\) and Allan J Collins\(^{1,2}\). \(^1\)Chronic Disease Research Group, Minneapolis, MN and \(^2\)University of Minnesota, Minneapolis, MN.

Body: Background: Acute kidney injury (AKI) is a serious complication of cancer and its treatment, causing delay or interruptions in cancer therapy and increased risk of adverse outcomes including premature death. Little is known from large population-based cohort studies about the association between chemotherapy (chemo) and risk of AKI in elderly cancer patients (pts).

Methods: This retrospective cohort study used the linked Surveillance, Epidemiology, and End Results (SEER)-Medicare data. Women diagnosed with stages I-III breast cancer (BC) at ages 66-89 years between 1992-2007 were included. We performed 1-1 sequential matching on time-dependent propensity score on the day of adjuvant chemo initiation within 6 months (mos) after the first surgery. Follow-up (F/U) began on the matching date and ended at AKI occurrence, chronic kidney disease diagnosis, death, change in enrollment status, or 6 mos after the matching date. For pts in the matched untreated cohort, F/U time was also censored at chemo initiation. Chemo was identified in claims through billing codes indicating drugs or administration. Regimens of interest included anthracyclines (A), CMF, taxanes (T, no anthracyclines), and others. AKI was identified in hospital claims. The cumulative incidence of AKI was assessed using the Kaplan-Meier method. The association between adjuvant chemo and risk of AKI was evaluated using a Cox proportional hazards model. The analyses were repeated by regimen type.

Results: The matched study cohorts included 28,048 pts. The mean (standard deviation) F/U time was 6.0 (0.8) mos for the chemo cohort and 4.3 (2.5) mos for the matched no-chemo cohort. The cumulative incidence of AKI at mos 1, 3, and 6 was 0.24%, 0.50%, and 0.80% for pts receiving chemo, compared with 0.05%, 0.17%, and 0.30% for no-chemo pts (\(P\)<0.001). Adjuvant chemo was associated with a 2.7-fold increased risk of AKI (HR 2.7, 95% CI 1.8-4.1; \(P\)<0.001), despite a very low overall incidence rate (16 and 6 per 1000 person-years in chemo and no-chemo pts, respectively). Further examination of distribution of other diseases coded on hospital claims in AKI pts showed that septicemia occurred in 40% of chemo-treated pts with AKI and in only 17% of untreated pts with AKI. Of chemo-treated pts, 53%, 31%, 7%, and 8% received an A-based, a CMF, a T-based, and other regimens, respectively. Each regimen was significantly associated with increased risk of AKI, with the strongest association for a T-based regimen (HR 4.2, 95% CI 2.2-7.8), the weakest for a CMF regimen (HR 2.2, 95% CI, 1.3-3.8), and intermediate associations for an A-based regimen (HR 2.5, 95% CI 1.6-4.1) and others (HR 3.0, 95% CI 1.5-6.2), but the effect of these regimens did not significantly differ from each other.

Conclusion: Adjuvant chemo is associated with increased risk of AKI in elderly women diagnosed with early stage BC. This association may be partially explained by septicemia caused by infection/neutropenia due to use of myelosuppressive chemotherapeutic agents, highlighting the importance of preventing serious complications of chemo in preventing AKI.
Title: Association between adjuvant chemotherapy and risk of chronic kidney disease in elderly women diagnosed with early stage breast cancer

Shuling Li1, Jiannong Liu1, Beth A Virnig2 and Allan J Collins1,2. 1Chronic Disease Research Group, Minneapolis, MN and 2University of Minnesota, Minneapolis, MN.

Body: Background: Chronic kidney disease (CKD) and cancer are major public health problems in the elderly population. With the development of cancer screening and efficacious treatments including chemotherapy (chemo), the number of cancer survivors has been increasing. In elderly cancer patients (pts), little is known about CKD as a late effect of chemo. This study examined the association between adjuvant chemo and risk of CKD in elderly women diagnosed with early stage breast cancer (BC).

Methods: This retrospective cohort study used the linked Surveillance, Epidemiology, and End Results (SEER)-Medicare data. Women diagnosed with stages I-III breast cancer (BC) at ages 66-89 years between 1992-2007 were included. We preformed 1-1 sequential matching on time-dependent propensity score on the day of adjuvant chemo initiation within 6 months after the first surgery. Follow-up (F/U) began on the matching date and ended at CKD diagnosis, death, change in enrollment status, or December 31, 2009. For pts in the matched untreated cohort, F/U time was also censored at chemo initiation. Chemo was identified in claims through billing codes indicating drugs or administration. Regimens of interest included anthracyclines (A), CMF, taxanes (T, no anthracyclines), and others. CKD was identified through diagnosis codes in Medicare claims. The cumulative incidence of CKD was assessed using the Kaplan-Meier method. The association between adjuvant chemo and risk of CKD was evaluated using a Cox proportional hazards model. The analyses were repeated by regimen type.

Results: The matched study cohorts included 28,048 pts. The mean (standard deviation) F/U time was 5.1 (3.4) years for the chemo cohort and 3.3 (3.6) years for the matched no-chemo cohort. CKD rate (standard error) was 29.0 (0.6) and 29.3 (0.8) per 1000 patient-years in chemo and no-chemo pts, respectively. Overall, there was no significant difference in the cumulative incidence of CKD between the two cohorts (chemo vs. no-chemo, 47.2% vs. 49%; \( P=0.91 \)). Adjuvant chemo was not associated with increased risk of developing CKD (HR 1.00, 95% CI 0.93-1.07). Of chemo-treated pts, 53%, 31%, 7%, and 8% received an A-based, a CMF, a T-based, and other regimens, respectively. Though the association between adjuvant chemo and risk of CKD varied across regimen types, these associations were not statistically significant (Table).

<table>
<thead>
<tr>
<th>Regimen Type</th>
<th>Total, n</th>
<th>Mean (SD) F/U time, years</th>
<th>Rate of CKD (1000 pt-yrs)</th>
<th>Adjusted HR (95% CI)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No chemo</td>
<td>14024</td>
<td>3.3 (3.6)</td>
<td>29.3</td>
<td>Reference</td>
</tr>
<tr>
<td>A-based</td>
<td>7465</td>
<td>4.8 (3.0)</td>
<td>27.7</td>
<td>0.96 (0.88-1.04)</td>
</tr>
<tr>
<td>CMF</td>
<td>4389</td>
<td>6.0 (3.9)</td>
<td>29.1</td>
<td>1.04 (0.95-1.14)</td>
</tr>
<tr>
<td>T-based</td>
<td>1030</td>
<td>2.8 (1.8)</td>
<td>39.4</td>
<td>0.91 (0.74-1.12)</td>
</tr>
<tr>
<td>Other</td>
<td>1140</td>
<td>5.1 (4.0)</td>
<td>31.2</td>
<td>1.04 (0.89-1.22)</td>
</tr>
</tbody>
</table>

*Adjusted for patient baseline characteristics and trastuzumab use

Conclusion: Adjuvant chemo in elderly women with BC may not impose additional risk for CKD. This finding suggests that patients’ underlying risk factors for CKD such as diabetes, hypertension, etc. should be considered in the discussions between clinicians and pts regarding potential risk of CKD development after chemo treatment and choice of chemo treatment.
Title: Case series of 21 patients with metastastic lobular breast carcinoma to the gastrointestinal tract

Noah J Switzer¹, Andrew Lim¹, Lillian Du¹, Katia Tonkin² and Dan Schiller¹. ¹University of Alberta, Edmonton, AB, Canada and ²University of Alberta, Edmonton, AB, Canada.

Body: Background: Invasive lobular carcinoma comprises 5-15% of all breast cancer cases, with its incidence gradually increasing. While it is uncommon for breast cancer to metastasize to the gastrointestinal tract, lobular carcinoma has a disproportionately higher incidence of spread to the gastrointestinal (GI) system in comparison to other types of breast cancer. To date, most studies of gastrointestinal metastatic lobular carcinoma have been case reports and small case series.

Aim: This study is a review of all cases of lobular breast cancer with gastrointestinal metastases seen at a University affiliated Tertiary Cancer Institute over a five-year period, examining demographic, epidemiological, medical, and treatment factors that may have an association with the risk of GI metastases.

Methods: This is a retrospective chart review of all patients seen at the Cross Cancer Institute in Edmonton, Alberta, Canada between 2005-2010, with lobular breast cancer spread to the gastrointestinal tract. The outcomes of interest were: age at diagnosis, receptor status, site of primary breast cancer, stage at initial presentation, pathology, hormone receptor status, site of gastrointestinal metastasis, time from diagnosis of breast primary to gastrointestinal metastasis, time from diagnosis to death, time to gastrointestinal metastasis to death, and treatment regimen for both primary and metastatic disease.

Results: 343 consecutive cases of lobular breast cancer were reviewed, and 21(6%) were found to have GI metastases. The mean age at initial diagnosis of primary tumor was 63 years. The site of primary breast cancer was most commonly in the outer upper quadrant. Stage at presentation of the breast primary was: Stage 1A = 17%, Stage 1B/2A = 17%, Stage 2B = 22%, Stage 3A = 11%, Stage 3B = 6%, Stage 3C = 17%, and Stage 4 = 11%. Receptor status of the primary breast cancer was as follows: HER2+ = 5%, PR+ = 76%, ER+ = 90%.

The mean age at time of diagnosis of metastatic disease was 67 years. The main presenting symptoms of GI metastatic disease were:: small bowel obstruction (12.5%), incidental finding on endoscopy (12.5%), and incidental finding on imaging (12.5%). Sites of gastrointestinal spread included the stomach (52%), peritoneum (14%), duodenum (4%), jejunum (4%), transverse colon (4%), and pancreas (4%). Five-year survival from initial diagnosis of lobular breast cancer averaged 46%. Five-year survival from diagnosis of gastrointestinal metastasis was 29%.

Conclusions: Approximately 1 in 20 patients diagnosed with a primary breast cancer of lobular pathology will have metastatic spread to the GI tract, presenting approximately 4 years after their initial primary diagnosis. The most common presentation of metastatic disease is small bowel obstruction, with the most common site of spread being the stomach. There remains a paucity of data in the literature and our project is one of the first to further characterize these patients. Future research is needed in developing treatment regimens for these patients, as the 5-year survival is only approximately 1 in 4.
Title: Breast cancer in Hong Kong, Southern China: The population-based, ten-year analysis of epidemiological characteristics, stage-specific, cancer-specific, & disease-free survival in breast cancer patients: 1997–2006

Ava Kwong1,3,4, Oscar WK Mang2, Anthony HP Tam2, Fidelia Wong1, The Hong Kong Breast Cancer Research Group3, Stephen CK Law2 and Roger KC Ngan2. 1University of Hong Kong, Hong Kong; 2Hong Kong Cancer Registry, Hong Kong; 3Hospitals of the Hospital Authority, Hong Kong and 4Hong Kong Hereditary Breast Cancer Family Registry, Hong Kong.

Body: Background: Breast cancer is the most common cancer and second leading cause of cancer death among women, after lung cancer in Asia. The age-standardized incidence rates of breast cancer in Hong Kong is 61.0 per 100,000 women, after Singapore and Taiwan. With such increase, it would be important to better understand breast cancer to guide health care professionals and health policy makers to plan clinical management. However, to date such information is still under-reported, this study provide a comprehensive ten-year analyses of breast cancer in Hong Kong.

Methods: A retrospective study on population database over 10-year obtained from Hong Kong Cancer Registry was performed. A total of 20,290 female breast cancers medical records, diagnosed between January 1, 1997 and December 31, 2006, were reviewed. Descriptive statistics were employed to describe the epidemiological, clinical, and diagnostic data. The prognostic information for diagnostic and pathological data of relative survival (RS) was estimated using the maximum likelihood approach with program Strel in STATA; while the overall survival (OS), cancer-specific survival (CSS) & disease-free survival (DFS) were estimated by the Kaplan-Meier method with SPSS. Chi-squared test and Student's t-test were employed to compare variables in the two 5-year-periods of 1997-2001 and 2002-2006, with plotted RS curves for diagnostic and pathological data between these two 5-year-periods.

Results: 18,110 invasive breast cancer medical records in 1997-2006 were eligible for analysis, after 2,180 cases were excluding due to incomplete data. The ages at diagnosis ranged from 16 to 105; and median age was 51 years old. There was a drop from 14.1% in 1997-2001 to only 10.6% in 2002-2006 for those were diagnosed with breast cancer at age 39 years & younger. 26.2%, 55.2%, 13.0%, & 5.6% in 1997-2001, versus 30.1%, 46.4%, 16.9%, & 6.6% in 2002-2006 had tumor staging of stages I, II, III, & IV cancers at diagnosis, respectively. In ten-year period, the 5-year OS, RS, CSS, & DFS for the whole cohort were 80.6%, 85.6%, 87.1%, & 90.5%, respectively. The 5-year tumor stage-specific RS were 97.8%, 90.4%, 70.4%, & 21.4% for stages I, II, III, & IV, respectively. Between the two time periods, all the stage-specific RS improved by about 1%, 4%, 6% & 2% for stages I, II, III, & IV, respectively. There were 2,670 (14.7%) triple negative cases in 1997-2006, the ER-positive, PR-positive, & HER2-positive cancers increased from 66.1%, 52.6%, & 25.5% in 1997-2001 to 72.0%, 57.1%, & 29.4% in 2002-2006, respectively.

Discussion: Comprehensive analyses of breast cancer with population database from the Hong Kong Cancer Registry were performed to provide detailed information of a baseline study cohort in Southern China for comparative studies with other Asian regions.
Title: Breast cancer pathologic subtype and fetal microchimerism

David A Mahoney¹, Veronica L Winget¹, VK Gadi¹², Christopher I Li¹³, Peggy L Porter¹⁵ and Jean A McDougall¹⁴. ¹Fred Hutchinson Cancer Research Center, Seattle, WA; ²University of Washington, Seattle, WA; ³University of Washington, School of Public Health, Seattle, WA; ⁴University of Washington, Seattle, WA and ⁵University of Washington, Seattle, WA.

Body: Background: Stably persistent offspring-origin cells in a woman’s circulation and tissues decades after pregnancy, also known as fetal microchimerism, is deficient in women with biologically unselected breast cancer and suggests a protective role. Although the finding is in harmony with the well-known association of parity with protection against future breast cancer, it has recently emerged that certain tumor types such as triple negative breast cancer are positively associated with parity. Here we tested the hypothesis that fetal microchimerism is positively associated with triple negative breast cancer in young women. Methods: Buffy coat specimens were obtained from a subset (n=450) of pathologically confirmed low-risk luminal and high-risk triple negative breast cancer patients and control participants from a population-based cohort study of all women aged 20-44 diagnosed with invasive breast cancer in the three county Seattle-Puget Sound metropolitan area from 2004-2010. Using quantitative PCR, DNA extracts from these specimens were tested for the presence and concentration of Y chromosome sequence DYS14, a marker of male fetal microchimerism.

Results: At this interim analysis of 405 specimens, 29.3% (56/191) of the healthy controls tested positive. Using controls as a reference group, 37.8% (34/90; OR .68; 95% CI .39-1.2) of women who developed luminal breast cancer tested positive for the presence of DYS14, while 26.6% (33/124; OR 1.14; 95% CI .67-1.96) of women who developed triple negative breast cancer tested positive.

Discussion: A preliminary analysis does not suggest an association between fetal microchimerism and breast cancer pathologic subtype in young women. Additional analyses are pending and will be presented at the meeting. Young women may regulate fetal microchimerism differently and surprisingly our data may suggest that as fetal microchimerism emerges later in life in the circulation, so does the protection against breast cancer – a finding consistent with population based studies of parity as a risk factor for breast cancer.
Title: Gamma-ray induced mutagen sensitivity and overall survival in young women with breast cancer

Michael C Stauder¹, Simona F Shaitelman¹, Pamela K Allen¹, Abenaa M Brewster¹, Banu K Arun¹, Wendy A Woodward¹, Thomas A Buchholz¹ and Li-E Wang¹. ¹University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background:
Hypersensitivity to radiation has been shown to be a risk factor for the development of breast cancer. We aim to determine whether the same hypersensitivity predicts for adverse clinical outcomes in patients diagnosed with carcinoma of the breast.

Methods:
465 young, female, non-Hispanic white patients diagnosed with carcinoma of the breast at our institution from 1/1997 to 12/2005 were included in this study. All cases were histologically confirmed and all blood was drawn prior to any systemic or local therapy. Patient age, body mass index (BMI), menopause status, tumor laterality, AJCC stage, ER status, nuclear grade, and receipt of chemotherapy and radiation were extracted from patient medical records. A gamma-ray-induced mutagen sensitivity assay was performed using standard published methods to evaluate individual responses to radiation. The number of simple chromatid breaks per sample was counted from 50 well-spread metaphases. Each simple chromatid break was counted as a single break and each isochromatid break, exchange figure, or interstitial deletion as two breaks. The mean value of chromatid breaks per cell (b/c) was then calculated and recorded. Cox multivariable proportional hazards model was used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for the association between b/c and overall survival.

Results:
A total of 402 patients had a b/c value recorded and were included in the final analysis. The patient median age was 46 years (range 22-55). 341 patients (84.8%) had invasive cancer and 253 patients (69.9%) had ER+ disease. AJCC stage distribution was stage 0 (15.2%), stage 1 (41.5%), stage 2 (33.5%), stage 3 (9.5%) and stage 4 (0.3%). The median follow-up for all patients was 97.2 months (interquartile range, IQR 83.3-119.6 months). The median b/c was 0.5 (IQR 0.38-0.62). The 5 and 10-year survival for all patients was 92.6% and 87.5%. A statistically significant decrease in 5 and 10-year overall survival was seen in patients with b/c greater than the median value of 0.5 (96.2% vs. 89.2%, p=.007 and 90.8% vs. 84.5%, p=.046, respectively). On multivariable analysis (MVA), age at diagnosis (HR 0.95, CI 0.91-0.99, p=.017), BMI (HR 1.07, CI 1.03-1.12, p=.003), ER status (HR= 0.31, CI 0.16-0.61, p=.01), AJCC stage (HR 1.91, CI 1.2-3.0, p=.006), and b/c level (HR 5.67, CI 1.5-18.2, p=.01) all predicted for overall survival. Excluding the 61 patients with in situ disease, there remains a significant difference in survival at both 5 and 7 years (95.5% vs. 88.5%, p=.017 and 93.5% vs. 86%, p=.021). A trend for decrease survival was seen at 10 years (p=0.09). On MVA for patients with invasive disease, age at diagnosis (HR=0.95, 95% CI 0.91-0.99, p=.026), BMI (HR=1.06, 95% CI 1.01-1.11, p=.023), AJCC stage (HR=2.41, CI 1.51-3.91, p=.0003), and ER status (HR=0.25, CI 0.12-0.49, p<.0001) and b/c level (HR=3.76, CI 1.39-8.06, p=.012) were associated with overall survival.

Conclusions:
In this cohort of young, female, non-Hispanic white breast cancer patients, a greater b/c level predicted for decreased overall survival. The use of a gamma-ray-induced mutagen sensitivity assay may be prognostic and help select for those at increased risk of death.
**Title:** Expression of lipid metabolism genes in tumor and contralateral unaffected breast are conversely associated with tumor estrogen receptor status

Ali Shidfar\(^1\), David Ivancic\(^1\), Megan E Sullivan\(^1\), Pranjal Patankar\(^1\), Seema A Khan\(^1\) and Jun Wang\(^1\). \(^1\)Northwestern University Feinberg School of Medicine, Chicago, IL.

**Body:** Background: The identification of women at risk for ER- cancer would allow optimization of breast cancer prevention strategies by guiding their recruitment to studies of agents with efficacy against ER- cancer and sparing them the toxicity of prevention agents effective only against ER+ cancer. In our previous studies, we identified lipid metabolism (LiMe) gene set in rFNA samples from contralateral unaffected breast (CUB) that was associated with tumor ER status. In the current study, we further validate LiMe gene expression in tumor and CUB at the mRNA and protein levels.

Methods: Tissue samples from 56 bilateral mastectomy cases (28 ER+ and 28 ER-) and 28 healthy reduction mammoplasty (RM) controls were used. The ER+ cases, ER- cases and controls were matched by age, race and menopausal status. We performed laser capture microdissection of epithelial cells in fresh frozen tissues from tumor and unaffected breast. Total RNA was extracted and LiMe genes were detected using Taqman low density gene expression arrays. The difference among groups was analyzed using ANOVA with Sidak multiple comparison adjustment. Three proteins (HMGCS2, ACSL3 and HPGD) were detected in FFPE sections of tumor and CUB tissues using immunohistochemistry.

Results: Among the 13 LiMe genes, 6 genes (DHRS2, HMGCS2, UGT2B7, UGT2B11, UGT2B28 and GLYATL1) were significantly higher in CUB of ER- cases compared to CUB of ER+ cases (2.2-2.9 fold, P<0.05). In contrast, the expression of 5 genes (DHRS2, HMGCS2, UGT2B11, UGT2B28 and GSTT2) was significantly lower in ER- tumor compared to ER+ tumor (0.11-0.37 fold, P<0.05). Immunohistochemistry of HMGCS2, ACSL3 and HPGD confirmed this pattern: protein levels were higher in ER- CUB than ER+ CUB, but lower in ER- tumor than in ER+ tumor. When CUB samples were compared with healthy controls, 7 genes (DHRS2, HMGCS2, UGT2B11, UGT2B28, GLYATL1, ALOX15B and SERHL) showed significantly higher expression in CUB of ER- cases (2.5-9.6 fold, P<0.05), but not in CUB of ER+ cases. Four genes (ACSL3, APOD, AKR1B15 and HPGD) did not show any significant difference among groups.

Conclusion: Differential expression of the LiMe genes in the CUB is associated with ER- index tumors and may characterize the environment leading to the development of ER- breast cancer. The converse patterns in tumor and CUB by ER status suggest that LiMe genes may be regulated by different mechanisms in benign and malignant tissues. These genes are potential risk biomarkers of ER- breast cancer and generate novel etiologic hypotheses regarding the development of ER- versus ER+ disease.

<table>
<thead>
<tr>
<th>Gene</th>
<th>ER-C vs ER+C</th>
<th>ER-T vs ER+T</th>
<th>ER-C vs RM</th>
<th>ER+C vs RM</th>
</tr>
</thead>
<tbody>
<tr>
<td>DHRS2</td>
<td>2.4 (0.034)*</td>
<td>0.16 (0.042)*</td>
<td>6.0 (0.014)*</td>
<td>1.3</td>
</tr>
<tr>
<td>HMGCS2</td>
<td>2.9 (0.050)*</td>
<td>0.15 (0.0095)*</td>
<td>6.5 (0.006)*</td>
<td>2.2</td>
</tr>
<tr>
<td>UGT2B7</td>
<td>2.2 (0.004)*</td>
<td>0.77</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>UGT2B11</td>
<td>2.4 (0.019)*</td>
<td>0.11 (0.0068)*</td>
<td>9.6 (0.013)*</td>
<td>2.0</td>
</tr>
<tr>
<td>UGT2B28</td>
<td>2.3 (0.009)*</td>
<td>0.15 (0.018)*</td>
<td>2.8 (0.029)*</td>
<td>1.5</td>
</tr>
<tr>
<td>GLYATL1</td>
<td>2.9 (0.010)*</td>
<td>0.37</td>
<td>4.3 (0.026)*</td>
<td>1.4</td>
</tr>
<tr>
<td>GSTT2</td>
<td>1.1</td>
<td>0.37 (0.040)*</td>
<td>1.5</td>
<td>1.3</td>
</tr>
<tr>
<td>ALOX15B</td>
<td>1.6</td>
<td>0.56</td>
<td>2.5 (0.016)*</td>
<td>1.5</td>
</tr>
<tr>
<td>SERHL</td>
<td>1.8</td>
<td>0.29</td>
<td>4.9 (0.0047)*</td>
<td>1.4</td>
</tr>
</tbody>
</table>

ER-C: ER- CUB; ER+C: ER+ CUB; ER-T: ER- tumor; ER+T: ER+ tumor; RM: reduction mammoplasty
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P3-08-03  
**Average Grade:** 6.75

**Title:** High quality population-based cohort of benign breast conditions (BBC): A foundational frame-work for breast cancer (BC) risk estimation

Azadeh Stark¹, Margaret Peppe², Jeffery Prichard³, Dhananjay Chitale¹, Christos Patriotis⁴ and Paul F Engstrom⁵. ¹Henry Ford Health System, Detroit, MI; ²Fred Hutchinson Cancer Research Center, Seattle, WA; ³Geisinger Health System, Danville, PA; ⁴National Cancer Institute/National Institute of Health, Bethesda, MD and ⁵Fox Chase Cancer Center, Philadelphia, PA.

**Body:**

**Background:** The Breast and Ovarian Collaborative Group at the Early Detection Research Network in collaboration with two large health care systems, Geisinger Health System (GHS) in PA and Henry Ford Health System (HFHS) in MI have established a 10,170 strong population-based BBC cohort.

**Method:** Pathology Information System, Co-Path, was used to identify women diagnosed with BBC between January 1, 1994 and December 31, 2005. Entry into the cohort was restricted to women between 40 and 70 years of age, with a minimum of 6 months stay with either GHS or HFHS. Women who had previous history of malignancy of the breast or other organs site except squamous or basal cell carcinoma of the skin were excluded. Additionally, women whose BCs were diagnosed within 6 months of the diagnoses of their BBCs were excluded. BBCs were detected because of routine screening mammography or because of physical signs and symptoms, i.e. pain and tenderness, lumps or nipple discharge. The minimum cohort data elements (date of birth, race/ethnicity, date of BBC, anatomic site of BBC, related procedure or treatment, date of biopsy and other procedure treatments, BBC histology, availability of BBC diagnostic tissues, availability of BBC tissue blocks, subsequent BC, and date and availability of BC diagnostic slides and blocks, pathologic diagnostic data including ER/PR, HER2, grade, stage, and date of last contact in the health system) were collected from EMRs.

**RESULTS:** A total of 3,048 women at GHS and 7,122 women at HFHS contributed to this cohort. The mean age at diagnosis of BBC was 51.8 (± 8.3) years. For 7,103 women (69.8%) BBCs were detected during their routine screening mammography, 1,623 women (16%) had experienced signs and symptoms of potential abnormalities and for 1,444 women (14.2%) we could not adequately discern the underlying reasons that lead to breast biopsy and detection of BBC. Women remained with the systems, after their diagnosis BBCs, for an average of 10.4 (± 4.5) years. Members of the cohort comprised of 2,604 African-American (25.6%), 6,563 White-American (64.5%) and 257 women (2.53%) from other racial/ethnic heritages. We were not able to identify racial/ethnic heritages for a total of 746 women (7.3%). A total of 422 women (4.1%) progressed to BC. Of these, 298 (70.5%) were diagnosed with invasive and 118 (28.1%) with in-situ. We were not able to accurately document histology of cancer for 6 women (1.4%).

**CONCLUSION:** Our work is a demonstration of an effective and productive partnership between community-based health systems and the traditional academic research centers. This partnership has provided an outstanding opportunity for coalescing resources and intellectual capacities and to overcome barriers, i.e. small sample size, absence or limited continuity in clinical care and no or limited clinic-pathology and demographic data. This exceptional national resource provides samples for discovery and/or validation of biomarkers. Information on how to access data and associated specimens can be obtained from the Early Detection Research Network Website.
Title: Aspirin and breast cancer risk for BRCA1 and BRCA2 mutation carriers

Naomi Kornhauser¹, Mary Beth Terry², Linda T Vahdat¹, Irene Andrulis³, Saundra Buys⁴, Mary Daly⁵, Esther John⁶, John L Hopper⁷ and Tessa Cigler¹. ¹Weill Cornell Medical College, New York, NY; ²Mailman School of Public Health, Columbia University, New York, NY; ³University of Toronto, Toronto, ON, Canada; ⁴Huntsman Cancer Institute, University of Utah Health Sciences Center, Salt Lake City, UT; ⁵Fox Chase Cancer Center, Philadelphia, PA; ⁶Cancer Prevention Institute of California, Fremont, CA and ⁷University of Melbourne, Melbourne, Victoria, Australia.

Body: Background: Although epidemiologic studies have found evidence that aspirin use may be inversely associated with breast cancer (BC) risk, little is known about whether this applies to BRCA1 and BRCA2 mutation carriers.

Methods: We compared aspirin use in 613 women with BRCA1 or BRCA2 mutations from the six centers of the Breast Cancer Family Registry (BCFR) who were recruited at baseline and completed a questionnaire at 10 year follow-up. We defined cases as carriers with BC (n = 215 with BRCA1 mutations and 137 with BRCA2 mutations) and controls as carriers unaffected with BC (n = 141 with BRCA1 mutations and 120 with BRCA2 mutations). We used logistic regression to estimate odds ratios and 95% confidence intervals separately by gene mutation type.

Results: Three cases (1.4%) and 27 controls (19.1%) among the BRCA1 carriers and 3 cases (2.2%) and 25 controls (20.8%) among the BRCA2 carriers reported ever use of aspirin-based medications before diagnosis. Aspirin use before diagnosis was inversely associated with BC risk for both BRCA1 (OR, 0.13; 95% CI, 0.04-0.46 for ever vs. never use) and BRCA2 (OR, 0.12; 95% CI, 0.03-0.41 for ever vs. never use) carriers, after adjusting for age and center for BRCA1 carriers and age for BRCA2 carriers.

Conclusion: If replicated by larger, prospective studies, aspirin use could become an inexpensive and acceptable risk-reducing measure for BRCA1 and BRCA2 mutation carriers.
Title: Association of mammographic density in high risk BRCA mutated tumors compared to average risk tumors and healthy controls: Analysis of Korean Hereditary Breast and Cancer Study (KOHBRA)

Jisun Kim¹, Jong Won Lee¹, Sung Won Park², Hee Jung Shin³, Hak Hee Kim³, Sae-Byul Lee¹, Jong Han Yu¹, Hee Jeong Kim¹, Beom Seok Koh¹, Byung Ho Son¹ and Sei-Hyun Ahn¹. ¹Asan Medical Center, College of Medicine, University of Ulsan, Seoul, Korea; ²Health Promotion Center, College of Medicine, Asan Medical Center, University of Ulsan, Seoul, Korea; ³College of Medicine, Asan Medical Center, University of Ulsan, Korea and 4Korean Hereditary Breast Cancer Study Group (KOHBRA).

Body: Introduction: Mammographic density is a well-known risk factor of breast cancer as a whole. Nonetheless only few studies have examined the association of density among high risk breast cancer regarding BRCA mutation. We examined mammographic density of 2019 breast cancer patients and 2029 healthy controls, regarding risk factors and BRCA mutation status.

Method: Total 2019 breast cancer patients diagnosed between 1980 to 2011 were divided into two groups- high versus average risk group. Women with 1) family history of breast/ovarian cancer or 2) younger than age 40 or 3) bilateral cases were considered high risk group and were participants of ‘Korean Hereditary Breast Cancer study’ (KOHBRA) whom undergone BRCA testing. Density of 2029 healthy women who took screening mammogram during the same period were analyzed for comparison. Density was measured of the unaffected contralateral CC view using computer-assisted method Cumulus by single observer (10% randomly selected, intra-class correlation coefficient=0.96). Percent density (PD, dense area/breast area, %) among three groups, association with BRCA mutation status and breast cancer subtypes were examined.

Results and Discussion: Percent density (PD) was significantly higher in high risk group compared to average risk and controls in a consecutive manner. This finding was consistent after adjusting age and BMI (p*<0.001).

<table>
<thead>
<tr>
<th>PD_median (%)</th>
<th>High risk cancers (N=1066)</th>
<th>Average risk cancers (N=953)</th>
<th>Control (N=2029)</th>
<th>High vs Average</th>
<th>High vs control</th>
<th>Average vs control</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;50</td>
<td>442 (41.5%)</td>
<td>567 (59.4%)</td>
<td>1414 (69.7%)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>50≤</td>
<td>624 (58.5%)</td>
<td>387 (40.6%)</td>
<td>615 (30.3%)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PD_quartile (%)</td>
<td>&lt;25</td>
<td>26 (2.4%)</td>
<td>106 (11.1%)</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>25≤&lt;50</td>
<td>416 (39.0%)</td>
<td>461 (48.3%)</td>
<td>1085 (53.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>50≤&lt;75</td>
<td>564 (52.9%)</td>
<td>360 (37.7%)</td>
<td>570 (28.1%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>75≤</td>
<td>60 (5.6%)</td>
<td>27 (2.8%)</td>
<td>45 (2.2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* adjusted p value by age and BMI

High mammographic density showed to be a significant risk factor throughout different subtypes.

Magnitude of mammographic density as a risk factor according to receptor status

<table>
<thead>
<tr>
<th>Receptor Status</th>
<th>HR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNBC (control(ref))</td>
<td>4.21, 2.12-8.38</td>
</tr>
<tr>
<td>Non-TNBC</td>
<td></td>
</tr>
<tr>
<td>Luminal</td>
<td></td>
</tr>
<tr>
<td>Non-Luminal</td>
<td></td>
</tr>
</tbody>
</table>
Among the 1066 high risk group, 81.5% (869) undergone BRCA testing and 70(6.6%) had BRCA1, 78(7.3%) had BRCA2 mutations without significant difference in density. Similar strong magnitude association of mammographic density was observed in both BRCA mutated/non-mutated tumors and among subtypes. The ongoing GWAS and whole exome analysis of this population-subset will give insight into the tumor etiology and how density could stratify breast cancer risk for personalized screening especially in high risk population.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-08-06
Average Grade: 5.75

Title: Demographics of breast cancer in a cohort of Afro-Caribbean women

Sophia HL George¹, Talia Donenberg², Mohammed Akbari³, Cheryl Alexis⁴, Gillian Wharfe⁵, Sook Yin⁶, Hedda Dyer⁷, Theodore Turnquest⁸, Vincent DeGennaro⁹, Steven Narod¹⁰ and Judith Hurley¹¹. ¹Campbell Family Institute for Breast Cancer Research, Toronto, ON, Canada; ²University of Miami, Miami, FL; ³Women’s College Hospital, Toronto, ON, Canada; ⁴University of West Indies, Wanstead, St Michael, Barbados; ⁵University of West Indies, Kingston, Jamaica; ⁶Cancer Society of Cayman Islands, George Town, Grand Cayman, Cayman Islands; ⁷Ross School of Medicine, Portsmouth, Dominica; ⁸Princess Margaret Hospital, Nassau, Bahamas; ⁹University of Florida, Gainsville, FL; ¹⁰Women’s College Hospital, Toronto, ON, Canada and ¹¹University of Miami, Miami, FL.

Body: Objectives: In Latin America and the Caribbean, non – communicable chronic diseases are now the leading cause of premature mortality. The incidence of cancer has increased in the region as a result of population aging and growth but also as more people adopt lifestyle choices like smoking, physical inactivity, and “westernized” diets. In women, fertility factors such as decreased parity, earlier onset of menses and later age at time of first pregnancy are known epidemiologically to increase incidence of hereditary and sporadic breast cancer. Recently there has also been a strong link to a genetic etiology of the breast and ovarian cancer in the Bahamas (27% in unselected breast cancer cases). A study was designed to address the prevalence and spectrum of BRCA1 and BRCA2 mutations in the Afro-Caribbean population.

Methods: Demographic and clinical pathologic data was collected from 347 women of Afro-Caribbean decent. The cohort included women with breast cancer from the following countries: the Cayman Islands, Jamaica, Barbados, Dominica, Trinidad and Haiti. Summary statistics and t-tests and ANOVA were used to analyze population characteristics. A Bahamian mutation panel was created and detailed analyses of samples are ongoing.

Results: The mean age of onset in the cohort is 48.1 yrs with a mean BMI of 27.7. 70% of breast cancer cases ER+ (n=241 informative cases) and in Jamaica 27% (n=105) of breast cancer cases were Her2+. 67.8% cases were diagnosed at stages II/III (n=90). TAH-BSO delayed invasive breast cancer from 48 to 53 years, p=0.005. Parity was a statistically significant factor (p<0.0001), which delayed age of onset by 8 yrs. Additionally, pregnancy alone delayed age of onset (p<0.005) also by 8 yrs in our cohort (n=379). Only three women out of 347 were found to have a mutation.

Summary demographics of Caribbean women with breast cancer

<table>
<thead>
<tr>
<th>Country</th>
<th>No. of Participants</th>
<th>Mean age at diagnosis (yrs)</th>
<th>ER+</th>
<th>Her2+</th>
<th>BRCA1/2 +</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cayman Islands</td>
<td>66</td>
<td>51.4</td>
<td>29/44</td>
<td>3/43</td>
<td>1/27</td>
<td>30.3</td>
</tr>
<tr>
<td>Barbados</td>
<td>89</td>
<td>46.6</td>
<td>52/73</td>
<td>9/73</td>
<td>4/87</td>
<td>27.5</td>
</tr>
<tr>
<td>Dominica</td>
<td>60</td>
<td>52.2</td>
<td>9/13</td>
<td>2/7</td>
<td>0/46</td>
<td>28.7</td>
</tr>
<tr>
<td>Jamaica</td>
<td>137</td>
<td>48.6</td>
<td>69/105</td>
<td>28/105</td>
<td>1</td>
<td>28.7</td>
</tr>
<tr>
<td>Trinidad and Tobago</td>
<td>6</td>
<td>45.5</td>
<td>4/6</td>
<td>1/5</td>
<td>1/5</td>
<td>27.4</td>
</tr>
<tr>
<td>Haiti</td>
<td>34</td>
<td>48.5</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>24.9</td>
</tr>
</tbody>
</table>

Reproductive Characteristics of Caribbean women with breast cancer

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean Age</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAH-BSO</td>
<td>71</td>
<td>53</td>
<td>0.005</td>
</tr>
<tr>
<td>Not Performed</td>
<td>304</td>
<td>48</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td>Nulliparous</td>
<td>60</td>
<td>42</td>
</tr>
<tr>
<td>------------</td>
<td>-------------</td>
<td>-----</td>
<td>----</td>
</tr>
<tr>
<td></td>
<td>Multiparous</td>
<td>319</td>
<td>50</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>None</td>
<td>48</td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>More than 1</td>
<td>331</td>
<td>50</td>
</tr>
</tbody>
</table>

Conclusions: This population-based study provides an insight into pattern of risk factors – both genetic and environmental of breast cancer incidence and subtype across the Caribbean. In conclusion 1) genetic causes of breast cancer appear rare outside of the Bahamas, 2) fertility factors appear important in the development of breast cancer, 3) TAH-BSO is common as both a form of contraception and because of the high incidence of fibroids in the Caribbean and it may be protective, 4) BMI may impact on breast cancer development and 5) screening mammography is rare and the vast majority of mammography performed is diagnostic in nature.
Title: A multicenter randomized study comparing the dose dense G-CSF-supported sequential administration of FEC followed by docetaxel versus docetaxel plus cyclophosphamide as adjuvant chemotherapy in women with HER2-negative, axillary lymph node-positive breast cancer

Dimitrios Mavroudis¹, Nikolaos Malamos¹, Pavlos Papakotoulas¹, Stylianos Kakolyris¹, Ioannis Boukovidas¹, Elias Athanasiadis¹, Nikolaos Kentepozidis¹, Nikolaos Ziras¹, Kostas Kalbakis¹, Charalambos Christophyllakis¹ and Vassilis Georgoulias¹. Hellenic Oncology Research Group (HORG), Athens, Greece.

Body: Background: The dose dense sequential administration of anthracycline and taxane is very effective as adjuvant therapy in node-positive early breast cancer. The non-anthracycline regimen docetaxel plus cyclophosphamide (TC regimen) was better than four cycles of doxorubicin/cyclophosphamide as adjuvant therapy. This study compared the dose dense sequential regimen versus the TC regimen as adjuvant therapy.

Methods: Women with axillary node-positive, HER2-negative early breast cancer were randomized following surgery to receive either dose dense G-CSF-supported FEC (5FU 500mg/m2, epirubicin 75mg/m2, cyclophosphamide 500mg/m2 every 14 days for 4 cycles) followed by Docetaxel (75mg/m2 every 14 days for 4 cycles) (arm A) or 6 cycles of Docetaxel 75mg/m2 plus Cyclophosphamide 600mg/m2 every 3 weeks (arm B). The primary endpoint of the study was the 3-year disease-free survival (DFS).

Results: Six hundred fifty patients were randomized; 326 on arm A and 324 on arm B. Of them 109 (33%) and 90 (28%) were premenopausal, 196 (60%) and 218 (67%) had 1-3 positive nodes, 284 (87%) and 288 (89%) were hormone receptor positive in arm A and B, respectively. Chemotherapy was completed in 97% and 93% of patients in arm A and B, respectively. After a median follow up of 46 and 47 months, there were 37 (11.3%) and 33 (10.1%) disease relapses and the median DFS has not yet been reached (p=0.5) while the 3-year DFS rate was 89.5% and 91.1% for arm A and B, respectively. Neutropenia grade III-IV was more common in arm B and anemia, nausea, vomiting and fatigue grade II-III in arm A. No toxic deaths occurred.

Conclusions: The 3-year DFS rate was similar between the dose dense sequential FEC/docetaxel combination and the TC regimen in women with node-positive HER2-negative early breast cancer.
Title: Association between definitive surgery and times to administration of adjuvant chemotherapy and outcomes in early breast cancer: Analysis of adjuvant studies conducted by NCIC Clinical Trials Group (NCIC CTG)

Ravi Ramjeesingh¹, Bingshu E Chen¹, Joseph L Pater², Liting Zhu¹, Margot Burnell³, Vivien H Bramwell³, Kathleen I Pritchard⁴, Lois E Shepherd¹ and Wendy R Parulekar¹. ¹NCIC Clinical Trials Group, Queen's University, Kingston, ON, Canada; ²Atlantic Health Sciences Corporation, Saint John, NB, Canada; ³Tom Baker Cancer Centre, Calgary, AB, Canada and ⁴Sunnybrook Odette Cancer Centre, Toronto, ON, Canada.

Body: Background: For early high risk breast cancer, adjuvant chemotherapy following definitive surgery is a current standard of care. However, the optimal time to commencement of therapy has not been well established. We evaluated the association between time to initiation of adjuvant chemotherapy after definitive surgery (sTTC) with survival.

Methods: We retrospectively analyzed 4 NCIC CTG-led breast cancer adjuvant clinical trials involving women diagnosed from 1984 and 2005 with stage 1 to 3 breast cancer, who received adjuvant chemotherapy. Patient data were categorized into four time groups: < 4, 4-8, >8-12, >12 weeks after definitive surgery. Outcomes measured were: overall survival (OS) and disease-free survival (DFS).

Results: 3837 patients were included in the final analysis. In univariate analysis, an improvement was identified for patients treated between >8 and 12 weeks:

<table>
<thead>
<tr>
<th>Time to Adjuvant</th>
<th>Number of Pts</th>
<th>10 yr OS(%)</th>
<th>p-value</th>
<th>10 yr DFS(%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4 weeks</td>
<td>774</td>
<td>69.6</td>
<td>ref</td>
<td>60.9</td>
<td>ref</td>
</tr>
<tr>
<td>4-8 weeks</td>
<td>2263</td>
<td>71.5</td>
<td>0.1351</td>
<td>64.7</td>
<td>0.1043</td>
</tr>
<tr>
<td>&gt;8-12 weeks</td>
<td>742</td>
<td>77.3</td>
<td>0.0002</td>
<td>72.6</td>
<td>0.0001</td>
</tr>
<tr>
<td>&gt;12 weeks</td>
<td>35</td>
<td>77.0</td>
<td>0.4531</td>
<td>73.9</td>
<td>0.2124</td>
</tr>
</tbody>
</table>

However, in multivariate analysis there was no significant association between any sTTC time periods and either outcome (see table below). Covariates which did show a significant association are listed in the table.

<table>
<thead>
<tr>
<th>Time to Adjuvant</th>
<th>OS p-value</th>
<th>DFS p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;4 weeks</td>
<td>ref</td>
<td>ref</td>
</tr>
<tr>
<td>4-8 weeks</td>
<td>0.7762</td>
<td>0.6764</td>
</tr>
<tr>
<td>&gt;8-12 weeks</td>
<td>0.4251</td>
<td>0.1396</td>
</tr>
<tr>
<td>&gt;12 weeks</td>
<td>0.6861</td>
<td>0.9312</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>BMI</td>
<td>0.0238</td>
<td>0.0298</td>
</tr>
<tr>
<td>Menopause Status</td>
<td>0.0126</td>
<td>0.1226</td>
</tr>
<tr>
<td>Performance Status 2</td>
<td>0.0256</td>
<td>0.1665</td>
</tr>
<tr>
<td>Surgery Type</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Pathological Stage 3</td>
<td>0.0037</td>
<td>0.0229</td>
</tr>
<tr>
<td>Positive Nodal Status</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ER Negative</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
The fact that the statistical significance of sTTC in univariate analysis was lost when covariates related to DFS and OS were accounted for in a multivariate analysis suggested there might be a relationship between sTTC and the risk of adverse outcomes. A disease risk score analysis was therefore carried out, but there was no indication of an advantage to an earlier sTTC within disease risk categories.

**Conclusions:** Within the context of chemotherapy given within 12 weeks, we were unable to demonstrate an effect on survival based on time to adjuvant chemotherapy in our multivariable analysis. Those treated later did not do significantly worse than those treated earlier. Significant covariates which effected survival were consistent with predictors of poor prognosis (younger age, poorer performance status, increased disease burden and receptor negativity). There is a potential relation of patient risk to time to treatment which requires further study.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-09-03
Average Grade: 6.20

Title: Comparative effectiveness of pegfilgrastim versus filgrastim in elderly breast cancer patients receiving chemotherapy: A SEER-Medicare based study

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Body: Adjuvant chemotherapy is a vital component of breast cancer treatment, but early-onset toxicities like neutropenia hinder its administration, especially in the elderly. Primary prophylactic (PP) use of granulocyte colony-stimulating factors (G-CSFs) prevents neutropenia and improves successful administration of adjuvant chemotherapy. PPG-CSF use has considerably increased since 2003, after the introduction of scientific-evidence demonstrating the benefits of PPG-CSF supported dose-dense chemotherapy in breast cancer patients, and also due to the FDA approval of easy to administer long-acting pegfilgrastim (used instead of the short-acting filgrastim). Consequently, PPG-CSF has become a vital component of breast cancer care, especially in the elderly. However, G-CSFs are expensive drugs. Every year approximately $3.4 billion are spent nationally on G-CSFs. In spite of their considerable use and cost burden certain clinical questions in real life clinical settings remain unanswered. First, there is ambiguity over the comparative effectiveness of pegfilgrastim and filgrastim, and debate over the exact filgrastim duration-equivalent of a single pegfilgrastim dose for preventing neutropenia. Second, the actual costs and long-term survival benefits associated with the two drugs are unknown. This NCI funded study addresses these questions. The study involves 20,227 women 66 years and above, newly diagnosed with stage I to III breast cancer between the years 2003 to 2009, and receiving first-course chemotherapy in the SEER-Medicare data. Among these women, 2,621 received PP pegfilgrastim and 542 received PP filgrastim. Covariate matching techniques, logistic regression, generalized linear models and survival analysis were used to examine the comparative effect of filgrastim and pegfilgrastim, and overall effect of any G-CSF use on neutropenia hospitalization rates, costs and survival. Our analysis demonstrates that using pegfilgrastim reduces the odds of neutropenia hospitalization by 40% and the odds of death by 50% as compared to filgrastim. Pegfilgrastim is equivalent to 6 days of filgrastim administration in terms of the ability to reduce neutropenia hospitalizations and mortality. Further simulations to examine the filgrastim duration equivalence and cost analysis are under way and will be completed before the December presentation.
Title: AML and MDS after adjuvant chemotherapy: A population based study among older breast cancer patients

Aron S Rosenstock¹, Sharon H Giordano¹, Jiangong Niu¹, Hui Zhao¹, Antonio C Wolff², Thomas A Buchholz¹ and Mariana Chavez-MacGregor¹. ¹University of Texas MD Anderson Cancer Center, Houston, TX and ²Johns Hopkins Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD.

Body: Background: Adjuvant chemotherapy for early breast cancer is associated with a small risk of developing MDS and AML. The aim of this study is to determine the risk of developing AML or MDS after modern adjuvant chemotherapy in older breast cancer patients and to further define the risk of individual chemotherapy regimens.

Methods: Patients diagnosed with stage I-III breast cancer from 2003 to 2009 were identified in the Surveillance, Epidemiology and End Results (SEER)-Medicare linked database. Development of AML or MDS, chemotherapy use, and comorbidities were identified using ICD-9 and HCPCS codes. A single inpatient or 2 outpatient codes 30 days apart were required for identification of AML or MDS. Analyses included descriptive statistics, Kaplan-Meier method for 5-year AML/MDS rates and Cox proportional hazards models to estimate the hazard of AML and MDS after adjusting for clinically relevant covariates.

Results: 68,702 patients were included, of them 166 (0.24%) developed AML and 385 (0.56%) developed MDS. The median time from breast cancer diagnosis to AML or MDS was 924 and 943 days respectively. The 5-year AML rates were 0.23% for the no chemotherapy group, 0.53% for anthracycline (A)-based regimens, 0.48% for anthracycline and taxane (AT)-based regimens, 0.39% for other (O) regimens and 0.09% for those who received docetaxel and cyclophosphamide (TC). 5-year MDS estimates were 0.62%, 1.07%, 0.72%, 1.08 and 0.38% respectively. In the multivariable model using no chemotherapy as the reference category, A (HR 3.03; 95%CI 1.75-5.24) and AT (HR 3.06; 95%CI 1.70-5.52) were the chemotherapy regimens associated with the highest risk for developing AML, followed by O (HR 2.05; 95%CI 1.13-3.69). No significant increase in risk was observed in patients who received TC (HR 0.91; 95%CI 0.26-4.60). Similar results were observed when evaluating risk of MDS among patients treated with A (HR 2.25; 95%CI, 1.50-3.37), AT (HR 1.67; 95%CI 1.06-2.65), O (HR 2.30; 95%CI 1.61-3.29) and TC (HR 1.22; 95%CI 0.60-2.66).

Conclusion: In this large cohort of patients we observed a small but significant increase in risk for AML and MDS with adjuvant chemotherapy. The highest risk was associated with A and AT-based chemotherapy. TC was the only regimen that was not significantly associated with an increased risk. Because of relative recent adoption of TC, longer follow-up is needed to better estimate the risk of MDS and AML in this group of patients.
Identification of optimal adjuvant chemotherapy regimen for early-stage breast cancer: A systematic review and bayesian network meta-analysis

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Body: Background: The National Comprehensive Cancer Network (NCCN) guidelines for treatment of breast cancer recommend several adjuvant chemotherapy regimens, including sequential or concurrent anthracycline-cyclophosphamide and taxane-based regimens (sequential AC-T or concurrent ACT, respectively); anthracycline alone; cyclophosphamide, methotrexate, and 5-fluorouracil (CMF); and docetaxel and cyclophosphamide (TC). Sequential AC-T is the most commonly prescribed standard regimen. Two types of regimens without anthracycline, TC and platinum-containing regimens, have been speculated to have similar efficacy to that of ACT-based regimens. Platinum-containing regimens have also demonstrated high efficacy in breast cancer with tolerable adverse effects in several previous studies. However, because of the limitations of conventional meta-analysis, we do not know how these regimens compare with one another in terms of survival or adverse events. Thus, the best adjuvant chemotherapy regimen for breast cancer is not known. Bayesian network meta-analysis allows comparison of treatments for which there has been no head-to-head comparison by using indirect comparisons.

Methods: We searched the MEDLINE, EMBASE, and Cochrane Library databases for articles published before January 2014; the American Society of Clinical Oncology annual meeting abstracts for 1983-2013; and the American Association for Cancer Research annual meeting abstracts for 1916-2013. We included only randomized controlled trials of adjuvant treatments for breast cancer that compared 2 or more of the following: observation alone; sequential AC-T; concurrent ACT; anthracycline alone; CMF; TC; and platinum-containing regimens. We compared regimens by using a network meta-analysis approach and random-effects models. Network meta-analysis allows derivation of hazard ratios (HRs) for death for each regimen and comparison of these HRs with each other even when there are no direct comparisons between 2 regimens.

Results: We identified 24 trials with a total of 28,853 patients. Network meta-analysis showed that OS with TC and platinum-containing regimens were similar to OS with sequential AC-T (TC: HR=0.94; 95% CI, 0.58-1.52; platinum: HR=0.90; 95% CI, 0.69-1.19). Patients receiving CMF or anthracycline alone had significantly worse OS than those with sequential AC-T (CMF: hazard ratio [HR]=1.62; 95% CI, 1.31-2.01; anthracycline: HR=1.23; 95% CI, 1.07-1.43). For overall adverse events, the mean number of adverse events per patient was higher for platinum-containing regimens (2.1) and concurrent ACT (1.22) than for sequential AC-T (1.17). The mean number of hematological adverse events was higher for concurrent ACT (0.67) and TC (0.63), than for sequential AC-T (0.48). The mean number of nonhematological adverse events was higher for platinum-containing regimens (1.76) than for sequential AT (0.7).

Conclusion: Our findings suggest that sequential AC-T should continue to be considered the optimal treatment for early-stage breast cancer because of the equivalent survival benefit of concurrent ACT, TC and platinum-containing regimens but superior adverse-event outcomes.
Title: Frequency of and reasons for deviation from therapy decisions in patients with primary breast cancer initially intended to receive chemotherapy – Results from the German prospective multi-center study BRENDA II

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¹University Ulm, Germany; ²University Mainz, IMBEI, Germany and ³University Würzburg, Germany.

Body: Objectives
This study examined the question, how often chemotherapy is not given to breast cancer patients when it is indicated according to guidelines and when the multi-professional tumour board (TB) decided to apply chemotherapy.

Methods
In a prospective multi-center cohort study, patients with primary breast cancer were sampled consecutively over a period of four years (2009-2012). Patients completed a questionnaire prior to surgery and prior to adjuvant therapy. This questionnaire assessed health related quality of life (QoL) using the EORTC QLQ-C30, psychiatric comorbidity with the Patient Health Questionnaire (PHQ), demographic characteristics (age, education), and the intensity of fear for chemotherapy. After surgery, a multi-professional team discussed recommendation for chemotherapy and this decision was documented in a database together with the indication for chemotherapy according to the German S3 guideline. The multi-professional team was blinded to that algorithm-based decision. Six months later, it was documented whether the patient had received adjuvant chemotherapy or not.

Patients were included in the analysis when chemotherapy was indicated (high risk) or possible (intermediate risk) according to the guidelines and when the multi-professional team had decided to recommend chemotherapy. Risk group stratification is based on St. Gallen 2007 classification.

Statistical analysis was performed with multivariate logistic regression, separately for high and intermediate subgroup.

Results
Altogether, 857 patients were included in the study of whom based on the guideline, 241 were indicated (high risk) for chemotherapy and in 537 it was possible (intermediate risk) to apply adjuvant chemotherapy. In only 391 of those patients (accrued from high and intermediate risk subgroup), the TB decided to recommend chemotherapy. The most important reason for not recommending chemotherapy was somatic comorbidity not allowing adjuvant chemotherapy. Of those 391 patients, 73 (19%) patients had not received chemotherapy (10% of high risk, 28% of intermediate risk subgroup).

If patients where CT was recommended (according to the tumorboard decision) deviations from initial therapy decision was likely if they were old (≥75 years) and had poor QoL, (OR 0.003, p 0.001). There was also some evidence that patients with higher education (OR 0.3, p 0.07) less frequently received CT.

If patients with intermediate risk (CT possible) were very afraid of chemotherapy, deviations from initial therapy decision was likely (OR 0.4, p 0.03). In that group of patients, age, QoL, education, and comorbidity were unrelated to deviations from initial decision.

Conclusion
Even in cases where chemotherapy was indicated or possible according to guidelines and after the decision of multi-professional team to recommend it, about 19% of patients eventually did not receive chemotherapy. In those patients, this mainly happened associated with poor QoL in elderly patients >75. In the intermediate risk group with chemotherapy recommendation, patient’s fear of chemotherapy is the main factor preventing patients from adjuvant chemotherapy.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-09-07
Average Grade: 5.60

Title: Prognostic value of immunophenotypically defined subtypes in patients treated with dose-dense sequential adjuvant chemotherapy in the trastuzumab era. A Hellenic Cooperative Oncology Group study

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Body: Background-Aim: The aim of the present study was to explore the efficacy of dose-dense sequential adjuvant chemotherapy followed by trastuzumab in HER2-positive patients according to the immunohistochemically (IHC) defined subtypes.

Patients and methods: A total of 771 formalin-fixed paraffin-embedded (FFPE) tumor tissue samples, prospectively collected from 990 eligible patients with high-risk N₀ or N₁ operable breast cancer participating in a phase III trial (HE10/05), were centrally assessed in tissue microarrays by IHC for 6 biological markers, that is, estrogen receptor (ER), progesterone receptor (PgR), HER2, Ki67, cytokeratin 5 (CK5) and EGFR. All cases were further evaluated for HER2 amplification by FISH. Patients were classified as: luminal A (ER/PgR-positive, HER2-negative, Ki67<sub>low</sub>, N=382, 49.5%); luminal B (ER/PgR-positive, HER2-negative, Ki67<sub>high</sub>, N=136, 17.6%); luminal-HER2 (ER/PgR-positive, HER2-positive, N=125, 16.2%); HER2-enriched (ER-negative, PgR-negative, HER2-positive, N=63, 8.2%); triple-negative (TNBC) (ER-negative, PgR-negative, HER2-negative, N=65, 8.4%); and basal core phenotype (BCP) (TNBC, CK5-positive and/or EGFR-positive, N=53, 6.9%).

Results: At a median follow-up of 60.5 months, the 3-year disease-free survival (DFS) and overall survival (OS) rates for the total patient population were 88.3% and 96.0%, respectively. The 3-year DFS rates for luminal A, luminal B, luminal-HER2, HER2-enriched, TNBC and BCP patients were 91.1%, 88.2%, 86.4%, 93.7%, 87.7%, and 89.4%, respectively, while the corresponding 3-year OS rates were 95.8%, 95.6%, 97.6%, 95.2%, 95.4%, and 95.0%, respectively. No significant differences were detected for either 3-year DFS or OS in the immunohistochemically defined subtypes, except a trend for significantly worse DFS in patients with luminal-HER2 tumors compared to patients with HER2-enriched tumors (log-rank, p=0.069).

Conclusions: In the post-trastuzumab era, at a relatively short follow-up, the luminal-HER2 patients show a trend for worse DFS compared to patients with HER2-enriched tumors treated with dose-dense sequential adjuvant chemotherapy followed by trastuzumab. No other significant differences were detected, with follow-up however being continued.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-09-08
Average Grade: 6.83

Title: Patterns of oncotype DX testing and adjuvant chemotherapy use in a tertiary care centre

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Body: Introduction:
The Oncotype DX Recurrence Score (RS) is a 21-gene signature, retrospectively validated prognostic marker and predictor of response to adjuvant chemotherapy (ACT) in estrogen receptor (ER) positive breast cancer (BC). In 2010 RS became publicly funded in Ontario for patients with estrogen-positive, HER2 negative and node negative breast cancer. The aim of our study was to explore the pattern of RS testing and use of adjuvant chemotherapy at the Cancer Centre of Southeastern Ontario (CCSEO) since the introduction of RS testing. Additionally we compared the RS tested patients with a matched cohort treated for early BC prior to 2010.

Methods:
A retrospective paper and electronic chart review was undertaken of patients with early BC (stage 1 and 2) with the following pathologic features: T1-T2, N0/N1mic, ER positive and Her-2neu negative. We collected patient demographics, co-morbidities, surgical data, tumour characteristics, ACT use, Adjuvant! mortality estimates and breast cancer outcomes. Cohort A included patients who underwent RS testing (2010 – 2013) and cohort B patients treated prior to 2010. The two cohorts were compared using chi-square tests for categorical data, and independent samples t-tests and the Mann-Whitney U for continuous data.

Results:
160 patients were included in our analysis of which 83 underwent RS testing. Compared to cohort B, cohort A was older (median age 60 versus (vs) 48, p<0.001); had higher postmenopausal status (77 vs 34%, p<0.001); higher rates of breast conserving surgery (88 vs 74%, p=0.024) and sentinel node biopsy (94 vs 26%, p<0.001). Cohort A also had larger tumors (T2 23 vs 5%, p=0.010), higher stage (stage 2 - 24 vs 6%, p=0.002) and higher Adjuvant! mortality estimates above 15% (18 vs 8%, p=0.054). Despite this the use of ACT decreased significantly (20 vs 98%, p<0.001). The majority of patients received adjuvant endocrine treatment (> 90% in both cohorts). Of the 83 patients in cohort A 55 (66%) had low risk RS (0-17), 18 (22%) intermediate risk (18-30) and 10 (12%) high risk RS. ACT was received by 2 of 55 patients with low RS; 6 of 18 patients with intermediate RS and 9 of 10 with high RS. Median follow-up was only 16 months in cohort A vs 74 months in cohort B. 92% of patients in cohort B remain recurrence free. To date only 2 patients in cohort A have recurred, both of which had high RS scores and received ACT.

Conclusion:
In patients with early BC undergoing RS testing at CCSEO we observed a higher proportion of low risk RS as compared to the literature. Additionally, despite larger tumors, higher stage disease and higher Adjuvant! mortality estimates above 15%, the proportion of patients undergoing ACT has significantly decreased since the introduction of RS testing. So far this has not translated into a negative long-term disease outcome although longer follow-up is needed for real-world validation.
Title: Eribulin mesylate plus capecitabine for adjuvant treatment in post-menopausal ER+ early-stage breast cancer: A phase 2, multicenter, open-label study using 2 different dosage regimens

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Body: Introduction: Eribulin mesylate is a novel non-taxane microtubule inhibitor. The primary objective of this study was to explore feasibility of administering eribulin plus capecitabine as adjuvant therapy in subjects with early-stage, estrogen receptor-positive (ER+) breast cancer.

Methods: In this phase 2, open-label, multicenter study, 67 postmenopausal women with early HER2-normal/HER2-negative, ER+ breast cancer received four 21-day cycles of treatment with eribulin mesylate (1.4 mg/m² IV on day 1 and day 8 of each cycle) in combination with capecitabine (900 mg/m² orally twice daily on days 1 through 14 of each cycle). A second dosage regimen for capecitabine was initiated after dose reductions and treatment discontinuations were noted and attributed to capecitabine-related toxicities, such as grade 3/4 GI events and hand-foot syndrome. As a consequence, capecitabine was administered to an additional cohort of 10 subjects at a fixed dose of 1500 mg given orally twice daily on a 7 days on/7 days off schedule for the 4 cycles; eribulin mesylate was administered on the same dosage schedule as the original regimen (1.4 mg/m² IV on day 1 and day 8 of each cycle). The 7 days on/7 days off regimen for capecitabine was based on mathematical modeling and has been shown to have an acceptable toxicity profile, including minimal gastrointestinal toxicity, when given in combination with bevacizumab to patients with metastatic breast cancer. Feasibility was assessed based on relative dose intensity (RDI) of the combination using prespecified criteria of 80% of subjects achieving an RDI of at least 85% of the regimen with lower 95% confidence boundary >70%; safety and tolerability were also assessed.

Results: Among subjects on the original eribulin plus capecitabine dosing schedule (n=64), the average (SD) RDI was 90.6% (11.94%) and the feasibility rate was 81.3% (95% lower CI: 71.4%), indicating that this dosage regimen is feasible. Dose reductions, missed doses, and withdrawals due to adverse events were ascribed more to capecitabine (36%, 85%, and 18%, respectively) than to eribulin (21%, 8%, and 12%, respectively), which led to higher RDI and feasibility rates for eribulin (93.5% and 82.8%, respectively) than for capecitabine (87.8% and 71.9%). Grade 3/4 hand-foot syndrome ascribed to capecitabine only, led to dose reductions in 11.9% of subjects under the original dosing schedule. The most common adverse events under the original dosing schedule were alopecia (77.6%), fatigue (58.2%), and nausea (52.2%). With the new dosing schedule (n=9), higher RDI and feasibility rates were achieved, the average RDI was 96% and the feasibility rate was 100%, indicating that this alternative regimen is also feasible, and probably better. (Detailed feasibility and safety data with new dosing regimen will be available at the time of the presentation.)

Conclusions: Adjuvant use of the combination of eribulin plus capecitabine is feasible in patients with early, HER2-normal/HER2-negative, ER+ breast cancer. The combination had an acceptable safety profile under the original dosing schedule and has the potential to be improved by use of a 7 day on/7 day off regimen of capecitabine.
Body: Background: Treatment goals of metastatic breast cancer (MBC) are to prolong survival and improve health-related quality of life (HRQOL). Current standard first-line chemotherapy for MBC are the taxanes or anthracyclines; however treatment-related adverse events greatly reduce HRQOL. S-1 is an oral 5-fluorouracil derivative, and phase II trials showed good clinical efficacy and tolerability. We conducted a phase III randomized controlled trial to establish non-inferiority of S-1 in overall survival (OS) and superiority in HRQOL to taxanes, when given as first-line chemotherapy for MBC.

Methods: Patients with HER2-negative non-life-threatening MBC, naïve to chemotherapy for metastatic disease, were randomly assigned to the taxane or S-1 groups. In the taxane group, patients received docetaxel 60–75mg/m² q3w, paclitaxel 80–100mg/m² q1w, or paclitaxel 175 mg/m² q3w according to institutional policy. In the S-1 group, patients received S-1 40–60 mg twice daily based on body surface area using a 28 days on;14 days off regimen. Treatment was repeated until tumor progression or for at least 6 cycles (taxane) or 4 cycles (S-1). After failure of the first-line protocol therapy, another cytotoxic agent was administered, based on the investigator’s discretion. HRQOL was assessed with the European Organization for Research and Treatment of Cancer (EORTC) QLQ-C30, the Patient Neurotoxicity Questionnaire (PNQ) and the EQ-5D at baseline and 3, 6, 12 months after the start of the treatment. The primary endpoint was OS. Secondary endpoints were time to treatment failure (TTF), adverse events, and HRQOL.

Results: A total of 618 women were enrolled. After a median follow-up of 34.6 months, median OS was 37.2 months in the taxane group (n=309) and 35.0 months in the S-1 group (n=309) (hazard ratio [HR] 1.05, 95% confidence interval [CI] 0.86–1.27, non-inferiority test p=0.015). Median TTF was 8.9 months in the taxane group and 8.0 months in the S-1 group (HR 1.10, 95% CI 0.93–1.30, p=0.022). The incidence of the following grade 3-4 adverse events, allergic reaction, edema and sensory neuropathy, were statistically significantly more frequent in the taxane group (p=0.038, 0.0013 and 0.0077, respectively). Hematologic and non hematologic toxicities except above did not differ significantly between the two groups. The results of the EORTC QLQ-C30 under study treatment indicated that the S-1 was better than the taxanes in global health status/QOL (p=0.044), physical functioning (p=0.002), role functioning (p=0.002), emotional functioning (p=0.004), cognitive functioning (p=0.026), social functioning (p<0.0001), pain (p=0.042) and financial difficulties (p=0.003). EQ-5D utility scores were significantly higher in the S-1 group (p=0.033) during the first year. PNQ sensory and motor scores were significantly better in the S-1 group (p<0.0001 and p=0.0002, respectively).

Conclusions: This study clearly demonstrated that S-1 was superior to taxanes in terms of HRQOL and toxicity, without compromising the prolonged OS. S-1 should be considered as a new standard for first-line chemotherapy for MBC. We are conducting another similar trial (UMIN000005449) that compares first-line anthracycline with S-1 in terms of OS and HRQOL.
Body: Background: There is still no standard chemotherapy for patients with metastatic triple-negative breast cancer (mTNBC). Our previous phase II pilot trial with first-line gemcitabine and cisplatin combination (GP) in patients with mTNBC (clinicaltrials.gov Identifier: NCT00601159) showed a median progression-free survival (PFS) of 6.2 months. In this Chinese Breast Cancer Study Group (CBCSG) 006 trial (clinicaltrials.gov Identifier: NCT01287624) we explored in a randomized trial the role of the less costly GP regimen versus the standard GT [Gemcitabine + paclitaxel] chemotherapy for the metastatic breast cancer as a first line treatment for mTNBC.

Trial objectives: progression free survival [PFS]; overall survival [OS]; and toxicity.

Methods: In the trial with a hybrid trial design incorporating a formal test of superiority as well as noninferiority, mTNBC patients with no previous chemotherapy for metastatic disease were randomly assigned to receive either GP regimen (G/P: 1250 mg/m² d1,8/ 75 mg/m² d1) or the GT regimen (same G; T: 175 mg/m² d1).

Results: Between Jan. 2011 and Nov., 2013, 236 patients were randomized [118 patients / arm], and all received at least one dose of assigned chemotherapy. As of Mar. 20, 2014, the intent-to-treat analysis showed 201 recurrences and 97 deaths. Objective response rates of GP vs GT were 67.9% vs. 50.4% (P= 0.008), with median PFS of 232 vs. 194 days (HR=0.692, 95% CI 0.523-0.915; P= 0.009). Overall survival of patients from the GP vs. the GT arms was median 672 vs. 556 days (HR=0.902, 95% CI 0.605-1.344; P= 0.611).

Significant differences in grade 3/4 adverse events were seen for nausea, vomiting, anemia and thrombocytopenia [GP vs. GT, 6.8 vs. 0.8%; 11.0 vs. 0.8%; 33.1 vs. 51.0%; and 32.2 vs. 2.5%, respectively].

In addition, assessment of adverse events of any grade showed the GP regimen had more anorexia, constipation, hypomagnesemia and hypokalemia, while GT regimen had significantly more alopecia and peripheral neuropathy.

The delivered relative dose intensity was > 90% for all three drugs, with the total number of delivered cycles of chemotherapy in GP and GT arms being 654 and 648 [average 5.54 and 5.49 /patient], respectively.

Conclusions:
1. The Gemcitabine + Platinum is superior to Gemcitabine + Paclitaxel in terms of objective response rates and duration of PFS.
2. While grade 3 / 4 nausea & vomiting, and anemia, were heavier for the GP combination, the delivery of chemotherapy and average number of cycles delivered were comparable between the two arms.
3. Overall survival data will be updated on the conference to indicate the long-term effect of the somehow more toxic GP regimen, which shows nevertheless superiority of response rates and of the PFS over the more costly GT regimen.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-10-03
Average Grade: 8.60

Title: Etirinotecan pegol target-specific pharmacodynamic biomarkers in circulating tumor cells from patients with metastatic breast cancer in the phase 3 BEACON study

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Body: Background: Etirinotecan pegol (EP) is the first long-acting topoisomerase 1-inhibitor providing sustained level of active metabolite that concentrate in the tumor for an entire chemotherapy cycle. In a Phase 2 study (Lancet Oncology 2013), EP demonstrated a 29% overall response rate in patients with metastatic breast cancer, leading to the Phase 3 global BEACON study in this population. Detection of circulating tumor cells (CTCs) using immunomagnetic EpCAM-based methods have been conceptually accepted as a "liquid tumor biopsy". To circumvent the limited recovery of CTCs these methods provide for molecular profiling applications, we isolated CTCs based on an antibody-independent, continuous flow dielectrophoresis (DEP) technology (ApoStream®). We developed quantitative multiplex immunofluorescent assays for target-specific pharmacodynamics (PD) biomarkers for EP in CTCs isolated pre- and post-treatment. Here we present the distribution of the PD biomarkers in CTC samples collected from BEACON patients.

Methods: Donation of blood samples for CTC analysis was voluntary. BEACON patients participating in the substudy had serial (pre-dose, Cycle 2 Day 1, Cycle 4 Day 1, End of Treatment) 7.5-mL whole blood samples drawn in EDTA tubes and shipped ambient to ApoCell (Houston, TX) within 96 hrs for processing. PBMCs were separated by Ficoll® gradient, and CTCs were isolated using ApoStream® technology. Isolated cells were deposited on three slides and stained for DAPI, CD45, and cytokeratin markers, as well as 2-3 of the seven PD markers, and analyzed by iCys® laser scanning cytometer equipped with image analysis software. CTC count, mean fluorescence intensity (MFI) of PD biomarkers, and % marker-positive cells were analyzed for their distribution.

Results: 75% of the 852 BEACON patients participated in the CTC substudy, yielding 649 pre-treatment CTC samples. As of Mar14, 538 Cycle 2 Day 1, 281 Cycle 4 Day 1, and 394 End of Treatment samples were collected. 98% of pre-treatment blood samples were successfully processed. 97% had detectable CTCs, with a median of 504 CTCs (interquartile range: 128-1432). PD markers could be analyzed in 83-92% of samples after implementing a minimum requirement of 10 CTCs analyzed. Staining was positive for Top1, Top2, μH2Ax, Rad51, ABCG2, Ki67, and TUNEL in 82%, 71%, 14%, 30%, 20%, 56%, and 94% of samples, respectively. Percent of marker-positive cells varied from 0.2-69%, with 6- to 37-fold interquartile ranges. Median MFI in these cells varied between 25x103 and 100x103, with 1.2- to 1.6-fold interquartile ranges.

Conclusions: CTC collection was successfully incorporated into the BEACON study, with 75% patient participation. CTC detection rate using ApoStream® was high, permitting evaluation of biomarkers at baseline and over time. EP target-specific PD biomarkers can be measured in CTCs isolated from patients participating in BEACON and will be assessed for their potential to predict clinical response. Final BEACON efficacy and safety results are expected in early 2015, which will allow further analysis of CTCs with patient outcome (response, PFS, OS) and change in CTCs at time of progression.
Title: A open-label, randomized, parallel, phase III trial to evaluate the efficacy and safety of Genexol®-PM compared to Genexol® (conventional paclitaxel with cremophor EL) in recurrent or metastatic breast cancer patients

Jung Sil Ro¹, Joo Hyuk Sohn², Sung Bae Kim³, Keun Seok Lee¹, Joo Seop Chung⁴, Jae Hoo Park⁵, Soo Hyeon Lee², Tae You Kim⁶, Kyung Hae Jung⁷, Eun Kyung Cho⁸, Yang Soo Kim⁹, Hong Suk Song¹⁰, Jae Hong Seo¹⁰, Hun Mo Ryoo¹¹, Sun Ah Lee¹², So Young Yoon¹³, Chul Soo Kim¹⁴, Yong Tai Kim¹⁵, Hwa Jung Sung¹⁶, Si Young Kim¹⁷ and Yong Jin Lee¹⁸. ¹National Cancer Center, Goyang, Gyeonggi-do, Korea; ²Severance Hospital, Seoul, Korea; ³Asan Medical Center, Seoul, Korea; ⁴Pusan National University Hospital, Busan, Korea; ⁵Seoul National University Hospital, Seoul, Korea; ⁶Gachon University Gil Medical Center, Incheon, Korea; ⁷Kosin University Gospel Hospital, Busan, Korea; ⁸Keimyung University Dongsan Medical Center, Daegu, Korea; ⁹Korea University Guro Hospital, Seoul, Korea; ¹⁰Daegu Catholic University Medical Center, Daegu, Korea; ¹¹Daegu Fatima Hospital, Daegu, Korea; ¹²Konkuk University Medical Center, Seoul, Korea; ¹³Inha University Hospital, Incheon, Korea; ¹⁴National Health Insurance Service Ilsan Hospital, Goyang, Gyeonggi-do, Korea; ¹⁵Korea University Ansan Hospital, Ansan, Gyeonggi-do, Korea; ¹⁶Kyung Hee University Medical Center, Seoul, Korea and ¹⁷Samyang Biopharmaceuticals Corporation, Seoul, Korea.

Body: Background: The rationale for developing an alternative paclitaxel formulation concerns Cremophor EL-related side effects, and a novel paclitaxel delivery system might augment its therapeutic efficacy. Genexol-PM is a novel polymeric micelle formulated paclitaxel free of Cremophor. The polymeric micelle formulation is composed of hundreds of low molecular weight, non-toxic, and biodegradable amphiphilic diblock copolymers which include monomethoxy poly (ethylene glycol)-block-poly (D,L-lactide). This multicenter phase III study was designed to evaluate the non-inferiority of efficacy of Genexol-PM compared to conventional CrEL-based paclitaxel.

Methods: In this phase III study, 213 patients were enrolled onto the study and randomly assigned (1:1) to treatment group according to prior recurrent or metastatic breast cancer chemotherapy. The study evaluated the objective response rate for the primary objective, and others including overall survival (OS), progression free survival (PFS), time to tumor progression (TTP), duration of overall response and adverse events. Eligible patients were randomly assigned to receive either Genexol-PM or standard paclitaxel. Genexol-PM or standard paclitaxel was administered on the first day of every 3 weeks. Measurable disease was assessed by imaging using the RECIST 1.0 criteria.

Results: The objective response rate (ORR) was higher by the administration of the study drug (39.05% v 24.30% in ITT, 56.92% v 39.29% in PP). One-sided 95% upper confidence limit was -4.36%, which is lower than the non-inferiority threshold (7%), indicating that the study group is not inferior to the control group. OS, PFS, TTP and duration of overall response were analyzed in the ITT population. The analysis of OS showed no significant difference (p=0.5878) (859 days, 95% CI : 732.00 – 1,025.00 v 726 days, 95% CI : 553.00 – ). Median PFS periods were 232 days (95% CI: 164.00 – 274.00) vs. 191 days (95% CI: 159.00 – 237.00). Median TTPs were 233 days (95% CI: 165.00 – 286.00) vs. 191 days (95% CI: 159.00 – 241.00) between the groups. Difference in PFSs and TTPs between the groups were not statistically significant. (p=0.2407, 0.2076, respectively) Genexol-PM was not significantly different from the comparator in terms of safety.

Conclusion: Genexol-PM demonstrated non-inferior efficacy and comparable safety profile compared with standard paclitaxel in this patient population. Of note, Genexol-PM permits the delivery of a higher paclitaxel dose without additional toxicity achieved with CrEL-based formulation. In the absence of CrEL, no filter or special tubing is required that conventional PVC infusion sets can be used.
Phase III trial evaluating paclitaxel plus carboplatin versus paclitaxel plus epirubicin as first-line treatment for metastatic breast cancer

Zhongsheng Tong¹, Shufen Li¹, Yehui Shi¹, Xu Wang¹, Chunfang Hao¹, Lihong He¹, Guolei Dong¹, Xiaorui Wang¹, Yongsheng Jia¹ and Li Zhang¹. ¹Tianjin Medical University Cancer Institute and Hospital, Tianjin, China.

Background: To compare survival and side-effects between patients with metastatic breast cancer (MBC) treated with paclitaxel/carboplatin (TP) or paclitaxel/epirubicin (TE) chemotherapy.

Patients and methods: From June 2009 to April 2012, 121 patients were randomly assigned, 61 of whom were randomized to TP and 60 to TE. Baseline characteristics were relatively well-balanced in the two treatment arms.

Table 1 Baseline patient characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>TP(n=61)</th>
<th>TE(n=60)</th>
<th>P(χ² test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age, years(range)</td>
<td>53.5(35-68)</td>
<td>51.8(32-69)</td>
<td>0.614</td>
</tr>
<tr>
<td>ECOG performance status &lt;2</td>
<td>61(100%)</td>
<td>60(100%)</td>
<td></td>
</tr>
<tr>
<td>Node positive at first diagnosis</td>
<td>27(44.3%)</td>
<td>25(41.7%)</td>
<td>0.773</td>
</tr>
<tr>
<td>ER/PR-positive</td>
<td>42(68.9%)</td>
<td>43(71.7%)</td>
<td>0.735</td>
</tr>
<tr>
<td>HER2-positive</td>
<td>18(29.5%)</td>
<td>15(25.0%)</td>
<td>0.578</td>
</tr>
<tr>
<td>Metastatic sites</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥2</td>
<td>33(58.1%)</td>
<td>35(55.2%)</td>
<td>0.639</td>
</tr>
<tr>
<td>Visceral</td>
<td>40(65.6%)</td>
<td>42(70.0%)</td>
<td>0.602</td>
</tr>
<tr>
<td>Lymph</td>
<td>25(41.0%)</td>
<td>28(46.7%)</td>
<td>0.529</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lobular</td>
<td>12(19.7%)</td>
<td>11(18.3%)</td>
<td>0.851</td>
</tr>
<tr>
<td>Ductal</td>
<td>44(72.1%)</td>
<td>42(70.0%)</td>
<td>0.796</td>
</tr>
<tr>
<td>Other</td>
<td>5(8.2)</td>
<td>7(11.7%)</td>
<td>0.523</td>
</tr>
<tr>
<td>Adjuvant endocrine therapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>28(45.9%)</td>
<td>25(41.7%)</td>
<td>0.639</td>
</tr>
<tr>
<td>No</td>
<td>33(54.1%)</td>
<td>35(58.3%)</td>
<td></td>
</tr>
<tr>
<td>Adjuvant radiotherapy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>13(21.3%)</td>
<td>11(18.3%)</td>
<td>0.681</td>
</tr>
<tr>
<td>No</td>
<td>48(78.7%)</td>
<td>49(81.7%)</td>
<td></td>
</tr>
</tbody>
</table>

Results: After a median follow-up of 21.3 months, there was no significant difference between the two treatment arms in objective response rate (ORR) (68.5 % vs. 73.3%, respectively). And both the progression-free survival (P=0.704) and overall survival (P=0.403) were very similar between the two arms. Both regimens were well tolerated. The main toxicities were myelosuppression, gastrointestinal reactions and alopecia. There was more grades 3–4 alopecia and more nausea with TE (p<0.05). For the QLQ-C30 questionnaire statistically significant changes after treatment were fatigue (0.031) in TP, nausea/vomiting (p=0.041) and lose of appetite (p=0.048) in TE.

Table 2 Summary of adverse events per patient; p-values are given overall
<table>
<thead>
<tr>
<th>Adverse events</th>
<th>TP (n=61)</th>
<th></th>
<th>TE (n=60)</th>
<th></th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Grades 1-4</td>
<td>Grades 3-4</td>
<td>Grades 1-4</td>
<td>Grades 3-4</td>
<td></td>
</tr>
<tr>
<td>Neutropenia</td>
<td>46</td>
<td>13</td>
<td>38</td>
<td>7</td>
<td>0.149</td>
</tr>
<tr>
<td>Leukopenia</td>
<td>50</td>
<td>11</td>
<td>40</td>
<td>7</td>
<td>0.054</td>
</tr>
<tr>
<td>Anemia</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0.981</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>4</td>
<td>1</td>
<td>3</td>
<td>0</td>
<td>0.714</td>
</tr>
<tr>
<td>Lymphocytopenia</td>
<td>2</td>
<td>0</td>
<td>4</td>
<td>1</td>
<td>0.391</td>
</tr>
<tr>
<td>Vomiting</td>
<td>15</td>
<td>3</td>
<td>20</td>
<td>6</td>
<td>0.289</td>
</tr>
<tr>
<td>Nausea</td>
<td>27</td>
<td>2</td>
<td>41</td>
<td>5</td>
<td>0.010</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>4</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>0.714</td>
</tr>
<tr>
<td>Alopecia</td>
<td>44</td>
<td>15</td>
<td>60</td>
<td>31</td>
<td>0.000</td>
</tr>
<tr>
<td>Cardiotoxicity</td>
<td>3</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0.523</td>
</tr>
<tr>
<td>Hypersensitivity reaction</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>ALT increase</td>
<td>5</td>
<td>0</td>
<td>7</td>
<td>0</td>
<td>0.523</td>
</tr>
<tr>
<td>Peripheral neurotoxicity</td>
<td>20</td>
<td>0</td>
<td>19</td>
<td>0</td>
<td>0.895</td>
</tr>
</tbody>
</table>

Overall p value for the comparison of grades 1-4 are given

Conclusion: This study did not found significant differences in ORR, PFS and OS between TP and TE arms. Both arms were well tolerated. TP has some advantages in less side-effects, especially alopecia and nausea. This combination can be safely prescribed to patients in which anthracyclines have the potential of being harmful such as those previously exposed to anthracyclines in the adjuvant setting.
Title: Real-world efficacy and safety outcomes of nab-paclitaxel (nab-P) in patients (pts) with metastatic breast cancer (MBC): Results from a US health insurance database

Debra A Patt¹, Caihua Liang², Ling Li², Amy Ko³, Cindy Duval Fraser³, Deyanira Corzo³ and Cheryl Enger⁴. ¹McKesson Specialty Health/US Oncology, Houston, TX; ²Optum Epidemiology, Waltham, MA; ³Celgene Corporation, Summit, NJ and ⁴Optum Epidemiology, Ann Arbor, MI.

Body: Background: nab-P, an albumin-bound formulation of paclitaxel, was approved in 2005 for the treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 mo of adjuvant chemotherapy based on clinical trials. nab-P demonstrated efficacy and safety when administered weekly in phase II trials and every 3 weeks (q3w) in an international phase III trial. Little is known about the treatment patterns and outcomes of nab-P in the real-world setting. Using health insurance claims data, this study was conducted to characterize efficacy and safety of nab-P in pts with MBC treated in US clinical practices.

Methods: This retrospective claims analysis used data in the Optum Research Database (United Health affiliate). Data were supplemented by Social Security Death Index sources. The analysis included women aged ≥ 18 y with MBC diagnosis (≥ 2 claims of BC diagnosis separated by ≥ 30 d and ≥ 2 claims of metastatic spread) prior to nab-P initiation.Pts had ≥ 6 mo of continuous enrollment in the health plan from January 2005-September 2012, complete medical coverage and pharmacy benefits, no other primary malignancy, and no prior chemotherapy. Cohorts were determined by line of therapy, nab-P regimen, and schedule. Endpoints included treatment patterns, time to next therapy or death (TNTD), overall survival (OS), and safety.

Results: Of the 664 eligible pts, most were between 40-69 y of age (88%) and had received nab-P as ≥ second-line therapy (74%), monotherapy (61%), and weekly dosing (71%). In combination, nab-P was most often given with bevacizumab (58%) or human epidermal growth factor receptor 2 (HER2)—targeted therapy (24%) vs another cytotoxic agent (19%). Median TNTD and OS were 6.1 and 17.4 mo, respectively. By line of therapy (first, second, and ≥ third), TNTD was 7.1, 6.6, and 5.3 mo, and OS was 22.7, 17.4, and 15.1 mo. The OS data are comparable with published clinical trial results (Table). In a subgroup of pts (n = 400) with aggressive disease features (≤ 50 y of age or having ≥ 3 metastases), median OS was 15.6 mo. These data are comparable with a retrospective analysis of pts with visceral dominant metastasis (VDM) or a short disease-free interval (SDFI; Table). Toxicities reported in healthcare claims were consistent with those previously published.

Conclusions: Consistent with clinical trial data, outcomes of this analysis demonstrated the efficacy and safety of nab-P across lines of therapy in a real-world population of patients with MBC.

Clinical Trial Experience in MBC for Pts Treated With nab-P

<table>
<thead>
<tr>
<th>Trial</th>
<th>nab-P dose (mg/m²) and schedule</th>
<th>n</th>
<th>Median OS, mo</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA012¹ (Ph III)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ITT (all lines)</td>
<td>260 q3w</td>
<td>229</td>
<td>15.0</td>
</tr>
<tr>
<td>≥ second line</td>
<td>260 q3w</td>
<td>131</td>
<td>13.0</td>
</tr>
<tr>
<td>CA024² (Ph II, first line)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>300 q3w</td>
<td>76</td>
<td>27.7</td>
<td></td>
</tr>
<tr>
<td>100 qw 3/4</td>
<td>76</td>
<td>22.2</td>
<td></td>
</tr>
<tr>
<td>150 qw 3/4</td>
<td>74</td>
<td>33.8</td>
<td></td>
</tr>
<tr>
<td>Subgroups With Aggressive Disease Features</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>VDM</td>
<td>n</td>
<td>SDFI</td>
</tr>
<tr>
<td>CA012³ (first line)</td>
<td>260 q3w</td>
<td>74</td>
<td>15.1</td>
</tr>
<tr>
<td>CA024³ (first line)</td>
<td>300 q3w</td>
<td>61</td>
<td>27.7</td>
</tr>
<tr>
<td>Dose</td>
<td>Count</td>
<td>%V</td>
<td>%D</td>
</tr>
<tr>
<td>------------</td>
<td>-------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>100 qw 3/4</td>
<td>60</td>
<td>19.6</td>
<td>21</td>
</tr>
<tr>
<td>150 qw 3/4</td>
<td>59</td>
<td>32.1</td>
<td>14</td>
</tr>
</tbody>
</table>

qw 3/4, weekly for the first 3 of 4 weeks.

Phase I trial of eribulin in combination with S-1 for advanced and recurrent breast cancer patients

Tsutomu Iwasa¹, Junji Tsurutani¹, Satomi Nishida¹, Yoshifumi Komoike² and Kazuhiko Nakagawa¹. ¹Kinki University Faculty of Medicine, Osaka-Sayama, Osaka, Japan and ²Kinki University Faculty of Medicine, Osaka-Sayama, Osaka, Japan.

Introduction: Eribulin is a non-taxane microtubule dynamics inhibitor that has been proved to prolong overall survival in patients with advanced and recurrent breast cancer compared to treatment of physician’s choice. S-1 is an oral fluoropyrimidine anticancer agent that combines tegafur as the effector drug with two modulators, gimeracil, and oteracil potassium, and has shown promising antitumor activity as a single agent for the treatment of advanced breast cancer. In the basis of these results, we aimed to assess the efficacy of the combination of these two drugs in patients with advanced and recurrent breast cancer. In this trial, we determined maximum tolerated dose (MTD) and phase II recommended dose of Eribulin and S-1 combination therapy. Methods: A traditional 3+3 dose escalation design was implemented. 12 patients pre-treated with anthracycline and taxane were enrolled. As an initiation level, patients received s-1 50 mg/m² from day1 to day14, and Eribulin 1.1mg/m2 on day1, and day8. In level 2, Eribulin dose was increased to 1.4mg/m2. In level 3, S-1 dose was increased to 80 mg/m2. Results: In level 1 or 2, no dose limiting toxicity (DLT) was observed. In level 3, grade 3 hypokalemia occurred in 1 case. The level 3 dose level was determined as phase II recommended dose. Neutropenia was observed in all cases, 3 of which was grade 3 febrile neutropenia. However, other non-hematological toxicity was mild, and the toxicity of the both drugs was not enhanced by the combination. Partial response

Efficacy (N=12)

| CR | 0 (0%) |
| PR | 5 (41%) |
| SD≥24 weeks | 2 (17%) |
| SD<24 weeks | 3 (25%) |
| PD | 2 (17%) |
| Clinical Benefit | 7 (58%) |
| Disease Control Rate | 10 (83%) |

is confirmed in 5/11 patients with measurable lesions. Conclusion: We have shown that treatment with Eribulin and S-1 is active and well tolerated in patients with advanced and recurrent breast cancer, suggesting that this combination therapy may have potential as a new treatment option. A phase II study is being conducted to evaluate the efficacy and safety.
Title: A Randomized phase II trial compared docetaxel with or without low-dose metronomic oral cyclophosphamide in first line treatment of non-triple-negative advanced breast cancer (ABC)

Zhonghua Wang¹, Leiping Wang¹, Jian Zhang¹, Biyun Wang¹, Fangfang Lv¹, Jun Cao¹, Sheng Zhang¹, Zhimin Shao¹ and Xichun Wang¹. ¹Shanghai Cancer Center, Fudan University, Shanghai, China.

Body: Background: Oral metronomic chemotherapy may target tumor cells indirectly via antiangiogenic activity, restoration of anticancer immune response, or induction of tumor dormancy. This phase II study (NCT01526499) aims to evaluate the efficacy of low-dose metronomic oral cyclophosphamide in addition to docetaxel as first-line therapy.

Patients and Methods: Eligible patients with ER or PR positive or HER-2-overexpressed ABC who previously untreated were randomly assigned to receive docetaxel 75 mg/m² on day 1 with or without continuous oral cyclophosphamide 50mg daily in a 21-day cycle. Patients with HER-2-overexpressed tumors should receive trastuzumab. All patients received docetaxel until disease progression or unacceptable toxicity or withdrawal of consent. Maintenance endocrine and/or trastuzumab were allowed. The primary endpoint was PFS.

Results: Between Dec 2011 and Nov 2012, 35 patients were randomized to cyclophosphamide plus docetaxel (Metro-TC) group while 31 to docetaxel (T) group. The majority of the patients (83.3%) were hormonal receptor positive; 31.8% were HER2 over-expressed; 84.8% had visceral metastasis and 48.5% had ≥3 metastatic organ sites. Patients' characteristics were well balanced between two groups. Median treatment cycles of docetaxel for both groups were eight cycles. In intention-to-treat population with median follow-up of 18 months, median PFS was statistically longer in the Metro-TC group (not reached) than it was in the T group (13.6 months, 95%CI, 7.0 to 20.2) (P =.04). Median OS had not been reached. The ORR were 51.6% (16/31) in the T and 71.4% (25/35) in Metro-TC group, respectively (P =.09). There was no significant difference of grade 3/4 toxicities between the two groups. Adverse effects were mainly docetaxel-related, including grade 3/4 neutropenia (100%) and febrile neutropenia (n=19, 29.2%). The only significant difference between the two treatment was mucositis (all grade, 10% versus 43%, P=0.003). There were no treatment-related deaths.

Conclusions: The addition of metronomic cyclophosphamide to standard chemotherapy as first-line treatment for non-triple-negative ABC shows a benefit in PFS without significant increase in toxicity.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-10-09
Average Grade: 0

Title: A Phase II trial of doxil, carboplatin and bevacizumab in metastatic triple negative breast cancer and molecular correlates of response

Kim M Hirshfield¹, Shou-En Lu¹, Serena Wong¹, Antoinette Tan¹, Laurie Kirstein¹, Thomas Kearney¹, Weichung Shih¹, Shridar Ganesan¹ and Deborah L Toppmeyer¹. ¹Rutgers Cancer Institute of New Jersey, New Brunswick, NJ.

Body: Background. Despite improved outcomes for other breast cancer subtypes, triple negative breast cancer continues to display the worst prognosis with conventional, standard of care chemotherapy. A growing body of evidence suggests that platinating agents may offer superior outcomes in a subset of triple negative breast cancers and that genomic alterations may identify those tumors most likely to respond.

Patients and Methods. Eligibility included previously untreated, stage IV ER, PgR, and Her2neu negative breast cancer with measurable disease. Thirty-one patients with ECOG PS ≤ 2 and adequate bone marrow, renal and hepatic function were enrolled to receive doxil (30 mg/m²), carboplatin (AUC 5) and bevacizumab (15 mg/m²) every 4 weeks in an open-label, multicenter, investigator-initiated Phase II trial from 2008-2012. The primary endpoint was median progression free survival (PFS). Secondary endpoints were response rate based on RECIST criteria, survival time, and toxicity profile.

Results. Thirty-one patients received a median of 5.6 cycles (range 1-13). Prior adjuvant or neoadjuvant anthracycline or taxane was given in 38.7% or 41.9%, respectively. Clinical benefit rate (CBR= CR+PR+SD ≥ 6months) was 38.7% where 9/18 (50%) with CBR<6 months and 4/13 (30.7%) with CBR>6 months had prior taxane. Median progression free survival (PFS) was 5.6 months, 95% CI [4.4-6.9] and 6-month PFS rate was 41.9%, 95% CI [24.6-59.3]. Median overall survival was 11.9 months, 95% CI [8.8-21.8] and 1-year survival rate was 47.3%, 95% CI [29.5-65.1]. Thirteen patients were alive at 10 months or longer. Grade 3 non-hematologic and hematologic toxicities included fatigue (n=1), hand-foot skin reaction (n=1), and neutropenia (n=1). Grade 4 hematologic toxicity included thrombocytopenia (n=1). Grade 4 hypertension (n=2) and thrombosis (n=1) were attributable to bevacizumab and this drug was discontinued from the protocol. Neither significant change in cardiac function nor alopecia were observed. Next-generation sequencing from tumors revealed p53 alterations as the most common alteration. For three patients with serial pre- and post-platinum specimens, gain of genomic alterations were observed at time of progression of disease occurring at 5 months, 8 months, and 20 months (eight months of these were off study but receiving doxil and carboplatin).

Conclusions. Results demonstrate that the combination of doxil, carboplatin, and bevacizumab is an active and well-tolerated regimen in previously untreated, metastatic, triple negative breast cancer. We anticipated presentation of additional genomic tumor profiling results that may yield insights into markers of sensitivity and mechanism of resistance to anthracycline and/or platinum.
Title: Neoadjuvant chemotherapy with or without bevacizumab or everolimus: Survival analysis of The HER2-negative cohort of the GEPARQUINTO study (GBG 44)

Bernd Gerber¹, Sibylle Loibl², Michael Untch³, Holger Eidtmann⁴, Mahdi Rezai⁵, Peter A Fasching⁶, Hans Tesch⁷, Holm Eggemann⁸, Iris Schrader⁹, Kornelia Kittel¹⁰, Claus Hanusch¹¹, Jens Huober¹², Christine Solbach¹³, Christian Jackisch¹⁴, Georg Kunz¹⁵, Jens-Uwe Blohmer¹⁶, Maik Hauschild¹⁷, Tanja Fehm¹⁸, Valentina Nekljudova² and Gunter von Minckwitz².¹³

¹Unifrauenklinik Rostock; ²German Breast Group, Neu-Isenburg, Hessen, Germany; ³Helios Klinikum Berlin-Buch; ⁴Unifrauenklinik Kiel; ⁵Luisenkrankenhaus Düsseldorf; ⁶Unifrauenklinik Erlangen; ⁷Chop GmbH Frankfurt; ⁸Unifrauenklinik Magdeburg; ⁹Henriettenstiftung Hannover; ¹⁰Praxisklinik Berlin; ¹¹Rotkreuzklinikum München; ¹²Unifrauenklinik Ulm; ¹³Unifrauenklinik Frankfurt/Mail; ¹⁴Sana Klinikum Offenbach; ¹⁵St Johannes Hospital Dortmund; ¹⁶St Gertrauden Berlin; ¹⁷Frauenklinik Rheinfelden and ¹⁸Unifrauenklinik Duesseldorf.

Body: BACKGROUND: The GeparQuinto study showed that adding bevacizumab to 24 weeks of anthracycline-taxane-based neoadjuvant chemotherapy increases pathological complete response (pCR) rates from 14.9% to 18.4% (P=0.04) overall; specifically in patients with TNBC (27.9% to 39.3% (P=0.003) (von Minckwitz et al, NEJM 2012). No difference in pCR rate was observed for adding everolimus to paclitaxel patients who had no early response to neoadjuvant chemotherapy (Huober et al, Eur J Cancer 2013). Here, we present disease-free (DFS) and overall survival (OS) analyses.

PATIENTS AND METHODS: Patients (n = 1948) with HER2-negative tumors of a median tumor size of 4 cm were randomly assigned to neoadjuvant treatment with 4xEC à 4x docetaxel with or without bevacizumab, 15 mg/Kg q3w before surgery. 408 patients not clinically responding to EC ± Bev were randomized to 12x weekly paclitaxel with or without everolimus 5mg/day. Patients with HR-positive tumors received endocrine treatment after surgery. 379 events are required to show a HR of 0.75 (α=0.05, β=0.8) between the bevacizumab arms. 397 relapses and 234 deaths were observed after a median follow up of 3.8 years overall, of those 115 relapses and 75 deaths occurred in the non-responding cohort.

RESULTS: Overall, 3-year DFS was 80.8% and 3-year OS was 89.7%. Outcome was not different for patients receiving bevacizumab (HR 1.03; P = 0.780 for DFS and HR 0.974; P = 0.842 for OS) compared to patients receiving chemotherapy alone. Patients with TNBC showed no improvement in DFS (HR 0.991; P = 0.948) and OS (HR 1.02; P = 0.891) when treated with bevacizumab. No other predefined subgroup (HR+/HER2-; locally advanced (cT4 or cN3) or not; cT1-3, cT4a-c, cT4d; pCR or not, CR, PR, NC after first 4 cycles chemotherapy) showed a benefit from bevacizumab. No difference in DFS (HR 0.997; P=0.987) and OS (HR 1.11; P=0.658) was observed for patients who had no early response to neoadjuvant chemotherapy receiving paclitaxel with or without everolimus overall as well as in subgroups.

CONCLUSIONS: Long-term results finally do not support the neoadjuvant use of bevacizumab in addition to an anthracycline-taxane-based chemotherapy or everolimus in addition to paclitaxel for patients who had no early response to neoadjuvant chemotherapy.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-11-02
Average Grade: 8.75

Title: Predictors of complete pathological response to neoadjuvant chemotherapy for breast cancer: 19,310 cases from the national cancer database treated in 2010 and 2011

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Body: Background: Complete pathological response following neoadjuvant chemotherapy for breast cancer indicates an excellent prognosis and may influence subsequent local or systemic treatment. However it is not clear which patients should undergo neoadjuvant chemotherapy.

Methods: The National Cancer Database is a joint project of the Commission on Cancer of the American College of Surgeons and the American Cancer Society and contains about 80% of the cancer cases in the United States. Patients undergoing neoadjuvant chemotherapy for breast cancer in 2010 and 2011 were identified and the pathological response was compared across multiple demographic and tumor associated variables. Complete pathological response (pCR) was defined as no invasive cancer remaining in the breast or lymph nodes at the time of subsequent surgery.

Results: Out of 334,447 cases of breast cancer diagnosed in 2010 and 2011, 29,534 (8.8%) underwent neoadjuvant chemotherapy. This included 2052 (6.9%) who also received neoadjuvant hormonal therapy and 616 (2.1%) who also received neoadjuvant radiation therapy. The pathological response was known for 19,310 (65%) and 6244 (32%) had a complete response (pCR), 11,522 (60%) had a partial response, and 1544 (8%) had no response. In logistic regression analysis, demographic and tumor related variables were significant (p<0.05) in both univariable and multivariable models. Estimated odds ratio of having pCR and p-values of demographic and tumor related variables are shown in Table 1, where a multivariable logistic regression was used to fit the data of 15686 patients.

Conclusions: These data can be used to construct a nomogram that estimates the chance of pCR based on patient age, tumor histology, grade, molecular type, clinical T stage, and clinical N stage. This may be useful to help select suitable candidates for neoadjuvant chemotherapy.

Predictors of Complete Pathological Response : Multivariate Logistic Regression

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</table>
Title: A randomized, phase 2 trial of preoperative MM-121 with paclitaxel in triple negative (TN) and hormone receptor (HR) positive, HER2-negative breast cancer

Frankie A Holmes¹,Kristi J McIntyre²,Ian E Krop³,Cynthia R Osborne⁴,John W Smith II⁵,Manuel R Modiano⁶,Manish Gupta⁷,Leona B Downey⁸,Rita Nanda⁹,Mansoor N Saleh¹⁰,Jonathan R Young¹¹,Kerry E Horgan¹¹,William Kubasek¹¹,Gavin MacBeath¹¹,Michael A Danso¹² and Joyce A O'Shaughnessy⁴.¹Texas Oncology, Memorial City, Houston, TX; ²Texas Oncology, Dallas Presbyterian Hospital, Dallas, TX; ³Dana-Farber Cancer Institute, Boston, MA; ⁴Texas Oncology, Baylor Charles A Sammons Cancer Center, Dallas, TX; ⁵Northwest Cancer Specialists, P.C., Portland, OR; ⁶ACRC/Arizona Clinical Research Center, Tuscon, AZ; ⁷Texas Oncology, Garland, Garland, TX; ⁸Arizona Oncology Associates, Tucson, AZ; ⁹University of Chicago Medicine, Chicago, IL; ¹⁰Georgia Cancer Specialists, Atlanta, GA; ¹¹ Merrimack Pharmaceuticals, Inc, Cambridge, MA and ¹²Virginia Oncology Associates, Norfolk, VA.

Body: Background: MM-121 is a fully human monoclonal antibody that targets ErbB3 and blocks heregulin (HRG), the principal ligand for ErbB3, from binding. HRG-driven ErbB3 signaling has been widely implicated in the development of resistance to conventional chemotherapies and targeted agents. Recently, as part of a broader Phase 2 program designed to identify responders to MM-121, HRG was identified as a key biomarker that correlates with poor response to standard-of-care therapies and benefit from co-treatment with MM-121 in the metastatic setting in patients with NSCLC, ovarian cancer and breast cancer. Here we present data on the impact of HRG and MM-121 co-treatment in the pre-operative setting in HER2- breast cancer.

Methods: Patients enrolled had locally advanced HR+, HER2-negative invasive breast cancer (Group 1) or TN (Group 2), no distant metastatic disease, no prior treatment for the disease under study, and ≥T2. Patients were randomized in a 2:1 fashion to receive MM-121 plus paclitaxel or paclitaxel alone followed by doxorubicin and cyclophosphamide, followed by surgery. The primary endpoint of this study was to describe pCR rates, defined as the absence of invasive cancer in the breast and lymph nodes (i.e. ypT0, ypN0). Residual cancer burden index (RCBI) was also recorded for each patient. Pre- and post-treatment tumor biopsies were collected in order to assess the levels of HRG and other potential biomarkers.

Results: 200 patients (101 HR+, 99 TN) entered the study. For Group 1 (HR+), 96 of the 101 patients were Efficacy Evaluable (EE) and the pCR rate in the MM-121 treatment arm was 7/66 (10.6%, 95% CI [5.2%, 20.3%]) compared to 1/30 (3.3%, 95% CI [0.6%, 16.7%]) on the control arm. For Group 2 (TN), 85 of the 99 patients were Efficacy Evaluable (EE) and the pCR rate in the MM-121 treatment arm was 23/56 (41.1%, 95% CI [29.2%, 54.1%]) compared to 14/29 (48.3%, 95% CI [31.4%, 65.6%]) on the control arm. Preliminary analysis of pretreatment biopsies (52% of samples analyzed) suggests a potential link between HRG levels and both pCR rates and RCBI. For both groups, the overall incidence of adverse events, regardless of relationship, was comparable between the two arms. A higher frequency of any grade AEs (Treatment vs. Control) was reported for diarrhea, rash, stomatitis, fatigue, epistaxis, nail disorder and dysgeusia. A higher frequency of Grade 3 or higher AEs (Treatment vs. Control) was reported for febrile neutropenia (HR+: 10.4% vs. 3.0%), diarrhea (HR+: 7.5% vs. 0%, TN: 7.8% vs. 0%), fatigue (HR+: 6.0% vs. 3.0%), anemia (HR+: 7.5% vs. 3.0%, TN: 7.8% vs. 3.1%), hypokalemia (HR+: 7.5% vs. 3.0%), infusion-related reactions (TN: 4.7% vs. 0%), pulmonary embolisms (TN: 4.7% vs. 0%), and hyperglycemia (TN: 4.7% vs. 0%).

Conclusion: Although a potential signal of benefit from MM-121 was observed in the HR+ group, the same was not observed in the TN group, and overall the results are inconclusive. Biomarker analyses, including pharmacodynamics studies, are ongoing. The observed safety profile is consistent with the expected toxicities associated with ErbB inhibitors and weekly paclitaxel, and did not impact exposure or compliance on treatment.
Title: Effects of neoadjuvant chemotherapy with or without zoledronic acid on pathological response: A meta-analysis of randomised trials

Judith R Kroep1, Ayoub Charehbili1, Rob E Coleman2, Rebecca L At3, Yoshiie Hasegawa4, Gerrit-Jan Liefers1, Matthew C Winter2, Katherine N Weilbaecher1, Kohei Akazawa5, Samantha Hinsley6, Hein Putter1, Hans WR Nortier1 and Norio Kohno7.

1Leiden University Medical Center, Leiden, Netherlands; 2University of Sheffield, Sheffield, United Kingdom; 3Washington University School of Medicine, St Louis, MO; 4Hirosaki Municipal Hospital, Aomori, Netherlands; 5Niigata University, Niigata, Japan; 6University of Leeds, Leeds, United Kingdom and 7Kobe Kaisei Hospital, Kobe, Hyogo, Japan.

Body: Background:
The addition of bisphosphonates to adjuvant systemic therapy reduces bone metastases and improves survival in postmenopausal patients with early breast cancer. We have conducted four randomised trials of neoadjuvant chemotherapy (CT) +/- zoledronic (ZA) in stage II/III breast cancer to investigate the potential for enhancing pathological response within the breast and axilla. We report here a meta-analysis of these studies to enable more reliable evaluation of treatment effects and investigate activity in pre-defined subgroups of interest.

Methods:
Individual patient data from four prospective randomized clinical trials reporting the effect of the addition of ZA on pathological response after neoadjuvant CT were pooled. Primary outcomes were pathological complete response in the breast (pCRb) and in the breast and lymph nodes (pCR). A fixed effects Mantel-Haenszel meta-analysis was performed using odds ratios (OR) from multivariate analyses in each study correcting for T-status and ER-status after testing for heterogeneity. Predefined subgroup analyses were performed for postmenopausal women and patients with triple-negative breast cancer.

Results:
pCRb and pCR data were available in 736 and 553 women respectively. A summary of results is shown in the table below. In the total study population, ZA addition to neoadjuvant CT did not increase pCRb/pCR rates (12.9% for CT only vs. 16.0% with CT+ZA; OR 1.34, 95% C.I. 0.86-2.08). However, in postmenopausal women, the addition of ZA resulted in a doubling of the pCRb rate (8.5% for CT only vs. 17.0% with CT+ZA; OR 2.72, 95% C.I. 1.15-6.42) and pCR rate (7.8% for CT only vs. 13.6% with CT+ZA; OR 2.48, 95% C.I. 0.80-7.69). There was significant interaction between menopausal status and ZA benefit (P for interaction=0.047). In patients with triple-negative breast cancer a trend was observed favouring CT+ZA.

Pathological response

<table>
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<tr>
<th>Pathological response</th>
<th>CT</th>
<th>CT + ZA</th>
<th>Odd's Ratio</th>
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<td></td>
</tr>
<tr>
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<td>48/373</td>
<td>12.9</td>
<td>58/363</td>
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<tr>
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<td>24/141</td>
<td>17.0</td>
</tr>
<tr>
<td>Pre/perimenopausal patients</td>
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<td>14.7</td>
<td>33/214</td>
<td>15.4</td>
</tr>
<tr>
<td>Triple-negative breast cancer</td>
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<td>18.6</td>
<td>21/67</td>
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<td>pCR in breast and lymph nodes</td>
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<td></td>
<td></td>
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<tr>
<td>Total population</td>
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<td>33/274</td>
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</tr>
<tr>
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<td>17.3</td>
<td>15/51</td>
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</table>
Conclusion:
This meta-analysis shows that no overall benefit from the addition of ZA to neoadjuvant CT. However, as has been seen in the adjuvant setting, the addition of ZA to CT in postmenopausal women with early breast cancer appears to specifically increase the effects of CT with increases in pCRb and pCR. Further translational research is warranted to explore the mechanism of these observations and the potential treatment interaction with menopause.
Title: Neoadjuvant chemotherapy in invasive lobular breast carcinoma: Comparison of response, surgery and disease free survival with invasive ductal carcinoma

Hilary L Martin1,2,3, Geraldine Walsh1, Komel Khabra1, Tabitha Skinner1 and Ian E Smith1. 1Royal Marsden Hospital, London, United Kingdom; 2Royal Perth Hospital, Perth, WA, Australia and 3University of Western Australia, Perth, WA, Australia.

Body: Background
Lobular breast cancer (ILC) has been reported to be associated with a lower rate of pathologic complete response (pCR) and breast conserving surgery following neoadjuvant chemotherapy compared with ductal carcinoma (IDC). ILC is predominantly oestrogen receptor positive, HER-2-ve and histologic grade 1 or 2. Oestrogen receptor positive breast cancer has been shown to have poorer responses to neoadjuvant chemotherapy. HER-2 positivity is associated with better responses to anthracycline based neoadjuvant chemotherapy than HER-2 negative, and substantially improved pathological complete response (pCR) rates relative to HER-2 negative disease with the use of HER-2 targeted agents in this patient group. This study investigates response and disease free survival of ILC compared with IDC.

Methods
Patients with ILC and IDC treated with neoadjuvant chemotherapy were identified from the prospectively collated breast unit database at the Royal Marsden Hospital. Demographic, tumour, treatment and outcome data was obtained from the database and hospital electronic patient record system. Statistical analysis was undertaken comparing IDC and ILC. Clinical response rates, pCR rates, breast conserving surgery rates, and disease free survival were analysed. Clinical response was defined as those with partial response (≥50% reduction in the product of breast tumour size from baseline measurement), or complete clinical response (no clinically palpable breast tumour). Subgroup analysis was performed on ER+ve, HER-2–ve and HER-2 unknown cases.

Results
A total of 1017 patients were identified. Of these, there were 920 IDC and 97 ILC cases. Median age at commencement of neoadjuvant chemotherapy was 47.9 years (range 23.6-74.9). Median follow-up was 7.6 years (range 0.2-26.5). Pathologic response rates were available for 717 patients. 119 (19%) IDC had pCR, and 1 (1.1%) ILC (p <0.001). Of the patients with clinical response data available, 762 (82.8%) of the IDC and 74 (76.3%) of the ILC had a clinical response (p 0.19). Of the 804 IDC patients who underwent surgery, 480 (59.7%) had breast conserving surgery, while of the 89 lobular patients 33 (37.1%) underwent breast conserving surgery (<0.0001). Median disease free survival was 10.9 years (95%CI 8.6-13.2) for IDC and 10.1 years (95%CI 7.3-12.9) for ILC (p 0.318).
Within the ER+ve, HER2-ve/unknown group there were 403 IDC and 82 lobular patients. pCR results were available for 148 (30%) of the patients. 41 (15.8%) in the IDC and 1 (1.3%) in the ILC group had pCR (p=0.001). 335 (83.1%) in the IDC group and 64 (78.0%) in the ILC group had a clinical response (p 0.27). Breast conserving surgery rates were 212 (57.6%) in the ductal group and 30 (39.0%) in the lobular group (p 0.003).

Conclusion
Within the cohort entire rates of pCR and of breast conserving surgery were lower for those with ILC compared with IDC. Despite lower rates of pCR and breast conserving surgery in ILC, there was no difference in clinical response rates or in disease free survival compared with IDC. Significant differences in pCR and breast conserving surgery rates between ILC and IDC persisted on subgroup analysis of those with ER+ve HER2-ve/unknown breast cancer.
Title: Bevacizumab in combination with docetaxel+trastuzumab +/- non-pegylated liposomal doxorubicin: Final results of ABCSG-32, a prospective, randomized phase II-study

Guenther G Steger1, Richard Greil2, Michael Hubalek3, Michael A Fridrik4, Christian F Singer1, Rupert Bartsch1, Marija Balic5, Peter Dubsky1, Daniel Egle4, Simon P Gampenrieder2, Georg Pfeifer1, David Mayr4, Theresa Czech1, Gabriel Rinnerthaler2, Ruth Exner1, Andreas L Petzer6, Paul Sevelda7, Alois Lang8, Margaretha Rudas1, Barbara Krause9, Michael Seifert1, Sophie Frantal9, Christoph C Zielinski1 and Michael Gnant1.

1Medical University of Vienna, Vienna, Austria; 2Paracelsus Medical University, Salzburg, Austria; 3Medical University of Innsbruck, Innsbruck, Austria; 4General Hospital of Linz, Linz, Austria; 5Medical University of Graz, Graz, Austria; 6Hospital of the Sisters of Mercy, Linz, Austria; 7Hietzing Hospital, Vienna, Austria; 8Feldkirch Hospital, Feldkirch, Austria and 9Austrian Breast and Colorectal Cancer Study Group, Vienna, Austria.

Body: BACKGROUND
Pathological complete response (pCR) after neoadjuvant systemic therapy is correlated with better prognosis in HER2-positive, early breast cancer. Bevacizumab (B) was shown to add efficacy to neoadjuvant systemic treatment in HER2-negative, early breast cancer in terms of pCR-rate but failed in the adjuvant setting of both, HER2-negative and HER2-positive disease. The role of B in the neoadjuvant treatment of HER2-positive, early breast cancer is not defined. Thus, ABCSG-32 was designed as a randomized phase II trial to evaluate the efficacy, the non-cardiac safety, and the cardiac toxicity of B when added to docetaxel + trastuzumab (DT) +/- non-pegylated liposomal doxorubicin (N) in the neoadjuvant setting of early, HER2-positive breast cancer.

METHODS
100 patients with biopsy-proven, invasive, early, HER2-positive breast cancer were stratified according to major risk factors including estrogen receptor (ER)-status, histology, tumor grade, and center and were randomized to receive 6 cycles (q 21 days) of either D100 mg/m2+T8/6mg/kg (DT, n=25), DT+B15mg/kg (DTB, n=25), D75T+N50 mg/m2 (DTN, n=26), or D75TN+B15 mg/m2 (DTNB, n=24). All patients received pegfilgrastim 6 mg sc on day 2. pCR was defined as the absence of invasive tumor cells in the breast and total pCR (tpCR) was defined as the absence of invasive tumor cells in the breast and axillary nodes after NST. Left ventricular (LV) ejection fraction (EF) was measured at baseline, before each treatment cycle, and before sugery. A cardiac toxicity event (CTE) was defined as the occurrence of either symptomatic LV dysfunction NYHA II-III, or an asymptomatic drop of EF (adEF) of >15% from baseline, or an adEF <50%, or the appearance of significant arrhythmias requiring treatment. The trial was designed to detect a difference in the incidence of CTE of 8% in the control group (DT) vs. 44% in each of the experimental groups (power: 80%, two-sided alpha: 0.05).

RESULTS
The overall rate of pCR in all patients was 52% (DT: 36%, DTB: 51%, DTN: 63%, DTNB: 62%). In the ER-negative subgroup (n=43) the overall pCR rate was 63% (DT: 31%, DTB: 70%, DTN: 75%, DTNB: 88%) and the overall tpCR rate was 60% (DT: 31%, DTB: 60%, DTN: 75%, DTNB: 88%). Cardiac toxicity was low with a CTE in only3 patients (DT:0, DTB:1, DTN:1, DTNB:1). Non-cardiac toxicity/patient as evaluated by the incidence of serious adverse events (SAE,n=50) and significant safety events (SSE, n=114) was acceptable (SAE: DT:8, DTB:12, DTN:14, DTNB: 16; SSE: DT: 23, DTB: 31, DTN: 29, DTNB: 31). No differences in the incidence of non-serious AE and no new safety signals for B and N were detected. In 8 patients the treatment was terminated early (DT: 0, DTB: 3, DTN: 2, DTNB: 3).

CONCLUSIONS
Our data show that all regimens tested are effective in inducing pCR in HER2-positive early breast cancer. The addition of B and/or N to DT may lead to pCR/tpCR-rates of 51-88% with the highest activity seen in the ER-negative subgroup. 6 cycles of these regimens can safely be administered to patients with HER2-positive early breast cancer and further phase III-evaluation appear to be warranted.
Title: Changes in PgR and Ki-67 in residual tumour and outcome of breast cancer patients treated with neoadjuvant chemotherapy

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Body: PURPOSE We assessed the frequency of the change in biological features between the diagnostic biopsy and final surgery in breast cancer patients with residual disease after neoadjuvant chemotherapy. We also valued the impact of change on outcome in terms of disease free survival (DFS) and overall survival (OS)

PATIENTS AND METHODS We collected information through the institutional clinical database on all consecutive breast cancer patients treated with neoadjuvant chemotherapy at the European Institute of Oncology (EIO), Milan, Italy, between 1999 and 2011. We selected patients who did not achieved pathological complete response (pCR) at final surgery. All patients had pathological evaluation, including ER, PgR, HER2 protein and ki-67 expression performed at EIO both at diagnostic core biopsy and final surgery.

RESULTS We identified a total of 904 patients. The 5% of patients with ER positive at diagnostic biopsy had ER negative residual tumor at final surgery. For PgR expression, 67% of patients, whose tumors had a PgR> 20% at diagnostic biopsy had a PgR<20% at final surgery. The ki-67 expression changes from >20% to <20% in 40% of patients. At the multivariate analysis the decrease of PgR -immunoreactive cells correlated with improved outcome in terms of DFS (HR 0.73; CI 0.54-1.00 p 0.046). In addition, the decrease of ki-67 expression to < 20% of the cells at final surgery was found to be associated with better outcome both in terms of DFS (HR 0.52; CI 0.40-0.68 p <0.0001) and OS (0.45 CI 0.32-0.64 p<0.0001)

CONCLUSION The decrease of PgR and Ki-67 expression after preoperative chemotherapy have a prognostic role in breast cancer patients with residual disease and should be considered in the adjuvant therapeutic algorithm.
Title: Eribulin/cyclophosphamide (ErC) versus docetaxel/cyclophosphamide (TC) as neoadjuvant therapy in locally advanced HER2-negative breast cancer: A randomized phase II trial of the Sarah Cannon Research Institute

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Body: Background: Pathologic complete response (pCR) following neoadjuvant chemotherapy for locally advanced breast cancer strongly correlates with improved disease-free survival, rates of breast-conserving surgery, and provides an early indicator of treatment efficacy. Standard taxane-containing neoadjuvant combinations result in pCR rates of 18% in unselected patient (pt) populations. Eribulin (Er) is a novel inhibitor of microtubule dynamics. In the EMBRACE trial, Er demonstrated a survival advantage in anthracycline and taxane pretreated breast cancer. This study evaluated non-anthracycline regimens of Er in combination with cyclophosphamide (ErC) and docetaxel/cyclophosphamide (TC) as neoadjuvant therapy in HER2-negative breast cancer.

Methods: Adult women with histologically confirmed invasive HER2-negative (defined as IHC 0-1+ or FISH/SISH negative), clinical stage T1-3, N0-2, M0 (pN3a disease allowed) adenocarcinoma of the breast were eligible. Additional eligibility criteria included: ECOG PS 0,1 or 2; normal cardiac function, known hormone receptor status for stratification; adequate hematologic, liver, and renal function. Following a 10 pt lead-in to confirm the safety and feasibility of ErC, pts were randomized in a 2:1 ratio: Arm 1, Er 1.4 mg/m^2 IV (Days 1 & 8) and C 600 mg/m^2 IV (Day 1); Arm 2, T 75 mg/m^2 IV and C 600 mg/m^2 IV on Day 1, both regimens administered q 21 days x 6 cycles followed by surgery. Locoregional radiation and/or antiestrogen therapy were prescribed postoperatively according to standard guidelines. Tumor samples were collected at baseline and from residual disease noted at the time of surgery. A pCR rate ≥18% in pts treated with ErC warranted further evaluation.

Results: Enrollment was completed April 2014 (76 pts); 66 pts were randomized (Arm 1, 44; Arm 2, 22). Median age was 52 years (range, 23-77); 88% ECOG 0; 79% invasive ductal adenocarcinoma; median baseline primary tumor size 3cm (range, 0.4-10cm; 30% were T3); axillary nodes clinically positive in 51%. Thirty-three percent were triple negative. 55 pts (72%) received neoadjuvant therapy and underwent surgery. 16 pts continue to receive study treatment. At this time, pts who underwent surgery have similar pCR rates with ErC and TC (14%, 5/37 vs. 11%, 2/18 respectively). 3 of 51 hormone receptor positive pts (6%) and 4 of 25 triple negative pts (16%) had pCR. Neutropenia (40%) was the most common grade 3/4 toxicity. Common treatment-related toxicities included: fatigue (66%), alopecia (57%), and nausea (54%). Peripheral neuropathy incidence is currently lower with ErC than with TC (30% vs 45%) with 3 grade 3 events on TC. Prophylactic growth factor use was higher in TC than with ErC (77% vs 24%).

Conclusions: Eribulin in combination with cyclophosphamide was well tolerated with no unexpected toxicities. At present, pCR rates with both regimens are within the range previously reported with docetaxel/cyclophosphamide and other standard taxane-containing neoadjuvant regimens. Final efficacy and toxicity data will be presented. A planned exploratory correlative tissue analysis may identify tumors more likely to derive benefit from eribulin-based therapies.
Title: Elevated peripheral blood lymphocyte-to-monocyte ratio predicts favorable response and prognosis in locally advanced breast cancer after neoadjuvant chemotherapy

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Body: Neoadjuvant chemotherapy (NAC) is the standard of care for locally advanced breast cancer (LABC). However, the lack of efficient method to predict the treatment response and prognosis limits the clinical evaluation of the patient eligibility. Elevated lymphocyte-to-monocyte ratio (LMR) has been reported to be associated with favorable prognosis in some hematology malignancies and nasopharyngeal carcinoma, but has not been studied in breast cancer. The purpose of this study is to evaluate whether the LMR is a predictor of the curative impact of LABC patients after NAC and short/long-term mortality. A retrospective cohort of 542 LABC patients who received NAC was recruited between May 2002 and August 2011. The counts for peripheral absolute lymphocyte and monocyte before NAC were retrieved, and the LMR was calculated. Receiver operating characteristic curve analysis, univariate and multivariate Cox proportional hazards analyses were applied to evaluate the associations of LMR with overall survival (OS) and disease-free survival (DFS) respectively. Univariate analysis revealed that higher LMR level (≥4.25) was significantly related to better overall response rate (P = 0.005). Univariate analysis revealed that higher LMR level (≥4.25) was significantly associated with superior OS (P = 0.014), DFS (P = 0.009). The higher lymphocyte count (≥1.5×10⁹/L) was significantly associated with better OS (P = 0.035), while the lower monocyte count (<0.4×10⁹/L) was associated with better OS (P = 0.007) and DFS (P = 0.01), respectively. Multivariate Cox proportional hazard analysis showed that higher LMR level was a significantly independent predictor for superior OS (hazard ratio or HR = 0.636, 95% confidence interval or 95% CI = 0.432-0.937; P = 0.022), DFS (HR = 0.666, 95% CI = 0.495-0.896; P = 0.007) respectively. The traditional predictive factors, including tumor size, lymph node status, hormone receptor status and HER2 status, were also independent prognostic factors of OS and DFS. The elevated pre-NAC peripheral LMR level was a significant favorable factor for NAC LABC prognosis and this easily accessed variable may serve as a potent marker to predict the outcomes of LABC patients after NAC.
Title: Response to subsequent therapies after failure to achieve pathologic complete response (pCR) after neo-adjuvant chemotherapy (NAC) in patients (pts) with triple negative breast cancer (TNBC)

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Body: Background: More than half of pts with TNBC do not achieve a pCR after NAC, which carries a poor prognosis. It is unclear whether these patients respond to subsequent therapies on relapse. We sought to determine the time to progression (TTP) on subsequent therapies for recurrent disease in TNBC patients who did not achieve pCR to neo-adjuvant therapy.

Methods: We retrospectively identified 117 TNBC pts who received NAC from 2009 through 2012 using an electronic database at the Magee Women's Breast Cancer Program at the University of Pittsburgh. Patient records were then assessed for pts who did not achieve a pCR for time to local or distant recurrence (TTR) and time to progression (TTP) on subsequent therapies.

Results: 86 of 117 TNBC pts did not achieve pCR to NAC defined as residual disease present in either the breast and/or axilla. Median follow up was 2 yrs (range 1-4yrs). Out of these 86 pts, 21 pts(25%) had recurrence in form of distant metastasis or local recurrence. Nearly all pts(95%) received anthracycline and taxane based NAC. Median TTR was 19 months (4-29 months). Therapies received in the metastatic setting most commonly included single agent capecitabine, paclitaxel, nab-paclitaxel, and eribulin. The most commonly received doublets included carboplatin with paclitaxel or gemcitabine. Median TTP on systemic chemotherapy was 15 weeks (3 -39 weeks), 6 weeks (1-24 weeks) and 6 weeks (2-9 weeks) in first, second and third line setting, respectively. Greater than 80% pts were able to receive two lines of systemic chemotherapy but <50% received third line treatment. Two pts died before initiating any systemic treatment.

Conclusions: Response to subsequent chemotherapeutic regimens in metastatic setting in TNBC pts who do not achieve pCR to anthracycline and taxane based NAC is limited. This highlights the importance of identifying new agents and targeted therapies for this poor prognosis sub-type.
Title: Dedicated breast PET (dbPET) a unique functional new tool to accurate quantify response to neoadjuvant therapy in breast cancer

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Body: Background: Breast cancer is one of the most common cancers in women. Approximately 232,670 new cases of breast cancer (14% of all new cancer cases) and 40,000 breast cancer deaths (6.8% of all cancer deaths) are expected to occur among US women in 2014. Since 1990, breast cancer death rates have dropped by 34%. Continued progress in the control of breast cancer will require sustained and increased efforts to provide high-quality screening, diagnosis, and treatment. Recently, the MAMmography with Molecular Imaging (MAMMI) dedicated breast PET (dbPET) has emerged as an additional imaging tool for breast cancer diagnosis, clarification of complex lesions and therapy follow-up. This study is aimed to determine whether correlations exist between physiological images with 18FDG of pre, post-2-cycles and post Neoadjuvant Chemotherapy, with a predictive value of response.

Methods: Twenty-five patients, and three scan points: pre, 2 cycle and post Neoadjuvant Chemotherapy were included in this analysis. A prone position high-resolution dedicated breast PET (MAMMI-dbPET) was performed 60 min after administration of 90-120 MBq of 18F-FDG. Maximum standardized uptake value (SUVmax) quantification, volume characterization, positioning in all three space-axes, distances to reference points (proximal breast limit, nipple areola complex) were registered.

Results: When treatment was successful, a significant difference was found between pre and post neoadjuvant chemotherapy status and the SUVmax (p < 0.001) of breast tumors. Pre Neoadjuvant (mean SUVmax, 11.4) demonstrated a significantly higher SUVmax than did post 2 cycles tumors (median SUV, 4.7) (p= 0.025). No statistical significant difference was found for SUVmax of post-2 cycles vs. post lesions with a mean SUVmax of 4.7 and 3.9 (p=0.25) respectively. A statistically significant difference was found for volume measurement of pre vs. post-2 cycles vs post Neoadjuvant therapy lesions. A clear qualitative difference by three different observers has been reported among dbPET and MRI volume characterization. A 21% of volume (measurement) discrepancies was found, always with a volume over-estimation by magnetic resonance (structural vs functional imaging). An exquisite -and unexpected- millimetric correlation with post-surgical pathology at the end of neoadjuvant theraphy has been found in dbPET images.

Conclusions: dbPET MAMMI has proven to be an excellent tool for quantification, 3D spatial localization and monitoring of neoadjuvant therapy, showing, thanks to its functional nature, earlier and better precision and accuracy than conventional techniques (MRI). Post 2-cycle and Post Neoadjuvant Chemotherapy breast cancer tumors consistently display lower 18FDG uptake than pre treatment tumors when treatment is successful. This data suggest that SUVmax measurements of 18FDG-dedicated breast PET can provide valuable information about therapy efficiency. Such an association might be of relevant importance to treatment continuity or adjustement.
Title: Vacuum assisted core-needle biopsy after neoadjuvant therapy in breast cancer to predict pathologic response

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Body: Introduction: Recently published neoadjuvant breast cancer therapy trials have documented impressive pathologic complete response (pCR) rates of up to 50% and above, depending on grade, hormone receptor and HER2 status and type of therapy. The purpose of our prospective study is to examine the predictive value of a standardized vacuum assisted core-needle biopsy (VAB) of the tumor site after neoadjuvant therapy with respect to pCR status. This is a presentation of the first case series of this single institution study.

Methods: As of 2011, all patients who had completed neoadjuvant breast cancer therapy at our institution were informed about this trial regardless of their clinical response status. In case of consent, a mammography-guided VAB of the initially tagged tumor site was performed directly before the operation. In case of breast conserving surgery, mammographic wire localisation was performed immediately thereafter. Final surgery (breast conservation or mastectomy) was performed according to German guidelines. Ethical approval was obtained from the institutional review board. Pathologic complete response was defined as ypT0 ypN0.

Results: Of 70 patients completing neoadjuvant therapy, 44 consented to the trial. The pCR rate was 47% (n=21). Pre-operative VAB correctly predicted residual disease in 19 of 23 cases (82.6%). We observed incorrect prediction of pathologic response if the tagging clip was not placed centrally, or if the radiologist did not perform the VAB in the tagged area. In 3 cases of pathologic partial response (pPR), all residual tumor (DCIS) was removed by VAB. In these cases, no further malignant lesions were detected in the resulting surgical specimens.

Conclusions: Preliminary observations of this ongoing study are encouraging, although the correct prediction rate of residual disease is nowhere near the sensitivity needed to change clinical practice. However, if these results can be improved and confirmed in a larger series, it may be possible to reduce operative procedures after neoadjuvant therapy based on the results of a preoperative standardized VAB.
**Title:** Change of Ki-67 after long course of neoadjuvant chemotherapy as prognostic factors in patients with stage II, III triple negative breast cancer

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**Body:** Background: Triple negative breast cancer (TNBC) shows poor prognosis despite their higher response rate to neoadjuvant chemotherapy (NAC). Response to NAC such as pathologic complete response (pCR) is a well-known useful prognostic marker for relapse and overall survival. Pre-chemotherapy baseline higher Ki-67 is a positive predictive marker for higher pCR rate but a negative prognostic marker for relapse free survival (RFS) and overall survival (OS). After short course of NAC, Ki-67 index changed in response to the treatment. The purpose of this study is to investigate the prognostic role of Ki-67 index change between before and after the long course of NAC in TNBC patients.

Patients and Methods: Patients with clinical stage II or III breast cancer treated with NAC at Seoul National University Hospital were enrolled and their clinicopathological findings including Ki-67 index were reviewed retrospectively. The definition of Ki-67 threshold of high was ≥14% in current study. TNBC definition was adopted from 2011 St Gallen Consensus Panel.

Results: From Jan 2009 to Dec 2010, a total of 183 patients received NAC. Among them, 46 (25.1%) were TNBC. The pCR rate (19.6% vs 9.5%, P=0.069) and the objective response rate (complete response + partial response) (76.1% vs 70.8%, P=0.153) were higher in TNBC group than in non-TNBC group. The RFS was not different between the two groups (3 year 77.3% vs 75.4%, P=0.877). The change of Ki-67 index from high (≥14%) to low (<14%) was significantly associated with longer RFS in only TNBC group. As for TNBC patients with high Ki-67 index at diagnosis, RFS of those whose Ki-67 index changed to low after NAC were significantly longer than that of whose Ki-67 index remained high (3 year RFS 87.2% vs 54.5%, P=0.036). In non-TNBC group, change of Ki-67 index was not significantly associated with RFS (P=0.245). The initial Ki-67 index itself (high vs. low) was not associated with RFS in both TNBC and non-TNBC group (P=0.726 and P=0.523 respectively).

Conclusion: The change of Ki-67 index from high to low is associated with longer RFS in TNBC. Ki-67 index change between before and after the NAC can be used for further discrimination of TNBC as a prognostic marker.
Title: Is pathologic complete response (pCR) a valid marker of outcome even in large breast cancer? Clinical results from a neoadjuvant trial using a combination of epirubicin, docetaxel and bevacizumab (PROMIX)

Methods: In total, 150 women 18 years or older with verified HER2 negative breast cancer suitable for primary medical treatment were included in the trial between September 2008 and December 2011. The patients received two courses of epirubicin and docetaxel (Taxotere®), both 75mg/m² for the 1st two courses, followed by the same treatment with addition of bevacizumab (Avastin®) 15 mg/kg for 4 additional courses. Clinical and radiological evaluations with mammography and ultrasound were performed before start and after courses 2, 4 and 6. Core biopsies were taken before start, after 2 courses, and at time of surgery. Blood samples were drawn before and 24 hours after the first 4 courses.

Results: Median age was 49 years, range: 27 to 70 years; 73% were reported as ductal, 15% as lobular, and 12% as rare histological types. Mean tumor size was 59 mm, median 55 mm, range: 20-180 mm; 3 tumors (2.0%) were reported as inflammatory, and 13 (8.7%) presented with skin involvement (T4b). Enlarged axillary nodes were found in 102 patients (68%) before start of treatment, 77 of these (64%) verified as metastatic. SNB in cases of normal axillary status was performed in 16 cases, in 9 cases (8%) with positive finding. Supra- or infracavicular node involvement was verified in 20 cases (13%). 25% of all tumors were ER- and/or PR-negative, tumor grade based on a diagnostic biopsy was evaluable in only 83 cases. Of these, 4 (5%) were grade I, 46 (55%) grade II, and 33 (40%) grade III. Mean proliferation count (Ki67) was 37%, median 30%, range 1-90%. Breakdown into intrinsic subtypes based on immunohistochemistry defined 68 (46%) as luminal A-like, 36 (24%) as luminal B-like, and 44 (30%) as TNBC. pCR was achieved in 20 cases, 3 (2%) luminal A-like, 5 (3.4%) luminal A-like, 5 (3.4%) luminal B-like and 12 (8.2%) TNBC. After 2.2 years of follow-up, 35 patients (23.3%) have experienced recurrence and 18 of these (12%) have deceased due to breast cancer, among these 6 despite pCR, 2 classified as luminal B-like, and 4 as TNBC. The molecular subtype of the tumor predicted outcome, but pCR was not in our material, even after adjustment for tumor size at diagnosis, a predictor of favorable outcome. The number of events in relation to molecular subtypes is however limited. Updated outcome data will be presented.

Conclusions: The present trial does not confirm previously reported observations that pCR is a marker of favorable prognosis. One possible explanation is that unfavorable biological characteristics, particularly heterogeneity, may increase with tumor burden. Genomic and proteomic analyses are currently ongoing.
**Title:** Different clinical usefulness of AJCC response criteria according to the subtypes in stage II/III breast cancer patients after neoadjuvant chemotherapy

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**Body:** Background: Response to neoadjuvant chemotherapy (NAC) is a useful prognostic marker for relapse and overall survival but their prognostic role is distinct in different breast cancer subtypes. Pathologic complete response (pCR) is a current gold standard for evaluating response to NAC. Despite its clinical usefulness, discrimination into pCR and non-pCR is too simple because non-pCR includes broad range of actual responses from very good partial response to even progressive disease. American Joint Committee on Cancer (AJCC) 7th edition proposed response criteria for NAC based on precise clinical stage before NAC and pathologic staging after NAC. The purpose of this study is to evaluate the clinical usefulness of AJCC response criteria in each breast cancer subtypes.

Patients and Methods: Patients with clinical stage II or III breast cancer treated with NAC at Seoul National University Hospital were enrolled and reviewed retrospectively. AJCC response criteria for NAC were adopted from the AJCC 7th edition: complete response (CR), absence of invasive carcinoma in both breast and lymph node; partial response (PR), decrease in either or both T or N stage; no response (NR), no change or increase in either or both T or N stage. The definition of each breast cancer subtypes were adopted from 2011 St Gallen Consensus Panel for current study.

Results: From January 2009 to December 2010, 183 patients were enrolled in the study with median follow up period of 38.0 months. AJCC response was significantly associated with relapse free survival (RFS) (P<0.001), whereas pCR was not (P=0.120). In subgroup analysis, AJCC response was a significant prognostic factor for RFS in luminal B (P<0.001), HER-2 enriched (P=0.035) and triple negative breast cancer (P=0.037) groups, but not in luminal A group patients (P=0.101). On the contrary, pCR was not significantly associated with RFS in any groups.

Conclusion: AJCC criteria is an easily reproducible tool for response evaluation for breast cancer patients in NAC setting compared with classically used pCR in all breast cancer subgroups except luminal A.
Title: Impact of treatment on quality of life (QOL) and menstrual history (MH) in the NSABP B-36: A randomized phase III trial comparing six cycles of 5-fluorouracil (5-FU), epirubicin, and cyclophosphamide (FEC) to four cycles of adriamycin and cyclophosphamide (AC) in patients (pts) with node-negative breast cancer

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Body: Background
NSABP B-36 compares 6 cycles of FEC-100 with 4 cycles of standard AC in pts with node-negative breast cancer. As reported separately, no significant differences between the two treatment arms were observed in the primary endpoint of disease-free survival or in the secondary endpoints of overall survival, recurrence-free, or distant recurrence-free intervals. Greater toxicity was reported in pts who received FEC compared to AC. We present here the results of the QOL and MH studies obtained prospectively in conjunction with the treatment study. We hypothesized that pts on FEC would experience greater treatment toxicity in the first 12 months of the study, and would have greater rates of amenorrhea at 18 months compared to pts on AC.

Methods
Among the 1,357 pts enrolled in the QOL study, 1,332 (675 AC, 657 FEC) submitted the baseline form and had QOL follow-up (fup) information. Pts completed: 1) the FACT-B instrument; 2) a symptom checklist; and 3) the SF-36 Vitality Scale, all at baseline, day 1 of cycle 4, and at 6, 12, 18, 24, 30, and 36 months after random assignment. FACT-B Trial Outcome Index (TOI), symptom severity, and vitality scores were compared between the two treatment arms using a mixed model for repeated measures analysis with adjustment for the baseline scores, type of surgery, and hormone receptor status, examining the first 12 months and the later time points separately. Menstrual status was collected at baseline for all enrolled pts, with subsequent assessments on day 1 of cycle 4, and at 6, 12, 18, 24, 30, and 36 months for pts with menstrual bleeding within 12 months prior to random assignment and not having had a hysterectomy and/or bilateral oophorectomy (1, 096 pts). Post-chemotherapy amenorrhea was defined as the lack of menstrual periods during the 12 months preceding the 18-month fup evaluation. Data from 921 pts (475 AC, 446 FEC) were available for analysis. Logistic regression, adjusted for type of surgery and hormone receptor status, was used to test for association of amenorrhea status and treatment.

Results
Both TOI and vitality scores were worse for pts on FEC compared to those on AC at 6 months (p<0.01) with no significant difference at 12 months and beyond. No significant differences in symptom severity between the two treatment arms were observed. The rates of post-chemotherapy amenorrhea were significantly different between FEC and AC (66.8% vs. 58.7%, p=0.01) with positive hormone receptor status as an independent risk factor (p=0.03).

Conclusions
Women receiving FEC had diminished QOL at 6 months after random assignment, but no difference at 12 months or later. Premenopausal women receiving FEC experienced a higher rate of post-chemotherapy amenorrhea than women receiving AC.

Support
NCI grants U10-CA-12027, -37377, -69974, -69651 and -44066-26, and Pharmacia & Upjohn Company, a subsidiary of Pfizer, Inc.
Lifestyle interventions combined with acupuncture-like transcutaneous electrical nerve stimulation in managing vasomotor symptoms induced by breast cancer treatment: Results of a phase 2 randomized controlled trial

Margaret Forbes1,2, Raimond Wong1,2, Stephen M Sagar1,2, Jim A Julian1,2,3, Mark N Levine1,2,3 and Joseph Hayward1,2. 1McMaster University, Hamilton, ON, Canada; 2Juravinski Cancer Centre/Hamilton Health Sciences, Hamilton, ON, Canada and 3Ontario Clinical Oncology Group, Hamilton, ON, Canada.

Body: Background: Women with breast cancer can experience significant treatment induced vasomotor symptoms (TIVS). Non-hormonal strategies for TIVS (e.g. acupuncture, Venlafaxine) provide some relief but may be intolerable because of the invasive nature of the treatment and possible side effects. As such, women often prefer lifestyle strategies (LS) that can be self-administered. Acupuncture-like transcutaneous electrical nerve stimulation (ALTENS) is a non-invasive needleless technique that uses specific electrical parameters to stimulate selected acupoints to achieve clinical response with minimal toxicity. ALTENS can be administered with minimal training. It is amenable to quality assurance and can allow for self treatment by women. This study aimed to evaluate the efficacy of ALTENS in addition to LS in relieving TIVS in women with breast cancer.

Methods: Eligible subjects were postmenopausal women with Stages 0-3 breast cancer who had completed cancer treatment and were experiencing hot flashes for ≥ one month with a Hot Flash Score (HFS) ≥15 in one week prior to consent. Anti-estrogen therapy was permitted. Non-hormonal drug therapies were prohibited. Subjects were randomized to either LS (control) or LS with concurrent ALTENS (combined). LS consisted of standardized lifestyle strategies (e.g. environmental control, managing hot flash triggers) counseling delivered by a specially trained nurse practitioner at week 0 with reinforcing counseling at weeks 12 and 24. ALTENS was given twice weekly for 12 treatments over an 8-week period. The HFS, Hot Flash Related Daily Interference Scale and the Short Form version 2 health survey were administered at weeks 0, 12 and 24. Heart rate variability was measured at weeks 0 and 12. The primary study endpoint was the number of responders, defined as women who had > 50% reduction in their HFS between weeks 0 and 12.

Results: 71 eligible subjects with a median age of 52 (range: 40-87) were randomized to combined arm (n=36) and control (n=35). At 12 weeks there were 11 (30.6%) responders in the combined arm versus 2 (5.7%) in the control (p=0.012). The results at 24 weeks were 14 versus 4, respectively (p=0.013). Arms were balanced for anti-estrogen use. Two subjects chose to discontinue ALTENS after experiencing symptoms improvement. There were no serious adverse events.

Conclusion: ALTENS in combination with lifestyle strategies is a promising non-pharmacologic approach that showed improvement in managing treatment induced vasomotor symptoms in women with breast cancer. Our trial results support its evaluation in phase 3 studies.
**Title:** Risk of infectious complications in breast cancer patients treated with trastuzumab: A meta-analysis

Tomohiro Funakoshi¹, Maya Suzuki² and Hyman B Muss¹. ¹UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC and ²Memorial Sloan Kettering Cancer Center, New York, NY.

**Body:**

**Background**
Serious infections related to the anti-HER2 monoclonal antibody trastuzumab has been reported in clinical trials. It is not yet clear whether trastuzumab increases infection risk or not. We performed a systematic review and meta-analysis to assess the risk of infections associated with trastuzumab.

**Patients and methods**
We searched PubMed and the ASCO online database of meeting abstracts up to January 2014 for relevant clinical trials. Eligible studies included randomized controlled trials (RCTs) of trastuzumab for breast cancer patients that reported adequate safety data for grade 3-4 infection, febrile neutropenia, neutropenia, or leukopenia. Grade 3 and 4 infection is defined as "IV antimicrobial agents, interventional radiology or operative intervention indicated" and "life-threatening consequences (e.g., septic shock)", respectively. The summary incidence, relative risk (RR) and 95% confidence intervals (CIs) were calculated. RRs and summary incidence were calculated using random-or fixed-effects models depending on the heterogeneity of included studies. Prespecified exploratory subgroup analyses were performed according to treatment setting (neoadjuvant or adjuvant vs. metastatic setting) and therapy used with trastuzumab (combination vs. best supportive care (BSC) or single agent therapy) with regard to the RRs.

**Results**
A total of 10,094 patients from 13 trials of trastuzumab were included. The use of trastuzumab was associated with an increased risk of high-grade infection (RR, 1.21; 95% CI, 1.07-1.38; P=0.002) and febrile neutropenia (RR, 1.28; 95% CI, 1.08-1.52; P=0.004). The incidence of grade 3-4 infection and febrile neutropenia related to trastuzumab and control was 8.5% (95% CI 4.5-15.4%) vs. 6.9% (95% CI 4.0–11.6%) and 12.0% (95% CI, 8.1-17.4%) vs. 9.9% (95% CI, 6.6-14.6%), respectively. There was no significant increase in a risk of high-grade neutropenia (RR, 1.07; 95% CI, 0.98-1.68; P=0.15) or leukopenia (RR, 1.07; 95% CI, 0.94-1.23; P=0.31) in patients receiving trastuzumab. The results of exploratory subgroup analyses were shown in table1.

**Conclusions**
Treatment with trastuzumab is associated with a 1.21-fold increased risk of high-grade infection and a 1.28-fold increased risk of febrile neutropenia compared with control. In order to obtain the maximum therapeutic benefit from trastuzumab, clinicians should be aware of the increased infection risk and perform careful monitoring especially when it is used with combination chemotherapy in the neoadjuvant and adjuvant setting.
Body: Background: P53 activation is a major pathway by which normal tissues respond to DNA damaging agents such as chemo and radiotherapy. We have shown that the use of very low dose arsenic (LDA) for 3 days before chemotherapy in animal models temporarily and reversibly suppresses p53 activation for about 5 days. LDA-mediated protection requires functional p53 and thus is selective to normal tissues, as essentially every cancer cell has dysfunctional p53. Arsenic Trioxide (ATO) is currently used to treat acute promyelocytic leukemia (APL) at much higher dose (50-fold higher than the dose we used for suppression of p53). The primary objectives of this trial were to: 1) define the lowest safe dose of ATO that blocks p53 activity in vitro as measured in patients’ peripheral lymphocytes and 2) assess the potential of LDA to decrease hematological toxicity in pts receiving chemotherapy.

Methods: Pts with malignancies other than leukemia who were to receive at least 4 cycles of myelosuppressive chemotherapy given at least 2 weeks apart were eligible. Pts had to have no baseline p53 activation in peripheral lymphocytes but p53 had to be responsive as measured by an in vitro assay. For objective 1, dose escalation was performed at the starting dose of ATO 0.005mg/kg/day for 3 days. For objective 2, ATO 0.005mg/kg /day x 3 was given prior to chemotherapy cycles 2, 4 and 6 only. WBC, ANC, Hgb, and platelet counts were obtained on chemotherapy days 1 (prior to chemotherapy), 8, 15 and 22, normalized to the corresponding counts from day 1 and compared between cycles with a paired t-test. The presented analysis compares cycles 1 and 2.

Results: Thirty-three evaluable pts were accrued. Chemotherapy was: TC 8, AC 7, 8 other regimens 18 pts. ATO at a dose of 0.005mg/kg/day for 3 days blocked p53 activity in vitro as measured in pts’ peripheral lymphocytes and was not associated with any toxicity. For WBC and ANC potential protective effect was most pronounced on day 8. The mean normalized WBC counts were 0.54 in cycle 1 and 0.72 in cycle 2 on day 8 (p=0.01). 64% of pts had higher normalized WBC in cycle 2 over cycle 1, 24% remained the same (±5%) and 12% lower value. The mean normalized ANC counts were 0.53 in cycle 1 and 0.85 in cycle 2 on day 8 (p=0.02). 59% of pts had higher normalized ANC in cycle 2 over 1, 31% same, and 9% lower. There was no significant change detectable for Hgb as less than 10% of pts had > grade 1 anemia. The mean normalized platelet counts were 0.91, 1.11 and 1.21 in cycle 1 and 0.77, 0.83 and 1.03 in cycle 2 on days 8, 15 and 22 respectively (corresponding p values=0.001, 0.001, and 0.04). It is worth noting that >96% of pts had normal platelet counts or grade I thrombocytopenia. On an explorative subset analysis using the exact Wilcoxon signed rank test, the chemotherapy regimen that gained the most protective effect was AC (doxorubicin and cyclophosphamide) used for breast cancer, with mean normalized WBC counts of 0.39 and 0.46 (p=0.03) and ANC counts of 0.39 and 0.49 (p=0.02) on day 8 of cycle 1 and 2 respectively.

Conclusions: Our data suggest that LDA pretreatment confers significant protection against chemotherapy induced leukopenia and neutropenia. Further study is needed to confirm its beneficial effect on AC chemotherapy.
Average Grade: 5.40

Title: Reduction of ovarian reserve in young early breast cancer patients: 24 months follow up of a prospective cohort trial

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Body: Background: Sideeffects of adjuvant chemotherapy on ovarian function are inadequately studied by now. Premenopausal women undergoing chemotherapy are at risk for sexual hormone deficiency and impaired fertility. This prospective cohort study searches for predictive parameters of ovarian reserve after chemotherapy. Methods: 51 premenopausal patients (average 38 years; 28-46 years) with primary breast cancer have been included. They all received neo- or adjuvant chemotherapy: anthracycline based "A"(FEC)(n= 18); anthracycline/taxane based "T"( TAC or FEC/Doc or EC/Pac)(n=30) or anthracycline- free "AF"(n=3). After chemotherapy patients received endocrine therapy (ET) for hormone receptor (HR) positive disease (tamoxifen (n=34) or goserelin/tamoxifen (n=7)). For HR negative disease (n=20) no ET was given. Before (visit 1) and 6, 12 and 24 months after initiation of chemotherapy (visit 2-4) age and chemotherapy related changes in hormone (LH, FSH, E2 and Anti-Muellerian hormone (AMH)) levels, antral follicle count and amenorrhea as parameters of endocrine function and fertility were assessed. Also quality of life (QL) and additional impact of parity, BMI and nicotine use on ovarian reserve were evaluated. Results: Antral follicle count is decreasing dramatically after chemotherapy and is not fully recovering after 2 years. Antral follicle count before and 1 year after chemotherapy are significantly correlated (p=0.012). Follicle count and age before chemotherapy are negatively correlated (p=0.004). At visit 4 the type of chemotherapy (A, T, AF) has no influence on antral follicle count or AMH levels. Tamoxifen is correlated with a significant decrease in follicle count (vs. no endocrine therapy) (p=0.039). Smoking is significantly detrimental on antral follicle count after 24 months (p=0.003). LH and FSH are increasing between visit 1 and 2 and decreasing at visits 3 and 4 (but stay above basic levels). At visit 4 LH is not and FSH is correlated with visit 1 levels (p=0.005). AMH levels at visits 2-4 are significantly correlated with levels at the 1 visit and are negatively correlated with age (p=0.032). Tamoxifen therapy has no impact on AMH levels. LH and FSH are increasing between visit 1 and 2 and decreasing at visits 3 and 4 (but stay above basic levels). At visit 4 LH is not and FSH is correlated with visit 1 levels (p=0.005). LH and FSH are correlated with age (p=0.0001) and are not influenced by type of chemotherapy or tamoxifen. E2 levels at visit 4 are not influenced by type of chemotherapy, but significantly decreased (p=0.003) by continued smoking. At visit 4 31/51 patients have stayed amenorrheic, 19 resumed their menstruation. This was not influenced by type of chemotherapy or age. Non-smokers are 13 times more likely to resume their menstruation (vs. smokers). Tamoxifen is correlated with a higher probability of permanent amenorrhea (p=0.058, n.s.). BMI, nicotine abuse, age, type of chemotherapy and tamoxifen have no influence on duration of amenorrhea. QL is significantly lower at visit 2, but recovers to basic values at visits 3 and 4. Conclusions: Our study contributes to a better prediction of ovarian reserve before chemotherapy. We suggest personalized counselling on fertility preserving measures before chemotherapy especially at higher age, with low AMH levels or low antral follicle counts. To stop smoking could enhance chances for ovarian preservation.
Subpopulations of peripheral sensory neurons are differentially sensitive to the microtubule-targeting agent, paclitaxel.

Peter M LoCoco¹, Teresa C Chavera¹, Raehannah J Jamshidi¹, Susan L Mooberry¹, Kelly A Berg¹ and William P Clarke¹.
¹University of Texas Health Science Center, San Antonio, TX.

Paclitaxel (PTX), a microtubule-targeting anticancer agent, produces a debilitating peripheral neuropathy that is accompanied by neuropathic pain. Patients develop localized pain in their distal extremities, and more frequently complain of increased sensitivity to cold rather than hot temperatures. These clinical observations led us to hypothesize that PTX differentially damages subpopulations of peripheral sensory neurons. The purpose of this study was to evaluate the effects of PTX on bradykinin (BK) and eicosanoid (EP) receptor-expressing sensory neurons. PTX (11.7 mg/kg) or vehicle (Cremophor EL/EtOH/PBS; 1:1:6) was administered intraperitoneally to Sprague-Dawley rats (250-300 g) every other day for 5 days. Following treatment, PTX-treated rats exhibited increased sensitivity to cold and mechanical stimuli, but decreased sensitivity to heat stimulation as compared to vehicle-treated rats. Intraplantar (i.pl.) injection with BK (5 µg) produced a prolonged allodynia to cold, but a diminished allodynia to heat in PTX-treated rats. Interestingly, following treatment with PGE2 (0.3 µg, i.pl.), PTX-treated rats displayed no statistically significant differences in the allodynia to cold or heat compared to vehicle-treated rats. Efficacy of BK (1 pM - 1 µM) to stimulate inositol phosphate (IP) production in cultured primary sensory neurons from PTX-treated rats was significantly reduced. By contrast, stimulation of cAMP production by PGE2 (0.01 pM - 100 nM) was unaffected by PTX treatment. Preliminary mechanistic studies treating naïve cultures of rat primary sensory neurons directly with PTX (5 nM, 0 - 48 hr) yielded a time-dependent, tri-phasic effect on BK-stimulated IP accumulation and reduced PGE2-stimulated cAMP production, suggesting PTX differentially disrupts BK- and PGE2-mediated signaling. Collectively, these data provide evidence that subpopulations of peripheral sensory neurons are differentially sensitive to PTX. Understanding why particular subclasses are less sensitive, or resistant, to damage could reveal novel approaches to protect sensory neurons from chemotoxicity and ultimately prevent chemotherapy-induced peripheral neuropathy.

Supported by USPS grant #8ULITR000149, Greehey Fellowship, and Translational Science Training Across Disciplines Program at UTHSCSA

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Title: A multicenter randomized double-blind phase III clinical trial to evaluate the efficacy and safety of GCPGC, a novel pegylated G-CSF in patients receiving DA or TAC chemotherapy for breast cancer compared to peg-filgrastim

Joo Han Lim1, Ki-Hyeong Lee2, Keon-Uk Park3, In-Hae Park4, Eun Kyung Cho5, Moon Hee Lee1, So-Young Yoon6, Jee-Hyun Kim7, In-Sil Choi8, Jae-Hoo Park9, Young-Jin Choi10, Hee-Jun Kim11, Kyung Hae Jung12, Si-Young Kim13, Do-Youn Oh14 and Seock-Ah Im14. 1Inha University Hospital, Incheon, Korea; 2Chungbuk National University Hospital, Korea; 3Keimyung University Hospital, Korea; 4National Cancer Center, Korea; 5Gachon University Gil Medical Center, Incheon, Korea; 6Konkuk University Medical Center, Seoul, Korea; 7Seoul National University Bundang Hospital, Korea; 8SMG-SNU Boramae Medical Center, Korea; 9Ulsan University Hospital, Korea; 10Pusan National University Hospital, Korea; 11Chung-Ang University Hospital, Korea; 12Asan Medical Center, University of Ulsan College of Medicine, Korea; 13Kyung Hee University Hospital, Korea and 14Seoul National University Hospital, Korea.

Body: Introduction

Treatment with cytotoxic chemotherapy is known to be associated with a significant risk of febrile neutropenia. GCPGC (Green Cross Co., Korea) is a novel long acting recombinant human granulocyte colony-stimulating factor (G-CSF) analog that reduces the severity and duration of neutropenia. We conducted a phase III trial to evaluate the efficacy and safety of GCPGC compared to pegfilgrastim.

Methods

A total of 117 patients were enrolled in this multicenter, phase III, double-blind randomized trial between Feb 2012 and May 2013. They were randomly assigned to receive either GCPGC or pegfilgrastim during the maximal 6 cycles of chemotherapy which consisted of DA (docetaxel and doxorubicin) or TAC (docetaxel, doxorubicin, and cyclophosphamide). Based on the results of the phase II dose-finding study for GCPGC, the dose of 6 mg was selected. Both medications were administered 24 hours after the completion of each cycle of chemotherapy. The primary efficacy endpoint of this study was the duration of grade 4 neutropenia (Absolute Neutrophil Count (ANC) <500/mm$^3$) during the first cycle of chemotherapy. Secondary endpoints included the time to ANC recovery during the first cycle of chemotherapy, which was defined as the number of days required for neutrophil counts to exceed 2,000/mm$^3$, the rate of febrile neutropenia, the rate of severe neutropenia which persisted for more than three days during first cycle of chemotherapy, the depth of ANC nadir, the ANC level on day 7 after each cycle of chemotherapy, the frequency of dose reduction or delay, the number of hospitalization related to febrile neutropenia after the first cycle of chemotherapy, and the number of treatment with intravenous antibiotics.

Results

A total of 116 patients were evaluable for safety and 115 patients were evaluable for efficacy. The intention-to-treat analysis showed that there was no statistical difference between GCPGC and pegfilgrastim in terms of the duration (days) of grade 4 neutropenia in chemotherapy cycle 1 (1.64±1.18 vs 1.80±1.05; difference -0.15±1.11 [97.5% C.I. -0.26]; the pre-specified non-inferiority margin of 1.0). Notably, patients treated with GCPGC had a statistically significant reduction in the time to recovery from neutropenia (ANC >2,000/mm$^3$), one of the secondary endpoints of the study, compared to those treated with pegfilgrastim (8.85 ± 1.45 vs. 9.83 ± 1.20 days; p <0.0001). There was no difference between groups regarding the other secondary endpoints. In addition, no significant differences were observed between the safety profiles of the two groups.

Conclusion

Collectively, GCPGC is not inferior to peg-filgrastim and represents an effective alternative for reducing the duration of neutropenia in breast cancer patients receiving TAC or DA chemotherapy.
Title: Association of serum prealbumin (transthyretin) with anthracycline chemotherapy toxicity in patients with invasive breast cancer

A Armengol-Alonso¹, K Zamudio-Osuna¹, I Cortes-Franco¹, P Milke-García¹ and E León-Rodríguez¹. ¹Instituto Nacional de Ciencias Médicas y Nutrición “Salvador Zubirán” (INCMNSZ), Mexico City, DF, Mexico.

Body: Background: In Mexico about 60% of invasive breast cancer (IBC) cases are diagnosed in advanced or locally-advanced stages that require systemic treatment. The chemotherapy toxicity (CTox) depends largely on the free fraction of the drug, i.e. the fraction unbound to plasmatic proteins. There is evidence that the Prealbumin (P-Alb) is a good marker of hepatic protein production. Some studies relate reduced serum proteins with CTox. The aim of the study was to correlate serum P-Alb with the degree of hematologic, gastrointestinal and skin toxicity.

Patients and Methods: Prospective cohort from January 2010 to December 2011 which recruited 41 patients with newly diagnosed IBC who received chemotherapy FAC-50 (5-fluorouracil, Adriamycin and Cyclophosphamide). Anthropometric measurements and serum determination of blood count, total protein, albumin and prealbumin were performed at baseline. The presence and degree of toxicity was evaluated on day +12 and +21 of first cycle. Toxicity was evaluated by CTCAE (Common terminology criteria for adverse events) v.4.0 We used Mann-Whitney’s U test and Spearman correlation. The cutoff points for P-Alb were calculated by ROC curves.

Results: Median age was 50 years, 56% and 17% were diagnosed in cTNM stage II-III. Eighteen patients (43.9%) had grade (G) 3-4 neutropenia and 2 (4.9%) had febrile neutropenia. Nine patients (21.9%) had G. 3-4 vomiting and 3 (7.3%) patients had G. 3-4 diarrhea. We classified the patients according to neutropenia, group 1 (N= 23 patients) (56%) without or G. 1-2 toxicity; and group 2 (N=18 patients) (43.9%) with G. 3-4 toxicity. No significant differences in age, cTNM stage, performance status, total body surface area, and body mass index between the groups were identified. Lower basal P-Alb (23.1 mg/dl) was observed in group 2 compared to group 1 (28.3 mg/dl). (p = 0.001). We observed significant differences between the P-Alb medians and degree of neutropenia, vomiting and diarrhea (p = 0.003, p = 0.001 and p = 0.025). The G. of neutropenia, vomiting and diarrhea were negatively correlated with P-Alb seric concentration (r = -0594, p = <0.0001, r = -0418, p = 0.006 and r = -0642, p =<0.0001) respectively. A cutoff of P-Alb ≤ 25 mg/dl for G. 3-4 neutropenia had a sensitivity (S) of 77% and (E) specificity 87%. (AUC 0.84). For diarrhea of any G. a cutoff of P-Alb ≤ 26.8 mg / dl had a S 78% and E 88% (AUC 0.80). A cutoff P-Albs≤ 26 mg/dl for vomiting of any G. had a S 90% and E 76% (AUC 0.86). HR for grade 3-4 neutropenia was 23.3 (95% CI 18.8-144) (p = 0.011); diarrhea any G. HR 28.5 (95% CI 3.03-268) (p = 0.0001) and vomiting any G. HR 28.8 (95% CI 4.89-169) (p = 0.011).

Conclusion: To our knowledge this is the first prospective study describing that P-Alb is negatively correlated with the presence and grade of hematologic and gastrointestinal toxicity secondary to anthracycline breast cancer chemotherapy. A cutoff value of prealbumin <26 mg/dl has a good diagnostic accuracy for predicting CTox. (neutropenia, vomiting and diarrhea), The baseline P-Alb can identify patients at increased risk of severe CTox. Our results should be validated in other cohorts with a larger number of patients.
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-12-09
Average Grade: 5.75

Title: Factors influencing aromatase inhibitor induced musculoskeletal syndrome: Roles of menopause timing and osteoporosis therapy

Clinton R Morgan¹, Zsolt Kulcsar¹, Jonathan D Jones¹, William F Rigby¹ and Peter A Kaufman¹. ¹Dartmouth-Hitchcock Medical Center, Lebanon, NH.

Body: Background: Aromatase Inhibitor (AI) therapy is the most effective hormonal treatment in post-menopausal estrogen receptor (ER) positive breast cancer. Side effects such as arthralgias - termed aromatase inhibitor induced musculoskeletal syndrome (AIMSS) - limit their use in some patients. We evaluated factors associated with AIMSS and explored possible therapeutic options in a large cohort of patients.

Methods: We performed an IRB-approved retrospective review of breast cancer patients seen in the Norris Cotton Cancer Center clinics from April 2011 to January 2013. 378 patients were included in our chart review on the basis of taking an AI for breast cancer with follow up documented in the electronic health record. Statistical analysis was performed by chi squared test for dichotomous variables and students t-test for continuous variables.

Results: In our cohort 91% of patients were taking an AI as adjuvant therapy (9% for metastatic disease) with 41% (n=153) reporting new or worsening arthralgias after initiation of an AI. AIMSS was 42.5%(95%CI: 0.375 to 0.478) in the adjuvant and 22.7%(95%CI: 0.101 to 0.434) in the metastatic groups. The median time to symptom onset was 120 days. 2.1% (n=8) discontinued AI therapy due to AIMSS. There was no association with prior chemotherapy, baseline arthralgia, BMI, or statin use. We found an increased risk of developing AIMSS with more recent menopause (p=0.055), and therapy in the adjuvant setting (p=0.067). We also note a potential association of baseline osteoporosis and osteoporosis therapies with lower rates of AIMSS. Treatments included temporary discontinuation of AI, switching between AIs, and non-steroidal anti-inflammatory therapy (NSAIDs). Of those attempting such treatment, all had improvement with temporary discontinuation, 25% improved after AI switch, and 85% had symptomatic benefit on NSAIDs.

Conclusions: The incidence of AIMSS in our review was 41%. Patients treated in the metastatic setting may have a lower rate of AIMSS. Our cohort revealed that more recent menopause did seem to be a risk factor. Baseline osteoporosis and osteoporosis treatments have a potential association to be explored. Management options included switching between AIs, temporary discontinuation, and NSAID treatment. Updated analysis will be presented.

Potential risk factors for the development of AIMSS

<table>
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<tr>
<th>Characteristic</th>
<th>N</th>
<th>(-)Arthralgia N(%)</th>
<th>(+)Arthralgia N(%)</th>
<th>p-value</th>
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<tr>
<td>Type of AI Used</td>
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<tr>
<td>Anastrozole</td>
<td>204</td>
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<td>Letrozole</td>
<td>131</td>
<td>83(63.4)</td>
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<td>Exemestane</td>
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<td>23(64.9)</td>
<td>13(36.1)</td>
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<td>Menopause timing</td>
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<td>LMP &lt; 5 years prior to AI start</td>
<td>151</td>
<td>79(52.3)</td>
<td>72(47.7)</td>
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<td>LMP 5-10 years prior to AI start</td>
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<td>17(42.5)</td>
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<td>LMP &gt; 10 years prior to AI start</td>
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<td>102(65.8)</td>
<td>53(34.2)</td>
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<td>Type of therapy</td>
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<td>Adjuvant</td>
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<td>200(57.5)</td>
<td>148(42.5)</td>
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<td>Metastatic</td>
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<td>17(77.3)</td>
<td>5(22.7)</td>
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<td>Baseline T-score by DEXA Scan</td>
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<td>Normal (T-score 0 to -1.49)</td>
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<td>55(49.1)</td>
<td>57(50.9)</td>
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<td>Category</td>
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<tr>
<td>Osteopenia (T-score -1.5 to -2.49)</td>
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<td>96 (56.1)</td>
<td>75 (43.9)</td>
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<tr>
<td>Osteoporosis (T-score &lt; -2.5)</td>
<td>40</td>
<td>29 (72.5)</td>
<td>11 (27.5)</td>
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<tr>
<td>On active osteoporosis therapy†</td>
<td></td>
<td></td>
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<tr>
<td>(-) Therapy</td>
<td>218</td>
<td>118 (54.1)</td>
<td>100 (45.9)</td>
<td></td>
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<tr>
<td>(+) Therapy</td>
<td>127</td>
<td>81 (63.8)</td>
<td>41 (32.3)</td>
<td></td>
</tr>
</tbody>
</table>

† Bisphosphonate/denosumab; LMP = Last menstrual period, AI = Aromatase Inhibitor, DEXA = Dual-energy x-ray absorptiometry
Title: Calcium and magnesium infusion for the prevention of taxane induced neuropathy in early stage breast cancer

Theresa Shao1, Joshua Kra1, Paula Klein1, Anupama Goel2, Stephen Malamud1, Tiffany Xing1, Johnny Chan2 and Michael L Grossbard1. 1Mount Sinai Beth Israel Medical Center, New York, NY and 2Mount Sinai St Luke’s and Roosevelt Hospitals, New York, NY.

Body: Background: Taxane is an active drug in the treatment of breast cancer, but peripheral neuropathy is a major dose limiting side effect. There are currently no effective drugs or treatment modalities for the prevention or treatment of taxane-related neuropathy. We examined whether calcium and magnesium (Ca/Mg) infusions can reduce the incidence of neuropathy in patients with early stage breast cancer who are treated with paclitaxel.

Methods: This was a pilot study evaluating the feasibility of Ca/Mg infusion to prevent taxane induced neuropathy in women with early stage breast cancer receiving adjuvant or neo-adjuvant paclitaxel treatment, either given every 2 weeks for 4 cycles or every week for 12 weeks. All patients received calcium gluconate and magnesium sulfate infusion, 1 g of each agent immediately before and after each dose of paclitaxel. The primary endpoint was paclitaxel-related neuropathy grade 2 or greater as measured by NCI Common Terminology Criteria Version 3 compared with historical controls. Secondary endpoints included other measures of neuropathy and quality of life such as the Functional Assessment of Cancer Therapy-Taxane (FACT-Tax) score, taxane-related neuropathic pain as measured by the Brief Pain Inventory-Short Form (BPI-SF). The endpoints were assessed in patients midway through treatment, at the end of treatment and 4 weeks after finishing taxane therapy.

Results: We enrolled 50 patients, 47 patients were evaluable, and 3 patients were taken out of the study due to non-neuropathy related side effects or progression of disease. Median age: 50.8 (range 27-71), White/Hispanic/Black/Asian/Other: 17/16/12/3/2. Two patients received paclitaxel every 2 weeks, while the remainder received weekly therapy. Eight of 47 patients (17%) had grade 2 neuropathy four weeks after treatment completed, while no patients had grade 3 or 4 neuropathy. This rate of neuropathy is significantly lower compared to that seen in historical control where approximately 30% of patients develop grade 2 or greater neuropathy. There were no significant changes in the quality of life measurements. There were no observed toxicities related to the Ca/Mg infusion.

Discussion: Our study showed a decreased incidence of paclitaxel-related neuropathy in patients receiving Ca/Mg infusions when compared to historical controls. The infusions are well tolerated without any side effects. Randomized studies are warranted to further evaluate Ca/Mg infusion for the prevention of paclitaxel-related neuropathy.
Title: Cardiac toxicity in breast cancer patients: A single centre, retrospective review

Moira Rushton¹, Freya Crawley², Jeffrey Sulpher² and Susan Dent². ¹Departments of Experimental Radiation Oncology; ²Hematopathology, Ottawa, ON, Canada; ³Experimental Therapeutics; ⁴Pathology; ⁵Biostatistics; ⁶Radiation Oncology; ⁷Baylor College of Medicine, Houston, TX, USA; ⁸Breast Medical Oncology, University of Texas MD Anderson Cancer Center, Houston, TX, USA and ⁹Morgan Welch Inflammatory Breast Cancer Research Program and Clinic, University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: While advances in breast cancer treatment, including targeted therapies (e.g. trastuzumab) have improved patient outcomes, such treatments may also be associated with both short and long term toxicity. In 2008, the Ottawa Hospital Cardiac Oncology Clinic (COC) was established with the goal of providing timely access and care to cancer patients experiencing cancer therapy-related cardiac toxicity. The purpose of this observational study is to review demographics, baseline characteristics, referral patterns and clinical outcomes of breast cancer patients referred to a dedicated COC.

Methods: Breast cancer patients referred to the COC between October 2008 and December 2012 were reviewed. Data collected included: age, sex, date of diagnosis, date of referral to COC, stage of breast cancer at diagnosis and referral, cardiac history, medications, cancer therapy, treatment delays and completion rates, cardiac complications, cardiac test results and cardiology interventions. Descriptive statistical analysis was performed.

Results: 263 breast cancer patients were assessed at the COC (median age = 57; range 23-87). The majority of patients (64%) had early stage (I-II) disease and 215 (81.4 %) of patients had at least one identifiable cardiac risk factor. The most common cardiac risk factors included: smoking (99/263, 37.6%), hypertension (87/263, 33.1%) and dyslipidemia (64/263, 24.3%). Reasons for referral to the COC included reduced left ventricular ejection fraction (LVEF) (137/263, 52.1%) followed by pre-treatment assessment (33/263, 12.5%) and cardiac arrhythmia (21/263, 8.0%). The majority of patients (243/263, 92.4%) received chemotherapy, 188 (77.4%) with first line anthracycline regimens, of those a minority 10/263 (3.8%) stopped chemotherapy for cardiac related toxicity, while 21/263 (8.0%) patients experienced cardiac related chemotherapy delays. The majority of patients 160/263 (60.8%) received first-line trastuzumab and of these, 66/160 (41.2%) suffered cardiac toxicity, 55/66 due to reduced LVEF. 23/160 (14.4%) discontinued therapy due to cardiac toxicity.

164/263 patients were on cardioprotective medications at time of COC referral. 100/263 referred patients had new cardiac medications initiated, most commonly ACE inhibitors (n=67), beta-blockers (n=54) and angiotensin receptor blockers (n =15). 15 patients (5.7%) had invasive testing/procedures, such as cardiac catheterization. After COC assessment/treatment, 117/263 (44.5%) of patients experienced full recovery of LVEF; 27/263 (10.3%) experienced partial recovery, 104/263 (39.5%) had no change, and 13/263 (4.9%) experienced continued decline. 39/263 (14.8%) patients were hospitalized for cardiac toxicity and 3/263 (1.1%) died of cardiac complications. Discussion: While the majority of breast cancer patients referred to a dedicated COC complete systemic therapy, there remains a significant population who require delay or discontinuation of treatment secondary to cardiac dysfunction. Tools to identify patients at higher risk of developing cardiac toxicity are urgently needed, so that appropriate monitoring and treatment can be initiated. Future studies will also determine the impact of this clinic on the delivery of cancer therapy and cardiac health, compared with non-COC referred patients.
Title: Lurbinectedin (PM01183) activity in BRCA1/2-associated or unselected metastatic breast cancer. Interim results of an ongoing phase II trial

Judit Balmaña¹, Cristina Cruz¹, Judy Garber², Jose A Perez Fidalgo³, Ana Lluch³, Nadine Tung⁴, Silvia Antolin⁵, Cristian Fernandez⁵, Carmen Kahatt⁵, Sergio Szyldergemajn⁵, Arturo Soto Matos⁵, Sonia Extremera⁵, Jose Baselga⁶ and Steven J Isakoff⁸. ¹Hospital Vall d’Hebrón, Barcelona, Spain; ²Dana-Farber Cancer Institute, Boston, MA; ³Hospital Clínico de Valencia, Valencia, Spain; ⁴Beth Israel Deaconess Medical Center, Boston, MA; ⁵Complejo Hospitalario Universitario A Coruña, A Coruña, Spain; ⁶ Pharma Mar S.A. Sociedad Unipersonal, Colmenar Viejo, Madrid, Spain; ⁷Memorial Sloan Kettering Cancer Center, New York, NY and ⁸Massachusetts General Hospital, Boston, MA.

Body: Background: Metastatic breast cancer (MBC) is a clinically heterogeneous disease in which selective approaches are needed to identify patients (pts) who will benefit the most from available therapies and avoid unnecessary toxicities. Lurbinectedin (PM01183) is a new anticancer drug that binds to the DNA minor groove inducing double-strand breaks and blocking transcription. It has significant in vitro and in vivo antitumor activity, particularly in breast cancer models. PM01183 is more active against homologous recombination-deficient cell lines. Hence, MBC pts with deleterious germline BRCA mutations might be more sensitive to PM01183 than those with sporadic tumors.

PM01183 activity was shown in different tumor types in clinical trials, especially in pts with platinum-resistant ovarian cancer (objective response rate [ORR]: 30%).

Methods: MBC pts < 75-years-old with ductal or lobular carcinoma pretreated with ≤3 chemotherapy regimens for MBC, measurable disease per RECIST v1.1, performance status (PS) ≤1 and adequate major organ function are being treated with PM01183 7.0 mg flat dose i.v. every 3 weeks.

The primary aim is to evaluate the clinical efficacy of PM01183 in two cohorts of MBC pts: cohort A: BRCA+ (known germline BRCA1/2 mutation) and cohort B: unselected (BRCA1/2 wild type or unknown mutation status).

The primary endpoint is confirmed ORR by RECIST v1.1. A futility analysis was planned when 20 and 30 pts were recruited in cohort A and B, respectively. If at least 4 pts in cohort A and 3 pts in cohort B, achieve a response, recruitment in that cohort will continue up to 53 and 64 total pts, respectively.

Results: As of June 2014, 56 pts had been enrolled. Cohort A/B (n: 21/35): Median age 40/52-years-old; Cohort A(%)/B(%): PS 0: 48/66; >2 metastatic sites: 58/40; most common sites of metastasis: lymph node 79/33, liver 48/60 and bone 42/48; triple negative: 57/46; hormonal receptor +: 38/49; prior anthracyclines: 95/91, taxanes: 100/94, platinum: 52/26, PARPi: 29/0; cohort A: BRCA 1/2 (%): 52/48.

<table>
<thead>
<tr>
<th></th>
<th>Cohort A-BRCA 1/2+ (n=21)</th>
<th>Cohort B-unselected (n=35)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median cycles (range)</td>
<td>4 (1-20)</td>
<td>3 (1-14)</td>
</tr>
<tr>
<td>Best overall response</td>
<td>(n= 17 evaluable)</td>
<td>(n= 34 evaluable)</td>
</tr>
<tr>
<td>CR</td>
<td>1 (6%)</td>
<td>0</td>
</tr>
<tr>
<td>PR</td>
<td>6 (35%)</td>
<td>3 (9%)</td>
</tr>
<tr>
<td>SD</td>
<td>6 (35%)</td>
<td>16 (47%)</td>
</tr>
<tr>
<td>PD</td>
<td>4 (24%)</td>
<td>15 (44%)</td>
</tr>
<tr>
<td>ORR (95%CI)</td>
<td>41% (18-67%)</td>
<td>9% (1.9-23.7%)</td>
</tr>
<tr>
<td>Median duration of response Monts (95%CI)</td>
<td>5.0 (0.1-12.8)</td>
<td>3.3 (1.4-5.1)</td>
</tr>
</tbody>
</table>

In an exploratory analysis, ORR in cohort A was higher in PARPi naïve pts: 64% (7/11 pts).

Grade (G) 3-4 related adverse events occurred in ≥5% pts were myelosuppression (neutropenia 69%, febrile neutropenia 7%,...
thrombocytopenia 14%); G3 fatigue 7%, transient transaminase increase 19% (G4: 2%), nausea 6% and dyspnea 6% (3 pts, 2 of them due to pneumonitis). One patient with massive liver involvement and impaired function died on C1D4 due to multiorgan failure possibly related to the study drug.

**Conclusions:** PM01183 has promising activity in pretreated MBC pts with *BRCA* mutation. Safety profile appears to be mostly predictable and with non-cumulative toxicity. Treatment-related neutropenia was manageable with G-CSF and/or dose reduction. After futility analysis, targeted activity has been met in cohort A (*BRCA+*), and recruitment will continue up to 53 evaluable *BRCA+* pts.
**Title:** Phase II randomised clinical study of first line chemotherapy plus metformin versus first line chemotherapy alone in HER2 negative, non diabetic, metastatic breast cancer patients: Final results of the MYME study

Alessandra Gennari¹, Oriana Nanni², Andrea DeCensi¹, Samanta Sarti², Andrea Freschi³, Alessandra Bologna⁴, Lorenzo Gianni⁵, Laura Amaducci⁵, Francesco Rosetti⁵, Filippo Giovanardi⁶, Anna Fedeli⁶, Massimo Ambroggi¹⁰, Paolo Bruzzi¹¹ and Dino Amadori².

¹SC Oncologia Medica, EO Ospedali Galliera, Genoa, Italy; ²IRCCS Istituto Scientifico Romagnolo per lo Studio e la Cura dei Tumori (IRST), Meldola (FC), Italy; ³Centro di Riferimento Oncologico, Aviano, Italy; ⁴Arcispedale S. Maria Nuova-IRCCS, Reggio Emilia, Reggio Emilia, Italy; ⁵Ospedale degli Infermi, U.O. Oncologia Medica, Rimini, Italy; ⁶Ospedale degli Infermi, U.O. Oncologia Medica, Faenza, Italy; ⁷AULSS n.13 di Mirano, U.O. Oncologia Medica, Mirano (VE), Italy; ⁸DH Oncologico, Ospedale di Guastalla, Guastalla (RE), Italy; ⁹P.O.M. Bufalini, U.O. Oncologia Medica, Cesena (FC), Italy; ¹⁰Ospedale Civile di Piacenza, U.O. Oncologia Medica, Piacenza, Italy and ¹¹IRCCS San Martino IST, Genoa, Italy.

**Body:**

Background: Epidemiological studies indicated that the presence of insulin resistance is an adverse prognostic factor in MBC. Recently increasing interest has focused on metformin, an oral insulin-sensitizing drug widely prescribed for type 2 diabetes; unexpensive and well tolerated, metformin has also been shown to have direct antiproliferative properties in breast cancer. We present here the final analysis of a phase II comparative multicentric study on the addition of metformin to first line chemotherapy in MBC non diabetic patients.

Methods:

This is a Phase II randomized study of HER-2 negative MBC patients with measurable or non-measurable disease; no prior chemotherapy for MBC was allowed. Patients were allowed to have had prior endocrine therapy for MBC and prior adjuvant chemotherapy if completed at least 1 year prior to study entry. Patients were stratified by HOMA Index (>2.5 vs <= 2.5) and center. Patients were randomly assigned to Arm A, non pegylated liposomal doxorubicin 60 mg/sqm plus cyclophosphamide 600mg/sqm (AC) plus metformin (M) 1,000 mg PO QD or to AC alone. Treatment was administered for 8 3-weekly cycles in both arms, M was administered until disease progression. The primary endpoint was progression free survival (PFS). Secondary objectives included activity, safety and evaluation of metabolic profile. Correlative studies evaluated 1) circulating tumor cells and 2) modulation of insulin-related genes in mRNA isolated from CTCs. Planned sample size was 112 patients (98 events).

Results:

As of June 8th, 2014, 108 patients had been randomised. Median age was 60 yrs (range 36-77); 87% of patients were ER+, 60% had received prior adjuvant CT, with antracyclines in 51% of patients. Prior endocrine therapy for MBC was used in 39% of the patients. Measurable disease was present in 74% of the patients. 48% of the patients were insulin resistant by HOMA Index >2.5 and 60% were overweight (BMI > 25: 16% were obese, BMI >30). At a median follow up of 16 months (range 1 – 48), median PFS (ITT) was 9 months (95% CI 8-14) with AC + M and 11 months (95% CI 7-16) with AC alone, p=.84. No significant interaction was detected between HOMA Index and treatment arm (p = 0.15). Median OS was 30 months (95% CI 14-NE) in Arm A versus 27 (95% CI 17-33) in Arm B, p = .58. The most common toxicities observed were G3/4 neutropenia in 51.5% of patients in arm A vs 69.6% in arm B, with Febrile Neutropenia observed in 2.2% and 5.4% of patients, respectively. As expected G2 diarrhea was reported by 11.1% of patients in Arm A.

Conclusions: The addition of M to AC in MBC patients receiving first line chemotherapy did not improve PFS compared with AC alone. M seems to have a protective effect on hematological toxicity. Final results including translational data will be available at SABCS 2014.
Title: Efficacy and safety of eribulin in combination with capecitabine in patients with metastatic breast cancer: an open-label, phase II dose-confirmation study

Chris Twelves¹, Muhammad Y Nasim², Alan Anthoney¹, Claudio I Savulsky³, Shuxin Yin⁴ and TR Jeffry Evans². ¹Leeds Institute of Cancer and Pathology and St James's Institute of Oncology, Leeds, United Kingdom; ²Beatson West of Scotland Cancer Centre, University of Glasgow, Glasgow, United Kingdom; ³Eisai Ltd, Hatfield, United Kingdom and ⁴Eisai Ltd, Woodcliff Lake.

Body: Background: Two phase III trials have demonstrated the efficacy of eribulin monotherapy in patients with metastatic breast cancer (MBC) who have progressed after previous anthracycline and taxane chemotherapy. Capecitabine is also an effective agent in patients with MBC. A phase Ib dose-escalation study has investigated two dosing schedules of eribulin in combination with capecitabine to determine the recommended phase II dose. The aims of this open-label, non-randomized, phase II, dose-confirmation study were to determine efficacy and safety of this combination in patients with MBC. Interim data to April 2014 are provided here; final results will be presented at the meeting.

Methods: Eligible patients were females with MBC who had received up to 3 previous chemotherapy regimens in any setting (including an anthracycline and a taxane, but excluding capecitabine), an ECOG performance status (PS) ≤ 1, and adequate hematological, renal and hepatic function (at study entry, 20 [47.6%] patients had liver metastases; 24 [57.1%] had lung metastases). Patients received 1.4 mg/m² eribulin mesylate (equivalent to 1.23 mg/m² eribulin [expressed as free base]) i.v. on days 1 and 8 plus oral capecitabine 1000 mg/m² b.i.d. on days 1–14 (21-day cycles) until disease progression, unacceptable toxicity or death. Disease assessments (CT scans) were performed every 6 weeks. The primary outcome was the objective response rate (ORR); secondary outcomes included clinical benefit rate (CBR), progression-free survival (PFS), and safety and tolerability.

Results: 42 females (median age: 52.5 years [range: 32–74]; ECOG-PS of 0: n = 18 [42.9%]) were enrolled; patients received study drug as a 1st-line (23.8% [n = 10]), 2nd-line (45.2% [n = 19]) or ≥3rd-line (31.0% [n = 13]) chemotherapy. A median of 8 (range: 1–30) treatment cycles were administered (59.5% of patients received ≥6 cycles), with 94.4% of eribulin doses and 94.5% of capecitabine doses given as planned. The ORR was 42.9% and the CBR was 57.1% (Table), with a median PFS of 7.1 months (95% CI: 4.4, 9.8). The most common grade 3/4 adverse events (AEs) were neutropenia (66.7%), leukopenia (14.3%), febrile neutropenia (7.1%) and anemia, fatigue, nausea and peripheral sensory neuropathy (each 4.8%). AEs of special interest (any grade) included: hand–foot syndrome (26.2%), peripheral neuropathy (23.8%), and diarrhea (21.4%). The incidences of AEs leading to study drug dose adjustment were: 47.6% of patients (dose interruption), 42.9% (dose reduction), and 2.4% (drug withdrawal).

Table: Tumor response (investigator assessment)

| Patients N = 42 |  
|----------------|----------------|
|                | n (%)          |
| Complete response (CR) | 1 (2.4)       |
| Partial response (PR) | 17 (40.5)     |
| Stable disease (SD) | 16 (38.1)     |
| Progressive disease (PD) | 3 (7.1)      |
| Not evaluable | 5 (11.9)       |
| Objective response rate (CR + PR) | 18 (42.9)     |
| 95% CI | 27.7, 59.0     |
| Clinical benefit rate (CR + PR + SD ≥ 6 months) | 24 (57.1)     |
Conclusions: This phase II study suggests that eribulin in combination with capecitabine is efficacious in patients with MBC, with a safety and tolerability profile consistent with previous data. Phase III studies of this combination in patients with MBC are warranted.
Title: Change in survival of stage III and IV breast cancer patients from an institutional cohort: 1990-2007

Henry G Kaplan¹, Judith A Malmgren² and Mary K Atwood¹. ¹Swedish Cancer Institute, Seattle, WA and ²HealthStat Consulting Inc, Seattle, WA.

Body: Background: Breast cancer (BC) survival has significantly improved over the last twenty years. Our investigation compares change in BC survival over time for patients presenting with Stage III and IV disease to evaluate the impact of treatment changes during this time period.

Methods: We examined breast cancer specific survival among a cohort of stage III and IV breast cancer patients age 40-64 years, identified and tracked for mortality from 1990-2007 at our institution (n=710). Patients were all biopsy proven BC with diagnosis, treatment and follow up recorded and tracked in our breast cancer specific registry database. Breast cancer staging was converted to AJCC 7 to remove inconsistency over time. Pearson chi square tests were used for bivariate comparisons. Kaplan Meier method was used for survival analysis with breast cancer death as the outcome for disease specific survival (DSS) and the log rank test for survival comparisons.

Results: Mean age of patients was 51.32 years with 85% stage III (n=603) and 15% stage IV (n=107). The percentage of stage IV patients presenting at our institution did not change over time (p = .197). Stage IV patients were less likely than stage III patients to have surgery, receive radiation or adjuvant chemotherapy (all p<.001) (table). Stage IV patients that did receive systemic therapy were less likely to receive taxane therapy (p = .005) and were more likely to receive non-anthracyline regimens (p = .001) (table). There was no difference in hormone therapy given for the two groups. Five year disease specific survival (DSS) for all years combined was 79% for stage III vs. 51% for stage IV [10 year DSS stage III = 62%, stage IV = 24%] (p<.001). When stratified by diagnosis year, stage III patients had significant change over time for 5 and 10 year disease free survival with a 13% improvement for 5 year DSS and 9% improvement for 10 year DSS over the 17 year time period [5 year DSS: 1990-94 = 71%, 1995-99 = 78%, 2000-07 = 84%; 10 year DSS: 1990-94 = 56%, 1995-99 = 60%, 2000-07 = 65% (p = .037). No change was observed over time for stage IV breast cancer [5 year DSS: 1990-94 = 50%, 1995-99 = 46%, 2000-07 = 52%; 10 year DSS: 1990-94 = 17%, 1995-99 = 33%, 2000-07 = 20% (p = .461)].

Conclusions: The significant improvement in stage III survival is most likely related to treatment improvements for breast cancer in the studied time period. The lack of improvement in survival for stage IV patients may be due to a biologic change in the nature of metastatic tumors that impacts response to treatment or possibly the less aggressive use of systemic therapy in stage IV relative to stage III patients.

<table>
<thead>
<tr>
<th>Treatment Characteristics stage III and IV Breast Cancer (n=710)</th>
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<tbody>
<tr>
<td>variables</td>
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<tr>
<td>chemotherapy = no</td>
</tr>
<tr>
<td>radiation = no</td>
</tr>
<tr>
<td>surgery = no</td>
</tr>
<tr>
<td>taxanes = no</td>
</tr>
<tr>
<td>non-anthracyline regimen</td>
</tr>
<tr>
<td>hormone therapy = yes</td>
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</table>
2014 San Antonio Breast Cancer Symposium

Publication Number: P3-13-06
Average Grade: 4.20

Title: Efficacy of eribulin in patients with invasive lobular carcinoma of the breast: data from a pooled analysis

Javier Cortés¹, José Pérez¹, Yi He² and Otto Metzger-Filho³. ¹Vall d’Hebron Institute of Oncology, Vall d’Hebron University Hospital, Universitat Autònoma de Barcelona, Barcelona, Spain; ²Eisai Inc, Woodcliff Lake, NJ and ³Dana-Farber Cancer Institute, Boston, MA.

Body: Background: Invasive lobular carcinoma (ILC) represents the second most common breast cancer (BC) subtype and is usually characterized as hormone-receptor positive, low-to-intermediate histologic grade, and human epidermal growth factor receptor (HER) 2-negative. In the early-stage setting, ILCs are associated with lower rates of pathological response to preoperative chemotherapy compared with invasive ductal carcinoma (IDC). This exploratory analysis investigated the magnitude of benefit of single-agent eribulin for the treatment of advanced ILC using data from three clinical trials in women with advanced BC. We also describe the patterns of response and survival outcomes compared with IDC.

Methods: Individual patient (pt) data from the experimental arms of two phase III studies (305 and 301) and a single-arm, phase II study were pooled for the present analysis. Study 305 (EMBRACE) randomized pts treated with ≥2 lines of chemotherapy for advanced BC to receive eribulin or treatment of physician’s choice. In study 301, pts treated with ≤2 lines of chemotherapy for advanced BC were randomized to receive eribulin or capecitabine. In the phase II study, pts who had received ≥3 lines of chemotherapy were treated with eribulin. Overall survival (OS) and progression-free survival (PFS) analyses were adjusted by study, estrogen receptor (ER) and HER2 status, and number of lines of therapy for advanced disease.

Results: The three studies included 1353 eribulin-treated pts. Of the 1152 pts included in the present analysis, 118 were classified as ILC and 1034 as IDC. Median age of ILC and IDC pts was 58 years and 55 years, respectively. ER and/or progesterone receptor (PgR) positivity was more common in ILC (ER = 69%, PgR = 55%) than IDC (ER = 60%, PgR = 48%), while HER2 positivity was less frequent in ILC than IDC (9% vs 16%). A total of 52.5% of ILC and 61.4% of IDC pts received ≥3 lines of chemotherapy (for any stage BC) prior to eribulin. Pts with ILC and IDC had similar median OS (13.4 vs 13.5 months; hazard ratio [HR] = 1.10; 95% confidence intervals [CIs] 0.87, 1.38) and PFS (4.1 vs 3.6 months; HR = 0.91; 95% CIs 0.72, 1.14). Investigator-evaluated tumor response rates are shown in the table.

Conclusion: In this exploratory, pooled analysis, magnitude of benefit from single-agent eribulin did not differ between the ILC and IDC cohorts. While there was a limited numbers of pts with ILC, response rates, PFS, and OS were similar for the two pt groups. The results with eribulin for advanced ILC contrast with data for other agents in early-stage settings, where ILC is generally less responsive to chemotherapy than IDC. These findings may, however, underline changes in the disease biology after exposure to previous therapies or changes inherent to disease progression.

<table>
<thead>
<tr>
<th>Response, %</th>
<th>Invasive lobular carcinoma, n = 110</th>
<th>Invasive ductal carcinoma, n = 985</th>
</tr>
</thead>
<tbody>
<tr>
<td>Objective response rate: CR + PR (95% CIs)</td>
<td>15.5 (9.3, 23.6)</td>
<td>14.8 (12.7, 17.2)</td>
</tr>
<tr>
<td>Complete response (CR)</td>
<td>0</td>
<td>0.4</td>
</tr>
<tr>
<td>Partial response (PR)</td>
<td>15.5</td>
<td>14.4</td>
</tr>
<tr>
<td>Stable disease (SD)</td>
<td>53.6</td>
<td>52.7</td>
</tr>
<tr>
<td>Progressive disease (PD)</td>
<td>26.4</td>
<td>28.9</td>
</tr>
<tr>
<td>Clinical benefit rate: CR + PR + SD ≥6 months (95% CIs)</td>
<td>29.1 (20.8, 38.5)</td>
<td>28.6 (25.8, 31.6)</td>
</tr>
<tr>
<td>Not evaluable</td>
<td>4.5</td>
<td>3.6</td>
</tr>
</tbody>
</table>
Title: Does treatment response to new agents differ between visceral and non-visceral metastatic breast cancer: A systematic literature review of registration trials

Rachel Würstlein¹, Maximilian Bardenhewer¹, Alexander König¹, Thomas Kolben¹, Caroline Gehring¹ and Nadia Harbeck¹. ¹Breast Center, University of Munich (LMU), Munich, Germany.

Body: Differential efficacy of newly registered therapies in subgroups of metastatic breast cancer (mBC) is an important consideration for their subsequent use in clinical practice. Unfortunately, such subgroup analyses are often exploratory, rarely statistically adequately powered and may thus be misleading. In a systematic literature review, we evaluated differences in outcome (taking into account the diversity of available study data, inhomogeneous in- and exclusion criteria and subgroup-analyses) regarding progression free survival (PFS), time to progression (TTP), overall survival (OS) and visceral versus non-visceral disease. The impact of HER2- and hormone receptor-status was also considered.

Methods: A systematic literature search (6362 hits) in the meta-Database PubMed (U.S. National Library of Medicine) was performed for the last 20 years. 257 studies (n=126,291) were included for further analysis. 69 studies had published data for visceral (i.e. presence of visceral metastases independent of the presence of other metastasis sites) vs. non-visceral (i.e. non-visceral metastases in the absence of visceral involvement) disease including phase III trials plus studies that had further used their data. Out of these 69 studies we selected n=16 studies (n=13,083) by considering (A) the information given in the medical product's professional information and (B) the decision of the U.S. Food and Drug Administration or the European Medicine Agency for the approval of the respective therapeutic agents which are now used in treatment of mBC. All selected 16 studies had looked at the endpoints mentioned above. In order to achieve comparability, we extracted the information of hazard ratios (HR), confidence intervals (CI) and times in weeks (if available) for PFS, TTP, OS of the entire study population, which was divided into three groups: HER2-positive, HER2-negative, unknown HER2 status.

Results: No statistically significant difference in treatment response was found in mBC patients with visceral vs. non-visceral metastasis looking at HRs and CIs. Relevant, yet not statistically significant differences were found in the specific response of visceral metastases to modern combination therapies, especially in HER2-positive breast cancer: There was an increased benefit regarding OS using Lapatinib combined with Trastuzumab or Trastuzumab and Docetaxel combined with Pertuzumab. Additionally, in two chemotherapy trials, there was a numerical difference between therapy response in visceral vs. non-visceral metastases regarding PFS in the unknown HER2 group, and regarding OS in the HER2-negative group.

Conclusions: Using a systemic literature search, we stratified published studies of the last 20 years considering HER2 and hormone receptor status with respect to metastasis pattern. In the subgroup analyses, we did not find any significant differences in response rates for visceral vs. non-visceral metastasis. For targeted therapies based on a biomarker, there seems to be a beneficial effect of combination therapies regarding OS in visceral disease. At the present time, metastasis localization should not be used as a predictive marker for choice of systemic therapy in mBC.
Title: Modification of the response to chemotherapy of HER2 negative metastatic breast cancer by lipids of marine origin: A controlled, randomized, double blind dietary supplementation trial

Philippe Bougnoux¹, Jacques Bonneterre², Anne Mercier-Blas³, Patrick Soulié⁴, Hélène Simon⁵, Franck Priou⁶, Christine Piprot-Choffat⁷, Christelle Levy⁸, Caroline Goupille⁹ and Virginie Berger¹⁰. ¹Inserm U 1069, CHU Bretonneau, Tours, France; ²Centre Oscar Lambret, Lille, France; ³CHP Saint Grégoire, Saint Grégoire, France; ⁴ICO Paul Papin, Angers, Pays de la Loire, France; ⁵CHU Morvan, Brest, France; ⁶CHD Les Oudairies, La Roche sur Yon, France; ⁷CHU, Amiens, France; ⁸Centre François Baclesse, Caen, France; ⁹Inserm U 1069, CHU Bretonneau, Tours, France and ¹⁰ICO Paul Papin, Angers, Pays de la Loire, France.

Body: Background: Long chain n-3 Polyunsaturated Fatty Acids (n-3 PUFA) of marine origin (docosahexaenoic acid, DHA and eicosapentaenoic acid, EPA) have been shown to increase sensitivity of cell lines to anthracyclins or taxanes. Preclinical studies and a phase II clinical trial have shown that increasing DHA level in the body tissues through a dietary supplementation improves chemotherapy efficacy. We have set up a phase III controlled, double blind study to determine whether a dietary supplementation with n-3 PUFA would increase PFS in patients receiving chemotherapy for metastatic breast cancer (MBC).

Methods: Inclusion criteria were patients with a ductal or lobular breast carcinoma, positive hormone receptors, Her2 (-), who have developed visceral metastasis, and were due to receive a 1st or 2nd line chemotherapy. Dietary intervention was carried out during chemotherapy. Patients had to take daily at each meal a can* (medical food) containing either fish oil (1.56 g/d of DHA and 2.64 g/d EPA, experimental arm) or coprah oil (short chains fatty acids, control arm). Principal endpoint was PFS, secondary were objective response, overall survival, dietary tolerance to cans, lipids plasma levels of n-3 PUFA.

Results: Sixty five patients with MBC have been prospectively enrolled in the DHALYA trial (NTC01548534). The first 45 patients who completed chemotherapy along with dietary supplementation are evaluable. Plasma fatty acid level was measured at baseline, at C1 after 10 days loading dose, at C3, and at C6 or at withdrawal. Level of EPA and DHA increased in half of the patients, thus allowing a putative allocation into either arm: arm A with induced elevation of PUFA levels (N=22), and arm B without any change in PUFA levels (N=23). No difference was observed in the quality of the dietary intervention (number of cans, duration) among arms. Distribution of patients according to demographics (age, menopausal status, histology, SBR grade), or type or quality of chemotherapy received (anthracycline- or taxane-based, number of cycles, length) was similar between arms. However a significantly greater proportion of patients with poor prognosis at staging (larger tumor size and greater axillary lymph nodes involvement) were observed in arm A. There was no difference in side effects (grade ≥2) between arms. In terms of efficacy, median PFS was 14.3 (arm A) and 12.5 (arm B) months, not statistically different yet.

Conclusion: A dietary intervention targeted on marine-derived PUFA during systemic chemotherapy for MBC is safe and feasible. A longer follow-up is required in order to know whether chemotherapy efficacy can be increased. This study was supported by a grant from the French Ministry of Health, PHRC-11-153. *Cans were graciously provided by Nutrialys Medical Nutrition, Saint Grégoire, France.
Body: INTRODUCTION
Cancer biology is characterised by genomic alterations. There is an ongoing revolution in cancer research due to the emergence of novel technologies based on next generation sequencing (NGS).

OBJECTIVES
To identify abnormalities in individual patients with the aim of providing targeted therapy matched to individuals genomic alterations.

METHODS
From April 2013, to February 2014, we included patients who had metastatic breast cancer with lesion accessible for biopsy. The tumor tissue stored in paraffin was evaluated by the method of next-generation sequencing (NGS) where 236 cancer-related genes were sequenced.

RESULTS
Fourteen patients were included and NGS was feasible. An actionable genomic alteration was identified in 12 (85.7%) patients, most frequently in TP53 (10 [83.3%], PIK3CA (8 [66.6%]), AKT (5 [50%]), FGFR1 (4 [33%]), CCND1 (3 [25%]), PTEN (3 [25%]) and BRCA2 (2 [16%]). Therapy could be personalized in 12 of 14 patients. Of the 8 patients who received targeted therapy, 5 presented objective response.

Results

<table>
<thead>
<tr>
<th>Patients</th>
<th>Target Therapy</th>
<th>Objective Response</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Everolimus</td>
<td>NO</td>
<td>ERBB2, PIK3CA</td>
</tr>
<tr>
<td>2</td>
<td>Everolimus</td>
<td>Yes</td>
<td>PI3KCA, FGFR, AKT</td>
</tr>
<tr>
<td>3</td>
<td>Everolimus</td>
<td>Yes</td>
<td>PI3KCA, FGFR</td>
</tr>
<tr>
<td>4</td>
<td>Everolimus</td>
<td>Yes</td>
<td>AKT1, PI3KCA</td>
</tr>
<tr>
<td>5</td>
<td>Everolimus</td>
<td>Yes</td>
<td>AKT1</td>
</tr>
<tr>
<td>6</td>
<td>Everolimus</td>
<td>Yes</td>
<td>AKT1, FGFR</td>
</tr>
<tr>
<td>7</td>
<td>Everolimus</td>
<td>Yes</td>
<td>AKT1, FGFR</td>
</tr>
<tr>
<td>8</td>
<td>Everolimus</td>
<td>No</td>
<td>ERBB2, PI3K</td>
</tr>
</tbody>
</table>

Table 1

Mutations Prevalence

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td>83.30%</td>
</tr>
<tr>
<td>PIK3CA</td>
<td>60.60%</td>
</tr>
<tr>
<td>FGFR</td>
<td>33%</td>
</tr>
<tr>
<td>CCND1</td>
<td>25%</td>
</tr>
<tr>
<td>PTEN</td>
<td>25%</td>
</tr>
</tbody>
</table>
CONCLUSION

- NGS was able to find genomic alterations in the majority of breast cancer tissues;
- Most patients did not have "actionable" mutations for which a specific therapy could be offered;
- We are just at the dawn of exploring this strategy and NGS can effectively contribute for increasing the understanding of the disease.

REFERENCES
Title: The maintenance treatment after xeloda contained regimens with xeloda or endocrine for HR positive and HER2 negative MBC patients

Zefei Jiang¹, Fan Qi¹, Shaohua Zhang¹, Li Bian¹, Tao Wang¹ and Lei Li¹. ¹Affiliated Hospital of Academy of Military Medical Science, Beijing, China.

Body: Objective To discuss the optimizing selection of maintenance treatment after xeloda-contained chemotherapy regimen being applicated in HER-2 negative, HR positive relapsing metastatic breast cancer(MBC) patients, and contrast the curative effect of maintenance treatment with xeloda or endocrine. Methods 119 patients with HER-2 negative, HR positive were enrolled during 2009-2013. All patients taking the xeloda contained chemotherapy regimens to obtain CR,PR or SD were randomly divided into group A(65 cases, maintenance capecitabine therapy 800-1000mg/ m2 bid, orally, Days 1 ∼ 14, q3w)and group B(54 cases, a switch to maintenance endocrine therapy). Endpoints include overall survival (OS) and progression-free survival (PFS). Results The ages, menopausal status, number of metastases and treatment line numbers had no statistical differences between two groups, and the p value of two therapy co-operative groups was 0.002. There were 37 patients(56.9%)using TX and 28(43.1%)using NX in therapy co-operative group A. There were 39 patients (72.2%)using TX, 9(16.7%)using NX and 6(6%) using regimen containing xeloda in therapy co-operative group B. Progression-free survival was 8 months in patients who follow by xeloda and 12 months in those who follow by endocrine (p<0.01).Conclusion Endocrine therapy after xeloda-contained chemotherapy regimen tend to be more effective than single xeloda therapy for patients with HER-2 negative, HR positive. This results request to be verified by advanced OS data, which was needed to in-depth study in clinic and explore relevant biological indicators.
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Average Grade: 0

Title: Response to treatment and prognosis of recurrent breast cancer patients with receptor discordance

Hanako Ueno, Hiromitsu Jinno, Takamichi Yokoe, Toshiaki Kurihara, Masaru Takemae, Aiko Nagayama, Maiko Takahashi, Tetsu Hayashida, Kaori Kameyama and Yuko Kitagawa. 1Keio University School of Medicine, Tokyo, Japan and 2Keio University School of Medicine, Tokyo, Japan.

Body: BACKGROUND
Estrogen receptor (ER), progesterone receptor (PgR) and human epidermal growth factor receptor -2 (HER2) statuses are clinically used to select treatments. Several studies reported that discordance between primary and metastatic lesions lead to detrimental outcome. Although biopsy of recurrent breast cancer has been recently recommended by international clinical guidelines, prognostic relevance remains to be elucidated. The aim of the present study is to evaluate response to treatment and prognosis of patients with receptor discordance, compared with patients with receptor concordance.

Patients and METHODS
We retrospectively identified recurrent breast cancer patients who had biopsies or resections of recurrent lesions between January 2007 and April 2012 at Keio University Hospital. HR status was assessed by immunohistochemistry (IHC) and determined using the Allred score. HR status was defined as positive when score was 3 and more. HER2 status was assessed by IHC and fluorescence in situ hybridization (FISH) analysis. We defined HER2 positivity as 3+ staining intensity by IHC or the presence of HER2 gene amplification by FISH. Tumors were classified as luminal (HR+ and HER2-), luminal/HER2 (HR+ and HER2+), HER2 (HR- and HER2+), or triple negative (HR- and HER2-). Treatment was decided according to the receptor status of recurrent tumors.

RESULTS
Among 38 patients undergoing biopsy or resection, 13.2% (5) were loco-regional recurrences and 86.8% (33) were distant metastases (lung 21; liver 6; brain 3; pleura 1). Overall, 10 patients (26.3%) changed subtypes at recurrent lesions (Table 1) and all of them had a change in treatment plan.

Discordance in subtype between primary tumor and recurrent lesion

<table>
<thead>
<tr>
<th>Recurrent lesion (%)</th>
<th>Luminal</th>
<th>Luminal/HER2</th>
<th>HER2</th>
<th>Triple negative</th>
<th>Discordance rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Primary tumor (%)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal</td>
<td>18 (66.7)</td>
<td>2 (8.3)</td>
<td>1(4.2)</td>
<td>3(12.5)</td>
<td>6/24 (25.0)</td>
</tr>
<tr>
<td>Luminal/HER2</td>
<td>1 (14.3)</td>
<td>6 (85.7)</td>
<td>0</td>
<td>0</td>
<td>1/7 (14.3)</td>
</tr>
<tr>
<td>HER2</td>
<td>0</td>
<td>1 (100.0)</td>
<td>0</td>
<td>0</td>
<td>1/1 (100.0)</td>
</tr>
<tr>
<td>Triple negative</td>
<td>1 (16.7)</td>
<td>1 (16.7)</td>
<td>0</td>
<td>4(66.7)</td>
<td>2/6 (33.3)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>20</td>
<td>10</td>
<td>1</td>
<td>7</td>
<td>10/38 (26.3)</td>
</tr>
</tbody>
</table>

Changes in management included the addition of trastuzumab in patients with gain of HER2 (n=3), the use of chemotherapy in those with loss of HR (n=6) and provision of endocrine therapy for those with gaining HR (n=3). Response rate of discordant group and concordant group were 10.0% and 21.4%, respectively (p=1.000). Clinical benefit rate of discordant group and concordant group were 40.0% and 67.9%, respectively (p=0.150). There is no significant difference of time to progression (TTP) between the discordant and concordant groups (169.5days vs.319.5days, p= 0.081).

CONCLUSION
Patients with receptor discordance tended towards worse response rate and shorter TTP, leading to the poor prognosis of the recurrent breast cancer patients with receptor discordance.
**Title:** Circulating tumor cell (CTC) enumeration and HER2 assessment as predictors of breast cancer outcomes in the ALTTO (BIG 2-06, Alliance N063D) Trial

Minetta C Liu¹, Brigitte Rack², Amylou C Dueck⁴, David W Hillman¹, Michael B Campion¹, Monica M Reinholz³, Kevin C Halling¹, Christos Sotiriou⁶, Françoise Rothé⁵, Marion Maetens⁶, Ghizlane Rouas⁶, Wolfgang Janni⁶, Antonio C Wolff⁷, Lyndsay N Harris⁸, Julie R Gralow⁹, Kathleen I Pritchard¹², Susan Ellard¹², Nguyet A Le-Lindqwister¹³, Frances Boyle¹⁴, Evandro De Azambuja⁵, Martine J Piccart-Gebhart⁵, Michail Ignatiadis⁵ and Edith A Perez¹¹.

¹Mayo Clinic, Rochester, MN; ²Ludwig-Maximilians-Universität München, Munich, Germany; ³Ventana Medical Systems, Inc, A Memberof the Roche Group, Tüscön, AZ; ⁴Mayo Clinic, Scottsdale, AZ; ⁵Institut Jules Bordet, Brussels, Belgium; ⁶Universitäts-Frauenklinik Ulm, Ulm, Germany; ⁷Johns Hopkins Hospital/Sidney Kimmel Comprehensive Cancer Center, Baltimore, MD; ⁸Case Western/Seidman Cancer Center, Cleveland, OH; ⁹Seattle Cancer Care Alliance, Seattle, WA; ¹⁰Sunnybrook Odette Cancer Centre and University of Toronto, Toronto, ON, Canada; ¹¹Mayo Clinic, Jacksonville, FL; ¹²British Columbia Cancer Agency, Southern Interior, Kelowna, BC, Canada; ¹³Illinois Cancer Care-Peoria, Peoria, IL and ¹⁴Patricia Ritchie Centre for Cancer Care and Research, University of Sydney, Mater Hospital, North Sydney, Australia.

**Body:**

**Background:** CTCs are associated with clinical outcomes in metastatic breast cancer irrespective of ER/PR/HER2 status. Some data support the prognostic relevance of serial CTC enumeration relative to adjuvant chemotherapy in early stage breast cancer. However, data from a large scale study focused on HER2 directed therapy for HER2+ disease have been lacking. We therefore sought to prospectively evaluate the effect of trastuzumab +/- lapatinib on CTCs and assess the prognostic/predictive value of CTC monitoring in HER2+ early stage breast cancer patients (pts).

**Methods:** The Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation (ALTTO; NCT00490139) Trial is an international, randomized, open-label phase III study of two targeted agents for HER2+ breast cancer. From June 2007 to July 2011, 8381 pts were randomised from 946 sites in 44 countries to 1 of 4 arms with sequential or concurrent chemotherapy: (i) 52 wks of trastuzumab (T); (ii) 52 wks of oral lapatinib (L); (iii) 12 or 18 wks of T followed by a washout and then 34 or 38 wks of L; or (iv) 52 wks of L+T. 540 (6%) pts provided optional informed consent and up to 30 mL peripheral blood suitable for CTC analyses at baseline with additional collections at 13 or 19 wks, 52 wks, 18 mos, 24 mos, and recurrence. CTC analyses are being conducted in three laboratories (Mayo Clinic Rochester, n=431; Institut Jules Bordet and University of Munich, n=109). 2-3 x 10 mL CellSave™ samples are pooled and processed at each time point for CTC enumeration and HER2 expression using the immunomagnetic/immunofluorescence assay (CellSearch™). A round-robin concordance project was done between Mayo Clinic Rochester and Institut Jules Bordet before embarking on the primary correlative work.

**Results:** At baseline, 20% pts had detectable (i.e., ≥1) EpCAM+/CK+/DAPI+/CD45- CTCs, and 16% pts had detectable EpCAM+/CK+/DAPI+/CD45-/HER2+ CTCs. Correlative analyses with clinical outcome are ongoing with plans for completion by Fall 2014.

**Conclusions:** CTCs were detected in 20% of pts with HER2+ early stage breast cancer. This is similar to the frequency of detection in mixed early stage breast cancer populations relative to ER/PR and HER2 status. Concordance of enumeration and HER2 assessments between the two experienced laboratories, and correlation between disease free survival and CTC findings (from serial samples collected at baseline, during the course of HER2 directed therapy, and at set intervals of follow-up) will be reported.
Title: Prospective characterization of HER2-positive circulating tumor cells in patients with HER2-negative metastatic breast cancer

Ian E Krop¹, Erin Macrae², Sarah R Galler¹, Farideh Bischoff³, Romeo Fauni³, Edgar Sales³, Lan Huynh³, Christine Mitchell³, Trisky Clarin-Tamayo³, Mark Anderson³, Leslie Abad³ and Eric Winer¹. ¹Dana-Farber Cancer Institute, Boston, MA; ²Ohio State University, Columbus, OH and ³Biocept, Inc, San Diego, CA.

Body: Background: We have previously shown feasibility by CLIA validation of HER2 FISH on circulating tumor cells (CTCs). Several small retrospective studies have identified HER2 amplification in CTCs in a subset of patients (pts) with clinically HER2 negative metastatic breast cancer (MBC). While these findings potentially have profound implications for CTCs as a predictive biomarker, prospective validation and functional characterization of this subgroup is necessary.

Methods: We enrolled a prospective cohort of pts with MBC that was HER2 negative by IHC and/or FISH on all available primary and metastatic biopsies. Pts had ≥1 line of prior chemotherapy for MBC. Blood samples were collected at study entry. CTCs were enumerated based on standard criteria (Cytokeratin (CK)+/CD45- staining) as well as by FISH for HER2 using the OncoCEE-BR® CTC test (Biocept, Incorporated). This test employs a proprietary antibody cocktail for capture followed by CK/CD45 staining, and FISH analysis directly within a microfluidic device. Samples were reported as positive if the HER2/CEP17 ratio was ≥2.0. HER2+ CTCs were classified into two categories: CK+/CD45-/HER2+ and CK-/CD45-/HER2-

Results: CTCs were observed in 208 out of 323 pts (64%). Median number of CTCs was 10 (range 1 to > 34195). 75 pts (23%) had HER2+ CTCs, with a median number of 3 HER2+ CTCs (range 1 to 21). 36% (27/75) of these pts had CK+/HER2+ CTCs and 43% (32/75) of pts had only CK-/HER2+ CTCs. The remaining pts (21%) had both CK+/HER2+ and CK-/HER2+ CTCs present.

Conclusion: HER2 amplified CTCs are present in a subset (23%) of pts with clinically HER2-negative breast cancers. Interestingly, we observed a high prevalence of pts with only CK-/HER2 amplified CTCs (32 out of 323; 10%). The unique multi-antibody CTC capture method used here thus allows for detection of a prevalent population of CK-/HER2+ CTCs that may be largely undetected by other current adopted technologies. The functional significance of CK-/HER2+ and CK+/HER2+ CTCs in patients with clinically HER2 negative breast cancer is currently being evaluated in a prospective study with HER2-directed therapy.
Title: Prognostic relevance of circulating tumor cells across different molecular subgroups in the adjuvant SUCCESS-A study


Body: Aim: The prognostic value of circulating tumor cells (CTCs) in the adjuvant setting has recently been demonstrated in the SUCCESS A Study (Rack et al. JNCI 2014). As breast carcinomas depend on partly different pathways for progression, the relevance of CTCs could differ between molecular intrinsic subtypes of breast cancer. Aim of this study was therefore to analyze the prognostic impact of CTCs in molecular subtypes of a large patient cohort.

Methods: Within the adjuvant SUCCESS A Study, patients were treated either with 5-Flourouracil, Epirubicin and Cyclophosphamid (FEC) followed by Docetaxel (D) or with FEC followed by D and Gemcitabine (DG). There was no restriction with regard to molecular type, however a high recurrence risk was required for study entry. In addition patients were assessed prospectively for the presence of CTCs before chemotherapy. Molecular subtypes were defined as: triple negative (TN), hormone receptor positive and grading 1/2 (LUM A like), hormone receptor positive and grading 3 (LUM B like), HER2 positive (HER2 like).

We studied whether the addition of CTC status (0 CTC vs > 0 CTCs) to well-known predictors such as age, BMI, tumor size, lymph node status improved the prediction of overall survival (OS) and disease free survival (DFS) across all patients and especially within molecular subtypes using likelihood ratio tests, which compared multivariable Cox regression models with and without CTC and the interaction between CTC and molecular subtype.

Results: Information about molecular subtype and CTCs was available in a total of 1994 patients. At least one CTC was seen in 422 (21.2%) of patients. 383 (19.2%) were TN, 798 were LUM A like (40.0%), 328 (16.4%) were LUM B like (16.4%) and 485 (24.3%) were HER2 like. The effect of CTC on overall survival had a HR of 2.50 (95%: 1.75 to 3.58) for the entire cohort. However as the effect was different across subtypes (p=0.04, likelihood ratio test), subtype specific HR were calculated. The effects on OS were most prominent in LUM B like patients (HR=3.96; 95%CI: 1.93 to 8.14) and LUM A like patients (HR=3.57; 95%CI: 1.81 to 7.03), less strong in HER2 like (HR=2.35; 95%CI: 1.04 to 5.32) and not present in TN patients (HR=1.18; 95%CI: 0.62 to 2.24). CTC status had a clear effect on DFS as well (HR=1.93, 95%CI: 1.48 to 2.52). It could not be shown that this effect was different across subtypes (p=0.07, likelihood ratio test). However, the effect size was similarly distributed like the ones for OS.

Conclusion: With regard to OS the prognostic effect of CTCs in this study cohort seems most prominent in patients with hormone receptor positive disease. It is still significant in HER2 positive, but not in TN breast cancer patients. Results with regard to DFS trended into the same direction, differences within subgroups could however not be shown, possibly due to power reasons.
Circulating tumor cells (CTC) are associated with defects in innate and adaptive immunity in inflammatory breast cancer (IBC) patients

Michal Mego1,2, Hui Gao1, Evan N Cohen1, Simone Anfossi1, Antonio Giordano1, Sanda Tin1, Tamer M Fouad1, Wendy A Woodward1, Ricardo H Alvarez1, Vicente Valero1, Naoto T Ueno1, Gabriel N Hortobagyi1, Massimo Cristofanilli1,3 and James M Reuben1. 1University of Texas MD Anderson Cancer Center, Houston, TX; 2Comenius University, Faculty of Medicine, Bratislava, Slovakia (Slovak Republic) and 3Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, PA.

Body: Background: CTCs play a crucial role in tumor dissemination and are prognostic factor in primary and metastatic breast cancer patients. Immune cells in peripheral blood (PB) contribute to an unfavorable microenvironment for the CTCs survival. As such, effective host innate and adaptive immune surveillance systems could adversely influence tumor dissemination whereas dysfunctional immune systems could provide a favorable microenvironment for the dissemination of CTCs and cancer progression. This study aimed to correlate CTCs with the functions of innate [natural killer (NK) cells] and adaptive (T-cells) immune effector cells in PB of IBC patients.

Methods: This prospective study included 65 IBC (21 non-metastatic, 14 de novo metastatic and 30 recurrent metastatic) patients treated between October 2008 and April 2012 at the MD Anderson Cancer Center. CTCs were enumerated before patients started a new line of chemotherapy using the CellSearch® system, 33 (50.8%) of patients were treatment naïve at the time of blood collection. The phenotype of T cells, their ability to secrete cytokines following activation through the T-cell receptor (TCR) and the NK cell subsets were analyzed by multiparameter flow cytometry and the results were correlated with CTCs and clinical outcome. For survival analysis immune cell counts were dichotomized to low or high category using the median count.

Results: At least 1 CTC (≥1) or ≥5 CTCs per 7.5 mL of PB was detected in 40 (61.5%) or 21 (32.3%) of patients, respectively. Patients with at least 1 CTC or ≥5 CTCs had a significantly inferior overall survival (OS) [HR=2.48, p=0.003 and HR=1.85, p=0.045] than patients with no CTCs or with <5 CTCs, respectively. There was no correlation between CTCs count and total lymphocytes; however, patients with at least 1 CTC or ≥5 CTCs had significantly lower percentages of CD3+ and CD4+ T-cells compared with patients with no CTCs or < 5 CTCs, respectively. Patients with ≥1 CTC, had a lower percentage of TCR-activated CD8+ T-cells producing TNF-α (p=0.03) and IFN-γ (p=0.08), and a higher percentage of T regulatory lymphocytes (p=0.05) compared to patients with undetectable CTCs. Moreover, CTCs ≥5 was inversely associated with the percentage of the following NK cells subsets: non-ADCC NK (Spearman rho’ = -0.30, p=0.02), ADCC NK (rho’ = -0.15, p=0.20) and exhausted NK (rho = -0.24, p=0.04). We also observed increased prognostic value of CTCs in the context of adaptive immune cells, with worse OS for patients with ≥ 5 CTCs and low count of TCR-activated CD8+ T cells producing TNF-α (HR=6.72, p=0.0007) compared with patients with < 5 CTCs and high count of TCR-activated CD8+ that produced TNF-α.

Conclusions: IBC patients with CTCs in PB had abnormalities in both innate and adaptive immunity as evidenced by low percentages of NK cell subsets, and low percentage of TCR-activated CD8+ T cells producing TNF-α, respectively. These data illustrate an inverse relationship between CTCs and both innate and adaptive immune cells in the PB microenvironment that could potentially impact tumor cell dissemination and initiation of the metastatic cascade. Moreover, immune cell profiling could add further prognostic value to CTCs in IBC patients.
Title: Circulating tumor cell count as early predictor of metastatic spread in breast cancer patients with limited metastatic dissemination

Antonio Giordano¹,², Mario Giuliano¹,³, Ricardo H Alvarez¹, Michal Mego¹, E Benjamin Clyburn², Sabino De Placido³, Gabriel N Hortobagyi¹, Naoto T Ueno¹, Vicente Valero¹, Massimo Cristofanilli⁴ and James M Reuben¹. ¹University of Texas MD Anderson Cancer Center, Houston, TX; ²Medical University of South Carolina, Charleston, SC; ³University of Naples Federico II, Naples, Italy and ⁴Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, PA.

Body: Introduction: Traditional factors currently used for prognostic stratification do not always predict adequately treatment response and disease evolution in advanced breast cancer patients. Therefore, the use of blood-based markers, such as circulating tumor cells (CTCs), represents a promising complementary strategy for disease monitoring. In this retrospective study, we explored the role of CTC counts as predictor of disease evolution in breast cancer patients with limited metastatic dissemination.

Methods: Four-hundred and ninety-two advanced breast cancer patients who had a CTC count assessed by CellSearch prior to starting a new line of systemic therapy were eligible for this analysis. Using the threshold of 5 cells/7.5 mL of blood, pretreatment CTC counts were correlated in the overall population with metastatic site distribution (lymph nodes, soft tissues, bone, visceral, and brain), evaluated at baseline and at the time of treatment failure, using the Fisher’s Exact test. Time to visceral progression, as well as, time to the development of new metastatic lesions and sites were estimated in patients with metastases confined to non-visceral sites (bone, lymph node, soft tissues) and to a single site, respectively, by the Kaplan-Meier method. Survival times were compared among groups according to pretreatment CTC count by log-Rank test.

Results: In the overall population, pretreatment CTCs ≥ 5 were associated with greater metastatic tumor burden and higher frequency of bone involvement, compared with CTCs < 5 (P<.0001). At the time of treatment failure, patients with CTCs ≥ 5 developed more frequently new metastatic lesions and sites compared to those with CTCs <5 (development of new lesions P=.0031; development of new sites P=.0002). Among patients with disease originally confined to non-visceral sites, CTCs ≥ 5 were associated with remarkably shorter time to visceral metastases (P=.001) and overall survival (P=.0006), compared with CTCs < 5. Finally, in patients with single-site metastatic disease, CTCs ≥ 5 were associated with a significant reduction of the time to development of new metastatic sites (P=.0002) and lesions (P=.0051), and with worse overall survival (P=.0101). Multivariate analysis confirmed that these differences were independent from other variables, such as estrogen receptor (ER) and human epidermal growth factor receptor 2 (HER2) status, and presence of visceral or bone metastases at baseline.

Conclusion: Our results suggest that baseline CTC counts can be used as an early predictor of metastatic potential in breast cancer patients. High CTC count can potentially direct patients with limited metastatic dissemination to combination regimens including therapeutic agents targeting various phases of the metastatic process such as invasion, migration, and immune escape. Prospective studies, based on CTC enumeration and molecular profiling, may further help to develop novel treatment decision tools for breast cancer patients with limited metastatic dissemination.
Title: Circulating and disseminated tumor cells from breast cancer patient-derived xenograft-bearing mice as a novel model to study metastasis

Introduction: Real-time monitoring of biological changes in tumors that metastasize may be possible by investigating the transitional cells such as circulating tumor cells (CTCs) and disseminated tumor cells in bone marrow (BM-DTCs). However, the small numbers of CTCs and the limited access to bone marrow aspirates in patients with localized disease pose major hurdles. The goal of this study was to determine if breast cancer (BC) patient-derived xenograft (PDX) mice could provide a constant and renewable source of CTCs and BM-DTCs, thereby representing a unique system for the study of metastatic processes.

Methods: CTCs and BM-DTCs, isolated from BC PDX-bearing mice, were identified by immunostaining for human pan-cytokeratin and nuclear counter staining of RBC-lysed blood and bone marrow fractions, respectively. The lung metastasis (LM) rate was previously reported in these lines. Associations between the presence of CTCs, BM-DTCs, and LM were assessed by the Fisher’s Exact and Cochran-Mantel-Haenszel tests. Two separate genetic signatures associated with the presence of CTC clusters and with lung metastatic potential were computed using the gene expression arrays of primary tumors from different PDX lines and were subsequently overlapped to identify common genes.

Results: A total of 18 BC PDX lines were evaluated. CTCs and BM-DTCs, present either as single cells or as clusters, were detected in 83% (15/18) and 62.5% (10/16) of the lines, respectively. There was a positive association between the presence of CTCs and BM-DTCs within the same mice. LM was previously found in 9 out of 18 (50%) lines, of which all 9 had detectable CTCs. The presence of LM was strongly associated with the detection of CTC clusters but not with individual cells or detection of BM-DTCs. Overlapping of the 2 genetic signatures of the primary PDX tumors associated with the presence of CTC clusters and with lung metastatic potential identified 4 genes (HLA-DP1A, GJA1, PEG3, and XIST). This 4-gene profile predicted distant metastases-free survival in publicly available datasets of early BC patients.

Conclusion: This study suggests that CTCs and BM-DTCs detected in BC PDX-bearing mice may represent a valuable and unique preclinical model for investigating the role of these rare cells in tumor metastases.
Title: Different prognostic value of circulating and disseminated tumor cells in primary breast cancer. Influence of bisphosphonate intake?

Sabine Kasimir-Bauer¹, Bahriye Aktas¹, Siegfried Hauch², Rainer Kimmig¹ and Oliver Hoffmann¹.¹University Hospital Essen, Essen, Germany and ²AdnaGen AG, Langenhagen, Germany.

Body: Background: Disseminated tumor cells (DTCs) in the bone marrow and circulating tumor cells (CTCs) in blood of breast cancer patients (pts) were shown to provide independent prognostic information and can be regarded as an early indicator of therapy failure. Here we demonstrate a different prognostic value of DTCs and CTCs and explain these findings by early bisphosphonate intake.

Patients and Methods: 10 ml blood and two bone marrow aspirates of 488 pts with first diagnosis of breast cancer between Aug 2006 and Dec 2010 were studied for DTCs and CTCs. CTCs were detected using the AdnaTest BreastCancer (AdnaGen AG, Hannover, Germany) for the detection of EpCAM, MUC-1 and HER2 transcripts. DTCs were analyzed by immunocytochemistry using the pan-cytokeratin antibody A45-B/B3. In addition to chemo-, radio- and anti-hormonal therapy, bisphosphonates were given to DTC-pos pts [clodronate (2×520 mg per day) for at least two years] and 69 DTC-neg, postmenopausal pts [zoledronic acid (4 mg, twice a year) for three years].

Results: After a median follow-up time of 48 months (range: 5 to 87 months), the overall survival rate was 93% (456/488 pts) and relapses occurred in 11% (56/488 pts) of cases. CTCs were detected in 109/488 pts (22%) and significantly correlated with reduced disease free survival (DFS; p=0.02). Negative prognostic relevance was predominantly related to the lobular subtype (p=0.0047), the ER-pos subtype, (p_{ER+;Her2-} =0.01; p_{ER+;Her2+} =0.25; p_{ER-/Her2+} =0.5) and pts who had received chemotherapy (p=0.015) or radiation therapy (p=0.0083) but not to pts with anti-hormonal regimens (p=0.06). DTCs were detected in 162/488 pts (33%). In contrast to CTCs, no prognostic significance could be shown with regard to DFS (p=0.49), all clinical parameters as well as treatment regimens. In contrast, only in CTC-pos pts, the presence of DTCs significantly correlated with DFS (p=0.04). We further investigated whether this lack in prognostic significance was due to bisphosphonate intake in case of DTC-positivity. Predominantly, in the CTC-neg group, bisphosphonate treatment significantly influenced DFS in both histological subtypes (p_{CTC-/ductal} =0.04; p_{CTC-/lobular} =0.0021). However, in the CTC-pos group of pts, no such effect was observed (p_{CTC+/ductal} =0.76; p_{CTC+/lobular} =0.51).

Conclusion: Here we show, that CTCs, rather than DTCs were significantly prognostic for early relapse. The ER-pos subtype was mostly affected which is in concordance with our previous studies, demonstrating that CTCs show EMT and tumor stem cell characteristics and, thus, down regulate ER which probably makes anti-hormonal treatment less effective. In addition, the presence of these CTC-subtypes might also explain the negative prognostic impact of CTCs in pts receiving chemo- or radiation therapy which we could verify in a subgroup of our pts (n=238) showing a significant correlation of CTCs and EMT/stem cell like CTCs (p=0.017; data not shown). The lack of prognostic significance of DTCs can be related to bisphosphonate intake in all DTC-pos pts, a subgroup of DTC-neg pts as well as in DTC-pos/CTC-neg pts, underlining our assumption that CTCs might be a high risk indicator for already ongoing metastasis not limited to bone metastasis.
Title: Persistence of circulating tumor cells immediately after and two years after systemic adjuvant chemotherapy in patients with early breast cancer – Results of the German SUCCESS trials

Bernadette AS Jaeger¹, Ulrich Andergassen², Julia K Neugebauer², Marianna Alunni-Fabbroni², Carola A Melcher³, Carsten Hagenbeck³, Susanne Albrecht¹, Ralf Lorenz³, Thomas Decker⁵, Georg Heinrich⁶, Tanja Fehm⁵, Andreas Schneeweiss⁷, Matthias W Beckmann⁸, Klaus Pantel⁸, Klaus Frieser², Peter A Fasching⁸, Thomas WP Friedl¹, Wolfgang Janni¹ and Brigitte K Rack⁹.

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Body: Background

There is growing evidence that circulating tumor cells (CTCs) have prognostic impact in patients (pts) with early breast cancer (EBC). In this study the persistence of CTCs immediately after and two years after chemotherapy (Ctx) was prospectively evaluated according to molecular subtypes within the German multicentre SUCCESS trials.

Methods

SUCCESS A and C were randomized Phase III studies including pts with node positive or high-risk node negative EBC. In each trial two different adjuvant Ctx regimen were compared: FEC-DOC (3 cycles of FEC followed by 3 cycles of Docetaxel) to FEC-DG (3 cycles of FEC followed by 3 cycles of Docetaxel/Gemcitabine) in SUCCESS A and in the SUCCESS C study FEC-DOC to an anthracycline-free Ctx regimen (6 cycles of Docetaxel/Cyclophosphamide). Both studies involved a second randomization after Ctx: 2 vs. 5 years of zoledronic acid treatment (SUCCESS A) or 2-years of an individualized lifestyle-intervention program vs. general lifestyle recommendations (SUCCESS C). Adequate endocrine treatment and treatment with trastuzumab as indicated were included in both trials.

As part of the translational research program, 23ml of peripheral blood were drawn to isolate CTCs using the CellSearch System (Veridex, USA). After immunomagnetic enrichment with an anti-EpCam-antibody, cells were labelled with anti-CK8/18/19 and anti-CD45 antibodies to distinguish epithelial cells from leucocytes. The cut-off for CTC-positivity was ≥ 1 CTC.

Molecular subtypes were defined as luminal-A-like (hormone-receptor positive, G1 or 2), luminal-B-like (hormone-receptor positive, G3), HER2-positive and triple-negative.

Results

CTC analyses were performed for 3344 blood samples collected immediately after Ctx and for 1352 blood samples two years after Ctx. After Ctx 17.5% (584/3344) of the pts were CTC-positive (range 1 – 124 CTCs), and two years after Ctx the positivity rate for CTCs was 17.2% (233/1352, range 1-99).

CTC positivity as assessed immediately after Ctx differed significantly among molecular subtypes (chi-square test, p < 0.001): Pts with HER2-positive tumors were more likely to have CTCs in the blood (26.3%, 105/400) as compared to pts with luminal-A-like tumors (15.4%, 283/1842), luminal-B-like tumors (17.7%, 142/802), or triple-negative tumors (18.0%, 54/300).

Two years after Ctx CTC-positivity did not differ significantly among molecular subtypes (chi-square test, p = 0.463). CTC-positivity rates were 15.7% (96/613) for luminal-A-like tumors, 19.1% (49/256) for luminal-B-like tumors, 17.2% (51/296) for HER2-positive tumors, and 19.8% (37/187) for triple-negative tumors.

Conclusions

The data of this study confirm previous findings that CTCs may persist after standard adjuvant therapy. Immediately after Ctx CTCs seem to be more frequent in pts with HER2-positive tumors as compared to other molecular subtypes, while two years after Ctx no differences in CTC positivity among molecular subtypes were detected. These results might indicate good efficacy of HER2-targeted therapies on CTCs.
Body: Introduction: Circulating tumour cells (CTCs) are a real-time reflection of the ad hoc relevant subpopulation in patients with progressive disease. The study comprises the clinical application of a semi-automated methodology to determine the PIK3CA mutational status at a single cell level.

Methods: Using CellSearch and DEPArray, we purified single (n=172) and groups (n=93, ranging 5-120 cells) of CTCs from peripheral blood in 18 patients with metastatic ER+/PR+/HER2- breast cancer, of whom archival primary tumour (PT) material was available. Isolation of WBCs served as internal negative control. Recovered cells were subjected to whole-genome-amplification (WGA). During CTC blood draw, an extra SST tube was filled for isolation of circulating cell-free DNA (cfDNA) from serum as comparator. All samples from different compartments underwent gene mutation analysis via targeted amplification of exons 9 and 20 of the PIK3CA gene and downstream 454 massive parallel sequencing (MPS) on the GS Junior system.

Results: WGA of single and group CTC samples had a success rate of 83±22 % and 96±8%, respectively. MPS resulted in an average throughput of 135.996±24.423 high quality reads with a normal-distributed mean fold coverage depth of 1257±703 and 1494±897 reads for exon 9 and 20, respectively. PIK3CA mutations were present at a relatively high frequency in archival PT (20/29 (68,9%)) and showed poor and moderate agreement with cfDNA (n=24; 33,3% disparity; kappa=0,03) and CTCs (n=14; 11,28% disparity; kappa=0,329), respectively. Comparison of CTCs and temporally matched cfDNA samples revealed substantial agreement (n=14; 14,3% disparity; kappa=0,536). A concordant PIK3CA status across all compartments (PT, cfDNA and CTCs) was observed in 11/15 (73,3%) samples. At the used sequencing depth, cfDNA failed to the detect PIK3CA mutations in 2 cases (13,3%), which were present in the respective PT and corresponding CTCs. CTCs failed to detect the mutant PIK3CA gene in one case (6,7%), which was present in PT and cfDNA. Gain of mutation was observed in 2/15 patients (13,3%), with a wild-type PT and mutant cfDNA and CTCs at progression. A wild-type PIK3CA sequence in recovered WBCs of all patients (n=15) indicates a high specificity and tumorigenic nature of the picked up variants. In depth intra-patient analysis of mutant CTCs on a single cell level reveals PIK3CA mutational heterogeneity with the presence of both mutant and wild-type CTCs (average %MT/%WT ratio of 50/50). Additionally, unique double-mutated CTCs were detected in 5/15 (30%) cases as well.

Conclusions: We report the frequent occurrence of PIK3CA hotspot mutations in metastatic HR+ breast cancer. Intra-patient mutational heterogeneity was observed in the single CTC samples of the same patient. Thereby, this study provides evidence of both concordance and discordance of the PIK3CA genotype in the intravascular compartment (down to the single cell level) with comparison to the temporally unmatched PT. The study presents the utilization of a liquid biopsy, thereby paving the way towards the application of a more personalized medicine in the management of patients with metastatic cancer.
Title: Predictive impact of circulating tumor cells with an epithelial-to-mesenchymal transition phenotype in patients with primary breast cancer treated with primary systemic therapy

Fanny Le Du\textsuperscript{1,2}, Dzifa Y Duose\textsuperscript{3}, Elisha J Dettman\textsuperscript{3}, Jackson A Summer\textsuperscript{1}, Mariana Chavez-MacGregor\textsuperscript{1}, Carlos H Barcenas\textsuperscript{1}, Abenaa M Brewster\textsuperscript{1}, Alvarez H Ricardo\textsuperscript{1}, Vincente Valero\textsuperscript{1}, Ana M Gonzalez-Angulo\textsuperscript{1}, James M Reuben\textsuperscript{1} and Naoto T Ueno\textsuperscript{1}.

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Background: Tumor cells with a mesenchymal phenotype include cancer stem-like cells (CSCs), which are known to contribute to metastasis. Circulating tumor cells (CTCs) are epithelial cells in peripheral blood that are detected using an anti-EpCAM antibody; however, CTCs undergoing epithelial-mesenchymal transition (EMT) may not be detected using this method. We have developed an antibody-independent CTC enrichment platform, Apostream®, which does not rely on EpCAM-based capture. We used this instrument to determine the clinical relevancy and feasibility of measuring EMT-CTCs in breast cancer patients.

Methods: Blood samples from newly diagnosed breast cancer patients were prospectively collected at baseline (T0), after primary systemic therapy (T1), and after definitive surgery (T2) and processed using the Apostream® system. Isolated cells were stained with antibodies to cytokeratin (anti-CK), leukocytes (anti-CD45), and the nuclear stain, 4,6-diamidino-2-phenylindole (DAPI), to identify CTCs. These CTCs were also stained with additional markers and examined on a laser scanning cytometer to measure protein expression levels of epithelial (EpCAM, E-cadherin), mesenchymal (β-catenin, vimentin) and CSC-markers (CD44, CD24). Pathological complete response (pCR) and residual cancer burden (RCB) statuses after preoperative treatment were obtained to correlate baseline CTCs and marker expression with treatment response.

Results: The study enrolled 33 patients (10 with early-stage, 19 with locally advanced, and 4 with metastatic breast cancer); 32, 12, and 10 patients provided samples at T0, T1, and T2, respectively. Of the 20 patients who underwent surgery, 6 patients achieved pCR. CTCs were detected (≥ 1 cell in at least one of the three samples) in 47%, 75%, and 80% of the T0, T1, and T2 samples, respectively. EMT markers (either vimentin or β-catenin) were detected in 67%, 11%, and 38% of these CTCs, respectively. The mean number of CTCs per mL detected at T0 was 0.43 (range, 0-3.7). CTC detection was correlated with a high histological Nottingham index (P=0.039). Conversely, vimentin-positive CTC detection was inversely correlated with clinical stage at T0 (P=0.034). No significant correlation was observed between CTCs detected at baseline and breast cancer subtypes. However, there was a trend for CTCs detected at T0 to be predictive of chemotherapy response, as 62% of patients with CTCs at T0 achieved a pCR, whereas only 11% of patients without CTCs at T0 had a pCR (P=0.057). The other EMT and CSC markers we tested did not predict response.

Conclusions: Apostream® was successful in detecting EMT-CTCs in this prospective study. There was a trend for the presence of CTCs at baseline to predict pCR. We will present our final data analysis of 50 patients.

<table>
<thead>
<tr>
<th>CK+CD45- CTCs</th>
<th>pCR (n=6)\textsuperscript{†}</th>
<th>RCB-I (n=2)</th>
<th>RCB-II (n=5)</th>
<th>RCB-III (n=4)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative (n=9)</td>
<td>1 (11)</td>
<td>2 (22)</td>
<td>3 (33)</td>
<td>3 (33)</td>
<td>0.146 0.057*</td>
</tr>
<tr>
<td>Positive (n=8)</td>
<td>5 (62)</td>
<td>0 (0)</td>
<td>2 (25)</td>
<td>1 (13)</td>
<td></td>
</tr>
</tbody>
</table>

\textsuperscript{†} All data are no. of patients (%). Two did not have an RCB status available, and 1 did not have a T0 sample. * Comparing pCR status (pCR vs no-pCR) between CTCs negative and positive.
Title: Circulating tumor cells (CTCs) detect HER2+ status and phenotypic heterogeneity in metastatic breast cancer (MBC)

Laura Austin¹, Zhaomei Mu¹, Tiffany Avery¹, Rebecca Jaslow¹, Carmela Paolillo¹, Angela Toss², Paolo Fortuna¹, Ye Zhong¹, Hushan Yang¹ and Massimo Cristofanilli¹. ¹Thomas Jefferson University Hospital, Philadelphia, PA and ²Universtiy of Modena and Reggio Emilia, Modena, Italy.

Body: Background
Circulating tumor cells (CTCs) are epithelial cells that can be found circulating in the blood of MBC patients and may represent a heterogeneous population including epithelial cells and cancer stem cells (CSC) shed from the tumor. Their detection and enumeration has prognostic significance and can be used for longitudinal monitoring of response to treatment. Recent advances have allowed for the detection of HER2 protein expressing CTCs using the CellSearch® platform. HER2 expression has been associated with CSCs phenotype in absence of gene amplification (Korkaya et al, Oncogene 2008) and particularly in Luminal B disease (Ithimakin et al, Cancer Res, 2014). We hypothesized that HER2+/CTCs are detectable in patients with MBC irrespective of their HER2 status and this information can be potentially be used for treatment selection. Targeting HER2+ CSCs may result in clinical benefit and improved outcome.

Methods
This is a prospective analysis of 40 patients with locally advanced or MBC whose blood was analyzed for the baseline detection of CTCs as part of their disease initial evaluation. Blood was drawn for CTC detection on eligible patients at initiation of a new line of therapy; CTCs monitoring was repeated at progression or change in therapy. The 7.5mL of whole blood was collected in a CellSave™ Preservative Tube, and CTC isolation, enumeration and characterization were performed using the FDA-approved CellSearch® System (Janssen Diagnostics, USA). The CellSearch® tumor phenotyping reagent HER-2/neu (Fluorescein-conjugated) was used to determine CTC with HER-2/neu expression using the CellTrack™ Analyzer II.

Results
Most patients in this study had metastatic disease (90%). According to disease subtype, 43% of patients were ER+/HER2- (Luminal A), 17% ER+/HER2+ (Luminal B), 20% ER-/HER2+ (HER2) and 20% ER-/HER2- (TNBC). Moreover, 55% had a clinical diagnosis of Inflammatory Breast Cancer (IBC). Twenty-two patients had CTCs detected (55%), the average number of CTCs was 8.6 (0-135) with a median follow up of two months. Of the patients who had CTCs detected and had HER2+ disease (IHC/FISH), 83% (5/6) had concordance in HER2+ CTCs. Interestingly, in the subset of patients who had HER2 negative disease (ICH/FISH) and had detectable CTCs, there was discordance in HER2 status: 44% (7/16) had HER2+ CTCs and all but one had Luminal A disease. Two patients have been started on HER2 targeted therapy based on finding HER2+ CTCs. One of these patients had 22 CTCs, 8 of which were HER2+, was initiated on HER-2 combined regimen and at a repeat evaluation in 3 months demonstrated 0 CTCs and clinical response.

Conclusions
CTCs offer a new and innovative approach to detect HER2+ cells in MBC. Tissue analysis with IHC and FISH has been the gold standard but these methods are unable to account for disease phenotypic heterogeneity and identify patients who have CSCs (HER2+) and therefore would benefit from HER2 targeted therapy. This warrants further investigation in a prospective trial in Luminal disease to formally compare these methods and correlate the results with clinical outcomes.
**Title:** Circulating tumor cells (CTC) and endothelial cells (CEC) changes in HER2 negative metastatic breast cancer (MBC) patients treated with first line weekly paclitaxel and bevacizumab: Preliminary results of a prospective cohort from the French Breast Cancer InterGroup Unicancer: COMET study

Jean-Yves Pierga¹, Isabelle Vaucher¹, Maya Gutierrez², Olivier Tredan³, Séverine Guiu⁴, Gilles Romieu⁵, Anthony Goncalves⁶, Marc Debled⁷, Christelle Levy⁸, Jean-Marc Ferrero⁹, Christelle Jouaunaud¹⁰, Elisabeth Luporsi¹¹, Marie-Ange Mouret-Reynier¹², Florence Dalenc¹³, Bernard Asselain¹, Jerome Lemonnier¹⁴ and Francois-Clement Bidard¹.

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**Body:**

**Background:** increased levels of circulating tumor cells (CTC) are associated with worse progression-free survival (PFS) and overall survival (OS) in patients (pts) with MBC (Bidard FC et al, Lancet Oncol 2014). The failure of chemotherapy to reduce CTCs to levels below five CTCs per 7.5 mL whole blood at first follow-up after initiating a new systemic therapy for MBC predicts shorter time to progression and OS. It has been hypothesized that bevacizumab could modify CTC prognostic value due to extravasation or epithelio-mesenchymal transition induction. CEC variations to predict benefit of anti-angiogenic treatment is still controversial.

**Patients & methods:** the French cohort COMET is a prospective study including first line HER2 negative pts receiving weekly paclitaxel and bevacizumab according to EMEA approved combination. The aim of this cohort is to evaluate clinical, biological and radiological parameters associated with pts outcome (CTC, CEC, VEGFA levels, ctDNA, pharmacogenomic polymorphisms, metabolomic parameters, visceral fat assessed by initial CTscan, serum estradiol level, and quality of life). We present here the first planned analysis on 206 patients evaluated for CTC and CEC using the FDA cleared CellSearch method.

**Results:** inclusions started in 09/2012. At time of analysis, 219 patients were included, 211 were evaluable for CTC at baseline (failure rate 4%) and 207 for CEC at baseline (failure rate 5%). Due to short follow-up, 173 pts and 166 pts were evaluable for both CTC and CEC at baseline and first day of second cycle of CT (D1C2) respectively. At baseline, 100/211 (47%) pts had ≥ 5 CTC (median 4 (range 0-30,000) and 30% had no detectable CTC (0 CTC). After one cycle of chemotherapy (D28) 38 pts (22%) had still ≥ 5 CTC: 37 pts with initial high level and only one patient with low CTC at baseline had increased CTC above 5. Median number CEC was 21 (0-2231) at baseline and 22 (1-881) at D1C2. CEC increased in 16% and decreased in 15% of the cases.

**CTC & CEC changes after one cycle**

<table>
<thead>
<tr>
<th>CTC Baseline</th>
<th>CTC at D1C2</th>
<th>CEC Baseline</th>
<th>CEC at D1C2</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 5</td>
<td>≥ 5</td>
<td>37 (21%)</td>
<td>&gt;20</td>
</tr>
<tr>
<td>≥ 5</td>
<td>&lt;5</td>
<td>45 (26%)</td>
<td>&gt;20</td>
</tr>
<tr>
<td>&lt;5</td>
<td>≥ 5</td>
<td>1 (&lt;1%)</td>
<td>≤ 20</td>
</tr>
<tr>
<td>&lt;5</td>
<td>&lt;5</td>
<td>90 (52%)</td>
<td>≤ 20</td>
</tr>
<tr>
<td>Total</td>
<td>173</td>
<td>166</td>
<td></td>
</tr>
</tbody>
</table>

CTC number at baseline line and CTC D1C2 were correlated (p<0.01) (Spearman test). There was no correlation between CEC at baseline or at D1C2 with CTC or CTC changes. Final analysis will be completed when 206 couples for both CTC and CEC at baseline and D1C2 will be available. Accrual is still ongoing.

**Conclusion:** this 22% rate of failure to reduce CTC < 5 after one cycle of first line CT in a homogeneously bevacizumab-treated cohort of MBC patients did not differ from previous series. As second lines of chemotherapy do not improve the poor prognosis of
this group of patients according to the SWOG 500 study results (Smerage et al, JCO 2014), trials of novel therapeutic agents should be considered at the time of progression.
Title: CTC enumeration and characterization has predictive and prognostic implications in patients with metastatic breast cancer treated with exemestane plus the mTOR inhibitor everolimus

Sofia Agelaki¹,², Dimitris Mavroudis¹,², Maria Spiliotaki², Eleni Politaki², Maria A Papadaki², Stella Apostolaki², Christos Nikolaou¹ and Vassilis Georgoulis¹,². ¹University Hospital of Heraklion, Heraklion, Crete, Greece and ²Laboratory of Tumor Cell Biology, School of Medicine, University of Crete, Heraklion, Crete, Greece.

Body: Background: The utility of CTC enumeration in predicting patient (pt) outcome has been demonstrated in metastatic breast cancer (MBC) treated with chemotherapy or endocrine therapy. In this study we evaluated the clinical impact of CTC assessment in terms of both enumeration and characterization in breast cancer pts treated with exemestane plus everolimus.

Patients and methods: Thirty-nine pts with hormone receptor (HR)-positive, HER2-negative MBC, received exemestane plus everolimus. CTC enumeration in peripheral blood (7.5 ml) was performed before treatment (n=39), post cycles 1 (n=39) and 3 (n=29), on disease re-evaluation and on relapse, whichever occurred first, using the CellSearch System. CTC characteristics were determined at the same time points by immunofluorescence (IF) analysis of PBMC cytospins (10⁶ cells), triple stained with pancytokeratin (CK) antibody along with Ki67 and M30 as proliferation and apoptosis markers, respectively, using the Ariol System. Patients were assessed by CT scans and bone scan, every 3 months or as clinically indicated. Results: At the cut-off of ≥ 1 CTC, 25 of 39 (64%) pts had detectable CTCs at baseline, 12 (31%) of 39 post-1st and 10 (34.5%) of 29 post-3rd cycle. Ten (25.6%) pts remained CTC(+) and 12 (30.8%) CTC(-) both at baseline and post-1st cycle; 15 (38.5%) CTC(+) pts turned to CTC(-) and 2 (5%) CTC(-) turned to (+). CTC positivity after the first cycle was associated with shorter median progression-free survival (PFS) compared to CTC(-) status (3.9 vs 8 mo, p=0.031). Shorter PFS was also recorded for pts that remained CTC(+) at both time points compared to all other (p=0.02). At the cut-offs of ≥ 2 and ≥ 5 CTCs, 16 (41%) and 9 (23%) pts were CTC(+) at baseline, respectively; post-1st cycle, 7 (18%) and 4 (10%) pts were CTC(+) (at ≥ 2 and ≥ 5 CTCs, respectively). Post-3rd cycle the positivity rate was 17% for both cut-offs and these pts had significantly shorter PFS compared to CTC(-) pts (3.7 vs 8.7 months, p=0.048). Efficacy assessment revealed partial response in 3 (7.7%) pts, stable disease in 27 (69.23%) and progressive disease (PD) in 8 (20.5%); 1 pt was non-evaluable for response. Among pts determined CTC(+) post-1st cycle (cut-off ≥ 2 CTCs), 57% progressed compared to 13% of CTC(-) pts (p=0.02). In addition, at the post-3rd cycle evaluation, pts with PD had significantly higher CTC counts compared to non-progressors (mean ± SEM; 10 ± 5.78/pt vs 1.62±0.83/pt, p=0.027). By the use of IF 43%, 44% and 40% of CTC(+) pts had proliferative [Ki67(+)/M30(-)] CTCs at baseline, post -1st and -3rd cycles, respectively (cut-off ≥ 1 CTC); 67%, 50% and 50% of those pts, respectively, experienced PD. Apoptotic [Ki67(-)/M30(+)] CTCs were detected in 14%, 22% and 60% of CTC(+) pts at baseline, post -1st and -3rd cycles, respectively; none of the pts with apoptotic CTCs experienced PD. Conclusions: CTC enumeration and characterization in terms of proliferation and apoptosis during the course of treatment has significant predictive and prognostic implications in patients with MBC receiving the combination of exemestane plus everolimus.
Title: Whole exome sequencing of circulating and disseminated tumour cells in patients with metastatic breast cancer

Dieter JE Peeters\textsuperscript{1,2}, Ken Op De Beeck\textsuperscript{1,3}, Anja Brouwer\textsuperscript{1,2}, Geert Vandeweyer\textsuperscript{3}, Patrick Pauwels\textsuperscript{1,4}, Marc Peeters\textsuperscript{1,2}, Peter B Vermeulen\textsuperscript{1}, Peter A van Dam\textsuperscript{1,2}, Steven J Van Laere\textsuperscript{1,5}, Guy Van Camp\textsuperscript{3} and Luc Y Dirix\textsuperscript{1}. \textsuperscript{1}Center for Oncological Research (CORE), University of Antwerp, Antwerp, Belgium; \textsuperscript{2}Antwerp University Hospital, Edegem, Belgium; \textsuperscript{3}Center for Medical Genetics, University of Antwerp, Antwerp, Belgium; \textsuperscript{4}Antwerp University Hospital, Edegem, Belgium and \textsuperscript{5}University of Leuven, Leuven, Belgium.

Body: Introduction:
Circulating tumour cells (CTC) found in the blood of patients with cancer offer the potential to provide a repeatedly accessible source of tumour cells for the real-time assessment of tumour characteristics in patients with metastatic breast cancer (MBC). Questions remain to what extent CTC are truly representative of the actually present tumour mass in a patient at a specific moment in time and the molecular heterogeneity within the CTC population is only now being explored. Here, we report on the first results of an ongoing comparative study of mutation profiles of CTC and synchronously isolated disseminated tumour cells (DTC) from metastatic effusions or biopsies of solid metastases of patients with clinically progressive MBC.

Materials and methods:
For this project CTC are isolated from 7.5 ml blood samples of patients with MBC using the CellSearch system. CellSearch enriched CTC samples are subsequently further purified and sorted into several batches of 1-125 CTC per patient using the DEPArray system. DNA is isolated and amplified using the Ampli1 whole genome amplification (WGA) kit and subjected to whole exome paired-end sequencing (WES). DTC from metastatic effusions, fresh frozen tissue from solid metastases or the primary tumour, or - in patients with extremely high CTC counts (>10,000/7.5 ml) - pooled CTC from the CellSearch Profile sample, are sequenced as a comparator for mutation profiles. DNA from the buffy coat of white blood cells are sequenced to enable somatic mutation analysis.

Results:
Eight samples of 1-125 CTC and a CellSearch Profile sample of one patient with MBC who had ca. 30,000 CTC/7.5 ml of blood (patient 1) and 4 CTC samples of 5-10 CTC, 2 temporally matched DTC samples of 10 and 20 DTC from a pleural effusion and a fresh frozen tissue sample of the primary tumour of a second patient (patient 2) have been sequenced so far. Average base coverages were 13.6x (patient 1) and 11.8x (patient 2) for CTC/DTC samples and 175x and 120x for the CellSearch profile sample (patient 1) and the primary tumour sample (patient 2) respectively. Between 29.64% and 53.57% of the exomes of amplification products of CTC/DTC DNA were uncovered, probably due to technical limitations of the WGA procedure. Overall, if adequately covered, good concordances were observed for variants identified with MuTect in 28 frequently mutated genes in breast cancer between samples of amplification products of 1-125 CTC and the CellSearch Profile sample of patient 1. In patient 2, the same H1047R PIK3CA mutation was identified in the primary tumour and all CTC and DTC samples. In-depth analyses of the full exome data are being conducted.

Discussion:
Our data provide insight into clinically relevant questions to what extent CTC reflect mutational profiles in temporally matched metastatic tumour cells, and – by analysing multiple CTC samples of the same patient – genetic heterogeneity between CTC in patients with MBC. Sample accrual and analysis is being expanded and updated results will be presented at the conference.
**Title:** Assay Development for detection of estrogen responsive gene histone acetylation in breast cancer circulating tumor cells

S A Litherland¹, Robert Reynolds¹, Louis Barr¹,², Alvin JO Almodovar¹ and David A Decker¹. ¹Florida Hospital Cancer Institute, Orlando, FL and ²Florida Hospital Center for Specialized Surgery, Orlando, FL.

**Body:**

**Research Objectives:** To develop a tumor-specific assay for assessment of histone acetylation and histone deacetylase (HDAC) effects in circulating tumor cells (CTC).

**Rationale:** Therapy-resistant tumor cells arise in breast cancer patients during hormone therapy. Anti-deacetylation drugs such as Entinostat, Vorinstat, and Romidepsin block histone deacetylases (HDAC), preventing removal of epigenetic acetyl chromatin modifications which allow for expression of estrogen receptor (ER). Clinical trials such as ECOG E2112 are investigating a histone acetylase inhibitor (HDACi) therapy in combination with standard endocrine therapy to overcome hormone resistance. A drawback for HDACi clinical trials in the past has been the lack of a sensitive assay to identify tumor specific HDAC effects contributing to hormone resistance.

**Methods:** We testing a histone acetylation assay using Fluorescence Activated Cell Sorting (FACS) peripheral blood CTC isolation and histone acetylation/HDAC specific chromatin immunoprecipitation (ChIP) with realtime PCR analysis to identify ERalpha and GREB-1 gene activation. We report the initial findings from 11 consented subjects (IRB approved protocol# 372522).

**Results:** Our findings indicate that GREB-1, a gene essential to breast cell ER expression in response to estrogen, can be regulated via a histone acetylation epigenetic control mechanism. Using our CTC-ChIP analysis, we found both ER+ and ER- CTC arising from ER+ tumors and that the proportion of ER- CTC mirrored clinical hormone resistance. GREB-1 activation and expression is detectable in both ER+/ER- CTC when enough CTC could be collected for ChIP analysis; however, ERalpha acetylation and mRNA expression was below the level of detection.

**Table of Preliminary Results**

<table>
<thead>
<tr>
<th>Patient Group/N</th>
<th>Tumor status</th>
<th>HT Status</th>
<th>CTC ER+/million (mean±SD)</th>
<th>CTC ER-/million(mean±SD)</th>
<th>CTC ER+GREB-1 Acetylation+(mean±SD)</th>
<th>CTC ER- GREB-1 Acetylation(mean ± SD)</th>
<th>PREDICTED ESTROGEN RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+ HST sensitive/4</td>
<td>Metastatic</td>
<td>Sensitive</td>
<td>130379±260109</td>
<td>336±594</td>
<td>0.95±0.08</td>
<td>0.015±0.02</td>
<td>Sensitive</td>
</tr>
<tr>
<td>ER+ HST resistant/2</td>
<td>Metastatic</td>
<td>Resistant</td>
<td>824±29.7</td>
<td>7334±6421</td>
<td>0.87±0.13</td>
<td>1*</td>
<td>Resistant</td>
</tr>
<tr>
<td>ER-metastatic/1</td>
<td>Metastatic</td>
<td>Resistant</td>
<td>18758</td>
<td>21498</td>
<td>1*</td>
<td>ND</td>
<td>Resistant</td>
</tr>
<tr>
<td>ER-primary/2</td>
<td>Primary</td>
<td>Unknown</td>
<td>277251±379512</td>
<td>112754±159451</td>
<td>34000000</td>
<td>89500000000±126600000000</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Healthy controls/2</td>
<td>None</td>
<td>None</td>
<td>0.5±0.7071</td>
<td>0</td>
<td>0*</td>
<td>0*</td>
<td>-</td>
</tr>
</tbody>
</table>

*Insufficient cells for accurate ChIP analysis

**Conclusions:** Our preliminary data suggest that by using a combination of CTC based epigenetic biomarker analyses; it is possible to detect the presence of hormone resistant ER+ and ER- CTC subpopulations arising from ER+ breast cancer tumors. Monitoring of longitudinal changes in ER+/ER- CTC and the epigenetic activation of GREB-1 and ERalpha responsiveness could provide support in deciding which patients may benefit from the use of anti-deacetylation drug therapy in combination with
hormonal therapy to promote ER alpha expression, reinstating estrogen dependency in both ER- and ER+ subpopulations; and thereby, re-sensitizing them to hormonal therapy.
Detection of EMT, anoikis and stem cell markers in metastatic breast cancer patients under different lines of treatment

Elisabeth K Trapp¹, Brigitte Rack¹, Leonie Majunke¹, Julian Koch¹, Simone Hofmann¹, Thomas WP Friedl², Julia Neugebauer¹, Julia Jückstock¹, Bernadette Jäger², Jens Huober², Wolfgang Janni² and Marianna Alunni-Fabbroni¹.
¹Ludwig-Maximilians-University, University Hospital, Munich, Germany and ²University Hospital, Ulm, Germany.

Body: Background: Metastasis are thought to be induced by occult spreading of tumor cells already during the early phases of the disease. Circulating Tumor Cells (CTCs) are regarded as precursors of distant metastasis, while detaching from the primary tumor and originating micrometastases in distant organs. Recent evidences pointed to CTC heterogeneity, showing that CTCs can present different phenotypes. Goal of this study was to identify in metastatic breast cancer (mBC) patients CTCs with EMT features and to further characterize them with respect to cellular heterogeneity.

Methods: This prospective ongoing study included mBC patients (n=12) with a median age of 58.5 years (range: 35-78 years), enrolled in a time frame of 7 months while undergoing therapy. The majority of patients were estrogen and/or progesterone receptor positive (11/12) and HER2 non-amplified (10/12). Patients had metastatic lesions in viscera as well as bone (6/12), only bone (2/12), only viscera (2/12), only viscera in combination with locoregional recurrence (1/12), or visceral and bone metastases in combination with locoregional recurrence (1/12). Current therapy was endocrine therapy (4/12), chemotherapy (3/12), chemotherapy and HER2-targeted therapy (2/12), HER2-targeted monotherapy (1/12), antiangiogenic therapy (1/12), or surgical therapy only (1/12). Blood samples, withdrawn at any time point during treatment, were depleted of EpCAM⁺ cells and CD45⁺ white blood cells (EpCAM/CD45 depleted fraction) (Mego et al., Int J Cancer 2012;130(4):808-816) and expression of epithelial markers (EpCAM, E-Cadherin, Cytokeratin 8,18,19), mesenchymal markers (n-Cadherin, Vimentin), EMT-inducing factors (Twist1, Snail1, SLUG, Zeb1 and FoxC2), anoikis markers (TrkB1, Bcl2) and stem cell markers (CD24, CD44, CD133) were analyzed by qRT-PCR. CTC counting with the CellSearch™ system (Veridex, Raritan NJ) was run in parallel. Healthy donors (n=10) were included in the study as negative controls.

Results: The data collected so far showed that 50% of the patients were positive for CTCs in the EpCAM⁺ fraction as detected with the CellSearch™ system, while 33% were still CTC positive in the EpCAM/CD45 depleted fraction. 50% of the patients, found CTC negative with CellSearch™, were nevertheless positive for the epithelial and EMT markers in the EpCAM/CD45 depleted fraction (EpCAM 25%, E-Cadherin 25%, CK8 16.6%, CK18 16.6%, CK19 16.6%, SLUG 9.3%, Zeb1 25%, Twist1 9.3%, Vimentin 58,3%). N-cadherin, FoxC2, Snail1, TrkB1, CD24, CD44 and CD133 were never detectable.

Conclusions: These preliminary results suggest that mBC patients undergoing different lines of therapy present heterogeneous CTCs. 50% of the patients with undetectable EpCAM⁺ CTCs, were found CTC positive in the EpCAM/CD45 depleted fraction. mBC patients might be insensitive to treatment due to a selection of resistant CTCs subpopulations with different phenotypes. Additional patients with the same clinical characteristics will be analyzed in the next 6 coming months and updated results will be included.
2014 San Antonio Breast Cancer Symposium

Publication Number: P4-01-17
Average Grade: 0

Title: Expression of circulating tumor cells (CTC) and CK-19 mRNA (CK19) as prognostic factors in heavily pretreated metastatic breast cancer

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Body: Introduction: The expression of CTC and CK19 holds prognostic value for patients with breast cancer but their clinical significance remains still controversial.

Methods: This clinical observational study included 58 preteated metastatic breast cancer patients who started a standard new treatment line. CTC and CK19 was measured with CellSearch® and RT-PCR respectively at inclusion time. Progression-free survival (PFS) was defined as the time elapsed between the initiation of the treatment and either the date of clinical or radiological progression or death or the last follow-up. Cox proportional hazards regression model was used to assess the univariate prognose value of CTC and CK19 on PFS, and Kaplan-Meier estimates. A multivariate Cox model was also performed to additionally account for ER and visceral disease. CTC and CK19 positivity was considered when a value of 1 or more was observed.

Results: Mean age was 59.68 (range 35-86), the average number of previous treatments was 2.98 (range 1-10), 38 patients (65.52%) were ER+ and 22 (37.93%) had visceral disease. Median PFS was 7 months (CI 95% 4-9). Univariate analyses showed a significant effect of the positivity of CK19 (HR=2.22, CI 95% 1.15-4.29, p=0.01) but did not reach statistical significance for CTC (HR=1.86, CI 95% 0.93-3.72, p=0.07). The estimate disease-free survival rate at 6 and 12 months were 63.6% and 43.4% for patients with CK19<1 and 47.1% and 10.6% for patients with CK19>=1, respectively. The estimate disease-free survival rate at 6 and 12 months were 77.8% and 28.8% for patients with CTC=0 and 43.6% and 21.5% for patients with CTC>=1, respectively. In the multivariate analysis the effect of CK19 and CTC were similar (HR=2.12 and 1.86, p=0.03 and 0.08 respectively), ER was statistically significant (HR=0.48, p=0.05, + vs -) but visceral diseases appeared not significant (HR=1.57, p=0.17, yes vs no).

Conclusions: The expression of CK19 appeared clinically meaningful in pretreated metastatic breast cancer patients, even when adjusting by ER and visceral diseases. CTC showed a similar but minor effect and not statistically significant. These results support CK19 as an interesting biomarker for predicting clinical response in metastatic breast cancer.
Title: The relationship between components of tumour inflammatory cell infiltration and hematogenous metastasis in patients with primary breast cancer

Naping Wu¹, Jue Wang¹, Tiansong Xia¹, Wenbin Zhou¹, Zhao Liu¹, Yi Zhao¹, Xiaoan Liu¹, Xiaoming Zha¹ and Shui Wang¹. ¹The First Affiliated Hospital with Nanjing Medical University, Nanjing, Jiangsu, China.

Body: Background: Tumors are infiltrated by inflammatory cells (e.g. macrophages, B and T lymphocytes), which may promote tumor growth and invasion. However, the clinical correlation between hematogenous metastasis and relative amount of inflammatory cells is still unknown.

Methods: The prevalence of circulating tumor cells (CTC) in peripheral blood of 133 breast cancer patients was detected by a multimarker comprised of cytokeratin 19 (CK19), small breast epithelial mucin (SBEM) and human mammaglobin (hMAM) real time quantitative RT-PCR (QPCR) platform. Meanwhile, CD68⁺macrophages, CD20⁺B lymphocytes and CD3⁺T lymphocytes at the peritumor and intratumor were also counted by immunohistochemical examination, in corresponding primary invasive breast carcinomas. The relationship between CTC in peripheral blood and inflammatory cell infiltration was analyzed.

Results: The positive rate of CTC was 39.8% (53 out of 133). The prevalence of CTC in peripheral blood was only observed in invasive breast carcinomas. There were more inflammatory cells at the peritumor than the tumor center (P < 0.001). In addition, higher total numbers of infiltrating both CD68⁺macrophages and CD3⁺T lymphocytes were associated with significantly the prevalence of CTC in peripheral blood (P < 0.05).

Conclusion: Our data support a hypothesis that CD68⁺macrophage and CD3⁺T lymphocyte infiltrating in the breast cancer microenvironment may promote tumor cells disseminating through circulation system.
Title: The combined detection of CTC and serum HER2 ECD predict PFS for HER2-positive advanced breast cancer patients

Zefei Jiang¹, Jinmei Zhou¹, Tao Wang¹, Yi Liu¹, Lei Li¹, Huiqiang Zhang¹, Shaohua Zhang¹, Li Bian¹ and Santai Song¹. ¹Affiliated Hospital of Academy of Military Medical Sciences, Beijing, China.

Body: Background: Circulating tumor cell (CTC) and serum HER2 ECD can all reflect an aggressive tumor behavior. We performed this prospective, monocenter, double-blinded study to investigate the potential clinical significance of combined detection of CTC and serum HER2 ECD for advanced breast cancer patients with histological HER2-positivity.

Methods: A total of 88 eligible patients were enrolled in the present study from April 2012 to October 2013. We used Cell search system and ADVIA Centaur System to detect CTC and serum HER2 ECD respectively. Patients received systemic treatment according to national and international guidelines.

Results: Twenty nine (33%) patients had ≥5 CTC, seventy three (83%) patients had serum HER2 ECD values of at least 15ng/ml, twenty seven (30.7%) patients had both elevated CTC and ECD values and fourteen (15.9%) patients had both normal CTC and ECD values. Patients with both normal CTC and serum HER2 ECD values exhibited a significantly longer median PFS than patients with both elevated values (9.0 months versus 2.8 months, p=0.023) and exhibited a trend toward longer PFS compared with patients with elevated CTC or ECD values (9.0 months versus 4.2 months, p=0.065), patients with both or one elevated values showed similar median PFS (2.8 months versus 4.2 months, p=0.211) (Figure1).

Conclusions: The combined detection of CTC and serum HER2 ECD showed prognostic significance for HER-2 positive advanced breast cancer patients, patients with both normal values exhibited longer median PFS than others.
Title: A novel microfluidic system for the detection, enumeration and molecular analysis of circulating tumor cells (CTCs) in metastatic breast cancer (MBC)

Carmela Paolillo¹, Zhaomei Mu², Angela Toss³, Priyadarshini Gogoi⁴, Saedeh Sepehri⁴, Yi Zhou⁴, Kalyan Handique⁴, Ye Zhong⁵, Hushan Yang², Ettore Capoluongo, Massimo Cristofanilli² and Paolo Fortina². ¹Catholic University of the Sacred Heart Rome, Rome, Italy; ²Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, PA; ³University of Modena and Reggio Emilia, Modena, Italy and ⁴DeNovo Sciences, Inc, Plymouth, MI.

Body: Background: In recent years blood testing for circulating tumor cells (CTC) has gained increasing interest in cancer research. CTC detection and enumeration can serve as a ‘liquid biopsy’ and an early marker of response to systemic therapy. Different analytical systems for CTC detection and isolation have been developed, but the CellSearch® is currently the only FDA-approved technology. We aimed to evaluate CTCs detection by a novel microfluidic technology, a size- and deformability-based capture system. This unique platform not only allows flexibility in the selection of antibody markers but also segregates the CTCs in their own chambers, thus, enabling morphological, immunological and genetic characterization of each CTC at the single cell level.

Methods: We performed a prospective study to compare the detection of CTCs using the CellSearch® (Janssen Diagnostics) vs. the new microfluidic platform. Enumeration by the CellSearch® was performed according to standard protocol. For the microfluidic-device capture (De Novo Sciences, MI) peripheral blood from MBC patients was diluted 1:1 with PFA 0.8% and PBS 1%. Prior to sample loading, the microfluidic device was coated with priming buffer. After cells were fixed using 4.0% PFA and were subsequently stained with pancytokeratin, Zym 5.2 and CD45. Nuclei were counterstained with Hoechst-33342. CTCs were identified as round and bright green cells not stained with red (CK+/CD45-/DAPI+). Data were analyzed using non-parametric methods: Cohen’s kappa, Chi² test, Spearman rank correlations and Mann-Whitney test. Associations with CTCs were calculated in two ways: CTCs as a continuous variable normalized for ml of blood and CTCs categorized as < 5 versus ≥ 5.

Results: The two methods was concordant in 88.2% of patients with a Cohen’s kappa of 0.743 when the detection of a single CTC is considered like positive. We also found a concordance of 85% (k=0.70) when we use the CTC cut-off level of ≥ 5 cells per 7.5 ml of blood to identify patient with higher risk for disease progression. Thirty-one patients with MBC were tested for CTCs using both methods. CTC detection by microfluidic platform was positively associated with Her2 positive patients if we consider as categorical variable; a weak association was seen also with the CellSearch®.

<table>
<thead>
<tr>
<th>CTCs Association</th>
<th>CTC/ml of blood Continuous</th>
<th>p-value</th>
<th>N</th>
<th>n (%)</th>
<th>OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DeNovo</td>
<td>median [min, max]</td>
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<td></td>
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<tr>
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<tr>
<td>No</td>
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<td></td>
<td>19</td>
<td>7 (36.8)</td>
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</tr>
<tr>
<td>Yes</td>
<td>1.75 [0, 4]</td>
<td></td>
<td>12</td>
<td>9 (75.0)</td>
<td>5.14 (1.03, 25.6)</td>
<td></td>
</tr>
<tr>
<td>CellSearch®</td>
<td>median [min, max]</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Her 2</td>
<td></td>
<td>0.704</td>
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<td>No</td>
<td>0.13 [0, 335.7]</td>
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<td>19</td>
<td>6 (41.2)</td>
<td>ref</td>
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<tr>
<td>Yes</td>
<td>0.95 [0, 3.33]</td>
<td></td>
<td>12</td>
<td>8 (34.7)</td>
<td>4.33 (0.93, 20.2)</td>
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</tr>
</tbody>
</table>

Conclusions: The enumeration of CTCs showed strong prognostic significance in MBC raising interest in a more accurate molecular characterization to achieve the possibility for a dynamic molecular monitoring. Introducing novel methodologies should demonstrate comparable detection rate for epithelial cells. This new microfluidic system showed accuracy and flexibility to move
to the next phase of molecular testing with the intent of assessing the value as liquid biopsy.
Title: Analysis and single-cell retrieval of circulating tumor cells to monitor treatment response and assess genotype in triple-negative breast cancer

Arturo Ramirez¹, Daniel Campton¹, Elisabeth Mahen², Sibel Blau³, Anthony Blau², Eric Kaldjian¹ and Jackie Stilwell¹. ¹RareCyte, Inc, Seattle, WA; ²Center for Cancer Innovation, University of Washington, Seattle, WA and ³Northwest Medical Specialties, Puyallup, WA.

Body: Introduction: We used a high-recovery rare cell analysis and single-cell picking system to enrich, visualize, and isolate circulating tumor cells (CTCs) for genomic analysis from the blood of patients with advanced triple-negative breast cancer (TNBC) undergoing treatment with cisplatin as part of a study to intensively characterize TNBC. CTCs were evaluated regularly during treatment to monitor CTC burden and characteristics that could be associated with treatment response or disease progression, and perform single-cell mutational analysis to inform clinical decision making. Methods: Patients were enrolled in the study at the University of Washington Center for Cancer Innovation after informed consent for participation in investigation of their disease, including molecular analysis of multiple biopsies of accessible tumor. CTCs were evaluated prior to treatment and tracked longitudinally. Density-based enrichment of blood cells was performed using the AccuCyte tube, float and collector system. Collected cells were processed and applied to microscopic slides. Fluorescently labeled antibodies to cytokeratin, CD45 and EpCAM, and a nuclear dye were applied to samples using an automated slide stainer. Slides were scanned on a digital microscope and candidate CTCs identified using image analysis software. CTCs were verified by appropriate morphology and expression of epithelial and nuclear stains without CD45 expression. Other antibodies used to characterize cells included Her2, EGFR, and Ki-67. A mutation hypothesized to lead to the activation of ROS1 was identified in the cancer cells isolated from the bone marrow of one patient. CTCs were picked using our integrated semi-automated system and evaluated for the ROS1 variant using whole genome amplification followed by nested PCR and Sanger sequencing. Results: Seven patients have been enrolled to date. At least 1 CTC/mL has been found in all patients. Pre-treatment CTC levels in the patient with the ROS1 mutation were extremely high (1500/mL). One week after treatment, CTC levels spiked to more than 5000/mL. CTC counts then dropped exponentially to 9/mL after 4 months. CTC clusters and Ki-67 positive cells also decreased during therapy. Treatment with cisplatin was discontinued in this patient due to toxicity and progression, and CTC levels increased to nearly 9000/mL over 4 months. The ROS1 mutation was found in approximately 50% of individually picked CTCs before treatment with crizotinib, a ROS1 inhibitor. A second patient was found to have somatic loss of BRCA1, and was therefore treated with the PARP inhibitor, veliparib. CTC levels increased during veliparib treatment up to 13/mL. The same patient was subsequently treated with ponatinib, an FGFR inhibitor, based on the identification of two linked somatic missense mutations involving FGFR2 (S252W and Y375C). After beginning ponatinib, CTCs fell to undetectable levels. Conclusions: Analysis of CTCs may provide a non-invasive measure of cancer progression/response and the molecular evolution of tumor cells in patients with TNBC. Single-cell CTC retrieval after slide-based immunofluorescent visualization is compatible with whole genome amplification and sequencing methods.
Title: High-depth sequencing of circulating tumor DNA to interrogate the genetics of residual micro-metastatic disease prior to relapse in early breast cancer

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Body: Background: The identification of early stage breast cancers that are at high risk of relapse after apparently curative treatment of the primary tumor would allow tailored adjuvant therapy approaches to prevent relapse. We sought to define whether high-depth targeted sequencing of circulating cell-free plasma DNA could be used to interrogate the genetics of residual micro-metastatic disease (RMD) persisting after neoadjuvant treatment in patients with early stage breast cancer.

Methods: In a cohort of 31 patients with early breast cancer receiving neoadjuvant chemotherapy, with no evidence of metastasis at presentation, we collected tumor tissue at baseline with the metastatic relapse if it occurred, and serial plasma samples at baseline, post-surgery, and every 6 months in follow-up. Serial plasma samples were subject to digital PCR mutation tracking to examine for circulating tumor DNA (ctDNA). Four patients had ctDNA detectable prior to clinical relapse. Primary tumor-derived DNA (from 4 cases), serial plasma DNA samples (from 4 cases), and metastases derived DNA (from 2 cases) were subjected to massively parallel sequencing (Illumina HiSeq2000) targeting all exons of 273 genes frequently mutated in breast cancers and/or a custom AmpliSeq cancer panel (IonTorrent).

Results: Targeted sequencing revealed the presence of 1 to 20 somatic mutations in the early breast cancers at baseline, including 3 PIK3CA H1047L/R and 2 TP53 pathogenic mutations, all of which were detected in the subsequent plasma DNA samples taken prior to relapse. However, in one case an ESR1 E380Q mutation found in the baseline primary breast cancer was undetectable by targeted sequencing in all 3 subsequent plasma samples and in the metastasis; this mutation could not be identified in the metastasis by digital PCR. In two cases plasma DNA sequencing revealed no additional mutations to those identified in the primary tumor, whereas in the other two cases, plasma DNA targeted capture sequencing revealed divergence in the genetics of RMD. In these cases, 1 and 5 somatic mutations were found in the plasma DNA but were not detected in the respective baseline tumors. In particular, an activating FGFR1 K656E mutation, which was not detected by targeted capture sequencing in the primary breast cancer at 355x sequencing depth, was found at a mutant allele fraction (MAF) of 2.6% in the plasma 12 months post-surgery, and was subsequently detected in the distant metastasis at a MAF of 43.4% by targeted sequencing and digital PCR.

Conclusions: Our results provide evidence of clonal shifts in response to neoadjuvant systemic therapy of early breast cancers. In addition, we demonstrate that high-depth targeted capture massively parallel sequencing analysis of plasma ctDNA may help predict the genotype of recurrence prior to the onset of clinically overt metastasis.
**Title:** Targeted molecular characterization of serum derived cell free DNA from metastatic breast cancer patients treated with first-line tamoxifen


**Body: Background:** Evaluation of cell-free DNA (cfDNA) is a very attractive tool to serve as "liquid biopsy" to define and establish mutational changes in circulating tumor DNA (ctDNA) non-invasively during therapy.

**Aim:** To identify tumor specific mutations in serum cfDNA associated with resistance against tamoxifen in metastatic breast cancer.

**Materials & Methods:** Ten metastatic ER-positive breast cancer patients treated with first-line tamoxifen of whom blood sera was available at start therapy, during treatment and at disease progression were selected. DNA was isolated from normal (nDNA) and primary tumor (ptDNA) tissue and from sera (cfDNA). DNA was analyzed with next generation sequencing by the ion-PGM system (Life Technologies) for a panel of 45 cancer genes. This panel included the most frequently mutated genes for breast, colon, prostate and ovarian cancer reported in the Catalogue Of Somatic Mutations In Cancer (Cosmic database, Release 67; cancer.sanger.ac.uk/). In total 1242 exons (∼255kb) were sequenced with 200 to 5000x reads depth coverage. The panel analyzed all exons for 39 genes and only hotspot exons for 6 oncogenes. Variant Caller software (Life Technologies; version 4.16) was applied to detect non-synonymous and stop-gain single nucleotide variants (SNVs) within the sequenced DNA. Hotspot mutations detected in PIK3CA exons 9 and 20 were validated with snapshot multiplex assays.

**Results:** Variant Caller analyses revealed in total 252 SNVs within the 40 DNAs from tumor tissue and/or serum analyzed which were not detected in normal tissue. Of these, 229 SNVs were novel and not yet reported in the Cosmic database. Mutations were detected for 10 genes in both ptDNA and cfDNA, which enabled us to characterize ctDNA in serum of nine out of ten patients. The discovered mutations were already reported in Cosmic for PIK3CA, TP53, and NF1, but not for CDH1, APC, SMAD4, MLL, AKAP, CREBBP and MLL2. The PIK3CA, TP53 and APC mutations were observed in tumor and sera for 2, 3 and 2 patients, respectively. Mutations in the other genes, except MLL, were unique for individual patients. The mutation for MLL was seen in almost all patients at low frequencies (1-5%). The hotspot mutations in PIK3CA were confirmed with snapshot assays in the sequenced samples and in additional sera. In four patients, Cosmic reported mutations in BRCA1, KAT6B, MAP3K1, MLL2, PTCH1 and PTEN occurred in ctDNA at disease progression while they were not found in the primary tumor nor in preceding sera. The mutation frequencies ranged from 3% for BRCA1 and MAP3K1 to 6% for MLL2. Moreover, mutations for MED12 (3%) and MLL3 (4%), not yet reported in Cosmic, were each observed in two sera at disease progression for two additional patients. The remaining SNVs are currently verified for their authenticity and occurrence in ptDNA and/or ctDNA.

**Conclusion:** Molecular characterization of tumor and serum derived DNA with targeted next generation sequencing enabled us to identify ctDNA in serum and to detect mutations at disease progression that might play a role in resistance to first-line tamoxifen.
Title: Detection of single nucleotide variations and copy number variations in breast cancer tissue and ctDNA samples using single-nucleotide polymorphism-targeted massively multiplexed PCR

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Body: Genomic instability, the hallmark of cancer, presents with a variety of mutation types, most commonly single nucleotide variations (SNVs) and copy number variations (CNVs), which traditionally have required different methods for identification. It has proven challenging to simultaneously achieve sufficient breadth to detect CNVs and depth to detect SNVs on samples of limited input amount. The objective of this study was to validate a new methodology for detection of SNVs and CNVs in a single assay. We used a massively multiplex PCR/NGS approach combining an SNV panel covering 585 point mutation hotspots in breast cancer (Cosmic) and a CNV panel targeting 28,000 SNPs designed to detect copy number at chromosomes 1, 2, 13, 18, 21, and X, and focal regions 4p16, 5p15, 7q11, 15q, 17p, 22q11, and 22q13. We applied these panels to breast cancer cell lines and fresh frozen (FF) breast tumor samples; the presence of CNVs in circulating cell-free tumor DNA (ctDNA) in the plasma of breast cancer patients was also investigated.

The CNV assay methodology was validated using genomic DNA isolated from 96 human samples with known karyotype; sensitivity to single region deletions or duplications was 100% (71/71) and specificity was 100% for normal regions in the same samples. Single-molecule sensitivity for the detection of CNVs was established by analyzing isolated single cells. Performance of the mutation assay was demonstrated with the analysis of 5 matched tumor and normal cell lines, with 24 out of 27 SNVs known to be present in these cell lines detected. The 3 undetected SNVs were determined to be a result of assay design failure. Also, multiple somatic CNVs (median: 13) were detected in all 5 tumor cell lines. Analysis of the normal cell lines found no cancer related SNVs or CNVs.

In 32 FF tumor samples, 78.1% (25/32) had SNVs detected; of samples with SNVs, 88% (22/25) had SNVs in TP53 or PIK3CA. Of the same 32 FF breast tumor samples, 96.9% (31/32) showed full or partial CNVs in at least 1 and up to 15 regions; of the 31 samples with detected CNVs, 93.5% had a CNV of either 1q or 17p, two of the three most prevalent breast cancer CNVs (the 16q region was not represented in this panel). Overall, a combination of SNV and CNV testing allowed identification of genetic changes in 100% of the breast tumor samples, a significant improvement in diagnostic yield than using SNV detection alone. Of the 12 breast cancer patients with matched tumor tissue and plasma samples, 83.3% (10/12) had CNVs detected in tissue. The CNVs present in each primary tumor sample were identified in corresponding plasma ctDNA samples (1 stage IIa, 7 stage IIb, and 2 stage III). The ctDNA fractions in these samples ranged from 0.58 to 4.33%; detection required as few as 86 heterozygous SNPs per CNV.

Analysis of ctDNA for cancer-associated mutations may allow earlier, safer and more accurate profiling and monitoring of breast cancer. Thus, this targeted PCR approach offers the promise of an assay able to detect both cancer-associated SNVs and CNVs in the same sample with good sensitivity and specificity, and improved detection rates compared to assays that only detect SNVs.
Title: The significance of serum HER2 levels at diagnosis on outcome of breast cancer patients by molecular subtype

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Body: Background/purpose: Increased serum HER2 levels have been shown to correlate with higher tumor burden and poorer clinical outcomes in metastatic breast cancer, but there is little data regarding the significance of serum HER2 levels at diagnosis in the operable breast cancer. We evaluated the association between baseline serum HER2 levels and clinicopathologic parameters, and the correlation of baseline serum HER2 levels with clinical outcome by molecular subtype in operable breast cancer patients.

Methods: We included patients with stage I-III breast cancer diagnosed at our center between October 2004 and December 2011. Baseline serum HER2 levels were measured by chemiluminescence immunoassay at diagnosis. Patients were categorized into 4 molecular subtype groups by their hormone receptor (HR) status and HER2 status: HR+/HER2-, HR+/HER2+, HR-/HER2+ and HR-/HER2-. HR and HER2 status were determined by immuohistochemistry (IHC) in all tumors, and fluorescence in situ hybridization (FISH) assay was performed whenever the HER2 status was equivocal.

Results: There were 439 consecutive stage I-III breast cancer patients, of which 271 (61.7%) were HR+/HER2-, 75 (17.1%) were HR+/HER2+, 30 (6.8%) were HR-/HER+ and 63 (14.4%) were HR-/HER2-. A total of 192 patients underwent neoadjuvant chemotherapy and the remaining 235 patients did not. Of these 235 patients, 204 underwent adjuvant chemotherapy. High serum HER2 levels (≥15 ng/ml) were reported in 51 patients (11.6%) and HER2-positive status in tumor tissue was observed in 105 patients (23.9%). High serum HER2 levels were significantly associated with tumor size > 2cm (p=0.032), high histologic grade (0.048), negative HR status and neoadjuvant chemotherapy usage. Patients who had high baseline serum HER2 levels had a worse disease free survival (DFS) (p<0.001). Especially, high baseline serum HER2 levels were associated with worse DFS in HR+/HER2-, HR+/HER2+ and HR-/HER2+ subtypes (p=0.005, 0.001 and 0.027 respectively). However there was no significant correlation between baseline serum HER2 and DFS in the HR-HER2- subtype (p=0.614).

Conclusions: This is the largest study in the evaluation of serum HER2 levels at diagnosis on the prognosis of patients with operable breast cancer. Our results showed that baseline serum HER2 level is a good prognostic marker in patients with operable breast cancer. High serum HER2 levels at diagnosis were associated with worse DFS, especially in patients of HR+/HER2-, HR+/HER2+ and HR-/HER2+ subtypes.
Title: A blinded multicenter phase II study of a panel of plasma biomarkers for the detection of triple negative breast cancer

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Body: Background: Triple negative breast cancers (TNBC) comprise 15-20% of all breast cancers and frequently present as interval cancers with high proliferative rates and increased risk of mortality. There is a clinical need for biomarkers for the early detection of TNBC to complement radiologic imaging. No plasma biomarkers for TNBC currently exist. The purpose of this study is to evaluate a panel of novel plasma biomarkers for TNBC for the discrimination of TNBC and benign breast disease as a crucial step in identifying a panel of plasma biomarkers for early detection.

Methods: In a multicenter collaboration between the NCI EDRN and the CPTAC consortium, we conducted a prospective blinded phase II biomarker study that evaluated 76 candidate TNBC plasma biomarkers. Plasma samples collected at the time of diagnosis from 65 TNBC cases and 195 matched controls with benign breast disease without atypia were identified from multiple clinical sites. The samples were distributed as blinded aliquots to the biomarker laboratories for protein and autoantibody detection. Candidate protein (n=54) and autoantibody (n=22) biomarkers were selected and ranked prior to evaluation. All results were centrally analyzed. The sensitivity at 95% specificity was calculated for each biomarker. Effects of age, race, and specimen source on biomarkers were evaluated. Logistic regression was used to assess complementarity of biomarkers. The top three biomarkers underwent verification with an independent set of 60 TNBC cases and 180 matched controls from women undergoing mammography.

Results: Statistically significant differences in case versus control signals were observed for 3 biomarkers with sensitivities of 17-23% at 95% specificity (p ≤0.008, unadjusted) and areas under the curve of 0.55-0.57. These three biomarkers were confirmed in the independent set of 60 cases and 180 controls with sensitivities 12-23% at 95% specificity and cross-validated combined sensitivity of 27%. In the subset of women ages 50-79 (cases n=38, controls n=119), five biomarkers were identified with sensitivities of 15.7-16.2% at 95% specificity (p<0.10).

Conclusion: We have developed a pipeline strategy for the validation of plasma biomarkers for detection of breast cancer. At least three biomarkers for TNBC were confirmed in this study. Further evaluation of these biomarkers for early detection is ongoing.
Title: Down-regulated circulating microRNAs after surgery: Potential noninvasive biomarkers for diagnosis of early breast cancer

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Body: BACKGROUND: The success of curing the breast cancer largely depends on the staging at diagnosis. However, there is a lack of biomarkers for the detection of early breast cancer, especially the noninvasive one. MicroRNA (miR) is a single-stranded noncoding RNA, which post-transcriptionally regulates gene expression and plays an essential role in tumor development and progression. Recently, microRNA is found to be released into the blood and exist there stably, reproducibly, and consistently among individuals of the same disease conditions. Therefore, circulating microRNA is becoming a promising novel noninvasive biomarker. It is reported that some circulating microRNAs might be down-regulated after certain treatments, such as chemotherapy or surgery. We postulate that post-operational declined circulating microRNAs might indicate the genesis of tumor in early breast cancer and the aim of this study is to identify their diagnostic value.

METHODS: Applying High-throughput microarrays, we screened the dysregulated circulating microRNAs in paired serum samples before and after surgery from 9 patients with early breast cancer (M0, classified into 3 subgroups, ER/PR positive and HER2 negative, ER/PR negative and HER2-positive, and triple-negative breast cancer, with 3 patients per subgroup). Compared to pre-operation, the obviously declined circulating microRNA in post-operation was selected. Using the quantitative TaqMan real-time PCR, we measured the relative concentrations of putative markers in the serum samples of early breast cancer (cancer group: \(n=24\)) and benign breast disease (control group: \(n=24\)) in the training set. Then, the same test were verified in the validation set of another larger independent serum samples (cancer group: \(n=56\), controls group: \(n=44\)). Sensitivity, specificity, and ROC curves were used to assess its diagnostic value.

RESULT: After applying the High-throughput screening chip and statistical analysis, 5 microRNAs significantly reduced after surgery (miR-130b-5p, miR-151a-5p, miR-206, and miR-222-3p, miR-943) were selected. In the training set, we found that the expression of serum miR-130b-5p (\(p=0.004\), area under the ROC curve (AUC) 0.7486), miR-151a-5p (\(p=0.03\), AUC 0.7599), miR-206 (\(p=0.0086\), AUC 0.7302), and miR-222-3p (\(p=0.0146\), AUC 0.7066) had a significant difference in breast cancer group as compared to the control group. In another large validation set, all results were validated (In validation set, AUC of miR-130b-5p 0.7276, miR-151a-5p 0.7959, miR-206 0.8605, miR-222-3p 0.8860). All of four microRNAs had the potential value of breast cancer diagnosis. The combined four microRNAs had better diagnostic value than every single microRNA (combined four microRNA, training set: AUC 0.8457, sensitivity of 85.00%, specificity of 65.22%; validation set: AUC 0.9309, sensitivity of 84.31%, specificity of 83.33%).

CONCLUSIONS: Our findings indicate that post-operation down-regulated circulating microRNAs may be potential specific biomarkers for breast cancer diagnosing.
Title: Cadherin-5 is a circulating glycoprotein biomarker of metastatic breast cancer

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Body: Background
Altered glycosylation is a hallmark of malignant transformation that is often overlooked in studies of circulating biomarkers. In a targeted glycoproteomic study cadherin-5 (CDH5) has emerged as a serological marker of metastatic breast cancer when both protein levels and glycosylation status were assessed (Fry SA, et al. Cancer Lett. 2013; 328(2):335-44). In this study we aimed to further determine the utility of CDH5 as a biomarker for breast cancer progression by investigating clinicopathological, treatment and lifestyle factors associated with metastasis and elevated biomarker levels.

Methods
A nested case-control study of serum collected 12 +/- 3 months post diagnosis as part of the DietCompLyf study (Swann R, et al. Maturitas. 2013; 75(3):232-40) was undertaken with; 112 breast cancer patients, of which n = 52 had developed a distant metastatic recurrence within five years post-diagnosis and n = 60 had remained recurrence free. Serum CDH5 levels were quantified employing an ELISA developed in-house, which was adapted to facilitate assessment of CDH5 glycosylation by binding of the lectin Helix pomatia agglutinin (HPA). Patient CDH5, HPA and CDH5 : HPA ratio values were analysed as continuous variables using the Mann Whitney test to assess biomarker discrimination between metastatic and non-metastatic samples.

Results
Elevated CDH5 levels and ratios of CDH5 : HPA binding distinguished patients with metastatic disease from those that remained metastasis free (Mann-Whitney test; P=0.028 and P=0.007, respectively). Clinicopathological variables of patients were built into multiple logistic regression models in a stepwise manner to determine their capability in prediction of distant recurrence. These analyses showed that the association between the CDH5 : HPA ratio and the formation of distant metastases was driven by patients with oestrogen receptor (ER+) positive breast cancer and tumours showing vascular invasion (VI+). The CDH5 : HPA ratio detected ER+ VI+ patients with recurrent breast cancer with 82% sensitivity and 74% specificity.

Conclusions
The assays developed to measure pg/mL levels of serum CDH5 and probe CDH5 glycosylation are sensitive, accurate and reproducible. This analysis of a new set of breast cancer patient serum samples (n = 112) validated previous work showing that CDH5 level and CDH5 glycosylation act as a biomarker test distinguishing patients with metastatic breast cancer from patients that remain metastasis free. The biomarker test reached optimal sensitivity and specificity in ER positive cancers with vascular invasion, indicating this sub-group of breast cancer patients for whom measurement of CDH5 is the most beneficial.
Title: Global quantitative measures using next-generation sequencing for breast cancer presence outperform individual tumor markers in plasma

Body: Background: Analytically and clinically validated non-invasive blood tests that quantify breast cancer burden and clinical drug response/resistance are greatly needed. Many groups have successfully detected tumor markers in blood using a variety of technologies, including next generation sequencing (NGS). We performed a comprehensive NGS study on a small number of patients to evaluate the value of global versus individual markers for the quantitation of tumor-derived cell free DNA (cfDNA) in plasma.

Methods: DNA isolated from formalin-fixed primary tumor, buffy coat cells, and plasma from 2 patients with metastatic breast cancer were characterized simultaneously for copy number aberrations (CNAs) and differentially methylated regions (DMRs) using whole genome bisulfite sequencing (WBGS), and targeted sequencing-based genotyping of 346 cancer-associated single nucleotide variations (SNVs). CNA and DMR regions were identified from log normalized, GC content corrected counts and DMR data using Poisson and binomial distribution theory and false discovery rate controlling methods. Percent tumor in cfDNA was estimated from the normalized ratio (plasma: primary tumor) of CNA or DMR compared to buffy coat, aggregating over genomic regions. Sample sets from 8 non-metastatic patients were also profiled using the targeted SNV panel in order to compare SNVs between samples and estimate percent tumor cfDNA.

Results: WGBS detected tumor specific alterations in each primary tumor compared to buffy coat. By analyzing the genome using 100 Kb bins, we observed over 1000 bins with detectable CNA signal and, among 56 million CpG sites, over 30,000 DMRs. As expected, 5 or fewer informative somatic SNVs were detected in each patient. Analysis of these somatic changes in plasma revealed that the tumor fraction estimated from SNV detected in cfDNA varied widely between sites originally discovered in the patient’s primary tumor. In contrast, similar estimates of tumor fraction in cfDNA were obtained using CNA and DMR profiles within each patient; both methods yielded similar estimates of over 50% in one patient and less than 10% in the other. For the patient with high tumor fraction, both CNA and DMR profiles contained examples of individual large genomic regions that displayed additional clear aberrations in the plasma compared to the original tumor, such as a striking loss of a >25 Mb region of chromosome 4.

Conclusions: Although individual somatic SNV in cfDNA can be detected in metastatic disease, calculated allelic fraction based on individual SNVs varies greatly within the same patient. Measuring and integrating CNA or DMR across the genome provided more consistent and reliable estimates of tumor DNA fraction in plasma, and also revealed alterations in plasma from patients with metastatic disease that were not prominent in the primary tumor.
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Title: RUNX3 hypermethylation in circulating tumor DNA is a biomarker of breast cancer distant metastasis

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Body: Breast cancer detection has not resulted in the expected decrease in mortality suggesting that current screening strategies are not finding metastasis-prone lesions before metastasis occurs and instead are detecting many lesions that will not progress to metastatic disease. Although several improvements in the management of breast cancer have been made, an estimated 90% of breast cancer related deaths are due to the development of distant metastasis to organs such as lung, bone, liver, and brain. Metastatic breast cancer affects 10-15% of breast cancer patients, is often refractory to therapy and associated with poor outcomes. However, a blood test indicating a high-risk population at the time of breast cancer diagnosis might shift the current strategy and improve outcomes. Efforts for identifying predictive markers of breast cancer metastasis have been impeded by a lack of data defining the genetic and epigenetic determinants of the disease. Numerous studies suggest that DNA methylation could be a useful biomarker for improving the clinical management. Recently circulating tumor (ct)DNA has attracted attention for clinical use in the context of risk prediction, prognostication and prediction in human cancer. Various types of DNA alterations have been reported in ctDNA but methylated DNAs are chemically stable and are among the most sensitive and specific ctDNA cancer markers. The objective of our study was to identify and validate a blood-based DNA methylation biomarker for breast cancer patients at high risk of distant metastasis. Using the HumanMethylation27K bead array, we recently identified RUNX3 hypermethylation involving 3 CpG probes in 32 breast brain metastasis tumor tissue compared to a series of 50 unmatched early stage breast cancer samples. We calculated the area under the curve (AUC) to exceed 0.8, with a higher AUC achieved in Her2-positive samples. In an independent, first of its kind analysis, we performed whole genome bisulfite sequencing (WGBS) of ctDNA from plasma samples representing three pools of 40 women with distant metastatic breast cancer to various organs, 40 women with metastasis-free breast cancer, and 40 non-breast cancer controls. In this unbiased analysis, these data demonstrated RUNX3 hypermethylation in the plasma of patients with metastatic breast cancer in the same region corresponding to the CpG probes of the methylation array. We are now performing bisulfite pyrosequencing of each individual plasma sample, and in an additional cohort of 50 archival primary/brain metastasis tumor pairs with matching serum and clinical outcomes data. To determine regional specific differences we have also selected two additional regions of RUNX3 not differentially methylated for validation by bisulfite pyrosequencing. Determining the extent to which RUNX3 hypermethylation occur in the context of brain metastasis versus other distant sites are ongoing studies. Together these studies point towards RUNX3-regional specific methylation as a potential blood-based biomarker of breast cancer metastasis. Such a biomarker will help establish surveillance methods and eventually preventative regimens that will increase the likelihood of long-term progression-free survival for breast cancer patients at risk of distant relapse.
Title: Detection of H1047R and E545K PIK3CA mutations from peripheral blood in ER positive breast cancer patients

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Body: Background: Digital polymerase chain reaction (PCR) is a new technology that enables detection and quantification of cancer DNA molecules from peripheral blood. Detecting tumor-specific mutations in circulating plasma DNA may potentially be useful to select systemic therapies for solid tumors. The aim of our study is to evaluate the feasibility of detecting PIK3CA mutations from plasma of breast cancer patients.

Methods: We have designed an allele-specific PCR assay and used a digital PCR system for the detection of PIK3CA mutations H1047R and E545K. Formalin-fixed paraffin-embedded (FFPE) tumor samples were analyzed by COBAS and by digital PCR. The matched plasma samples were then analyzed by digital PCR. Kappa Cohen’s coefficient (κ) was used to test agreement between methods.

Results: 37 ER positive breast cancer and matched plasma were evaluated. 31 (84%) patients were stage IV disease. E545K mutation was detected in 4 out of 37 (11%) tumor specimens and H1047R mutation was identified in 8 out of 37 (22%) tumor samples. The proportion of observed agreement between H1047R and E545K detection in tumor samples by COBAS and digital PCR was 100%. Regarding E545K mutation assay we found that the proportion of observed agreement between mutation status in FFPE samples and circulating tumor DNA (ctDNA) was 94.6% (kappa= 0.770; Sensitivity=100%; Specificity=93.9%). In H1047R assay the proportion of observed agreement between FFPE samples and ctDNA was 83.78% (Kappa=0.561; Sensitivity=60%; Specificity=92.59%).

Conclusions: The agreement between methodologies when assessing the PIK3CA status in tumor samples was perfect. However, a moderate-to-fair agreement was found between FFPE samples and ctDNA which might be due to the heterogeneity of the disease. The methodology presented in this study is a feasible approach for PIK3CA mutation detection in blood derived samples.
Title: Role of disseminated tumor cell (DTC) in bone marrow (BM) detected at primary diagnosis on overall survival and central nervous system (CNS) recurrences in a cohort of 620 early breast cancer patients after 11 years follow up

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Body: Background: BM DTC detection is known to be a prognostic factor for distant metastasis and overall survival in early breast cancer. However, distant relapses may occur in patients with no DTC detected at time of primary cancer. In this study, we studied the impact of early breast cancer pathological features (including BM DTC status) on the incidence of CNS metastases.

Methods: In a cohort (1998-2005) of 620 early breast cancer patients in whom BM DTC were detected using an anti-cytokeratin antibody (A45B/B3) (Bidard et al., 2008, Clinical Cancer Research, outcome updating (median follow up 11y) on overall survival (OS) and distant metastasis free survival (DMFS) was assessed by log rank test and uni- and multivariate analyses. 137 patients of this cohort have a later metastatic relapse. Chi2 test were performed to compare patient with CNS metastasis and patients with other metastases locations. CNS metastasis survivals were estimated using Kaplan-Meier method and compared by log-rank test.

Results: At eleven-year median follow-up, the prognostic value of DTC detection was confirmed in the 620 patients cohort for OS (p=0.03 in multivariate analysis), but DTC were probably less involved in late metastatic events.

Distant metastases were diagnosed in 137 (22\%) patients. In the course of the metastatic disease, 55 of these patients (40\%) have been diagnosed with CNS metastases (parenchymal=30, leptomeningeal=10, both localizations=15), as first metastatic or later event. Patients with CNS metastasis were mostly less than 50 years old (60\% vs 38\%, p=0.01), pN0 (31\% vs 10\%, p=0.002), hormonal receptor status negative (54\% vs 30\%, p=0.007) and HER2 status positive (50\% vs 22\%, p=0.01). Strikingly, although DTC detection was associated with development of distant metastasis in the whole cohort, the occurrence of CNS metastasis was found to be higher in patients who had no DTC detected at primary diagnosis (p=0.016). From CNS metastasis, median survival was 7.8 months (8.3 months for parenchymal and 2.4 months for leptomeningeal recurrences). For HER2\textsuperscript{+} patients, the median survival after diagnosis of CNS metastasis was 16.6 months (N=15) and 4.1 months for Her2\textsuperscript{-} patients (N=15).

Conclusions: After a long-term follow up, DTC remain an adverse prognostic factor in early breast cancer. Our study suggests that cancer cells with epithelial differentiation, as detected in the BM, are less prone to CNS dissemination. This study also confirmed previously reported determinants of CNS metastases (age, RH-, HER2+) in the pre-adjuvant trastuzumab era, but also showed that the outcome of HER2\textsuperscript{+} patients with CNS relapse was longer, probably due to HER2 targeted therapy during metastatic course.
Title: A new breast cancer classification scheme based on novel classes of tumor stroma

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Body: A major challenge in cancer treatment is the heterogeneous nature of the disease. This is particularly evident in breast cancer where gene expression profiling of whole tumours has identified multiple intrinsic subtypes of breast cancer. These subtypes are associated with differential prognoses and are correlated with previously identified clinical biomarkers (i.e., ER and HER2 status) used to stratify patients for targeted therapy. Despite recent studies demonstrating that elements within the tumour microenvironment can affect breast cancer progression and outcome, and that information contained within this compartment carries significant prognostic information for patient stratification above and beyond the information supplied by the intrinsic subtypes and existing therapeutic biomarkers, a limited understanding of stromal heterogeneity across the population has hindered the development of effective prognostic tools and targeted therapies directed against these processes. Here we perform expression profiling of the microdissected tumour-associated stromal components of 49 human breast tumours, and demonstrate that stromal heterogeneity can be captured by categorization into six classes which bear distinct molecular phenotypes. These stromal classes exhibit distinct biological functions and carry prognostic information independent of existing tumor-intrinsic biomarkers and molecular breast cancer subtypes. Specific combinations of stromal class and tumour subtype are significantly over- and under-represented; furthermore, simultaneous stratification of tumors in external datasets by both tumour subtype and stroma class identifies good- and poor-outcome cohorts within four of the five molecular breast cancer subtypes. The stroma classes identified here form the basis for an improved breast cancer classification scheme which takes the contribution of the microenvironment into account.
Cell death and efferocytosis generate a pro-metastatic landscape during mammary gland involution that increase dissemination of post-partum breast cancers

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Body: Although pregnancy at a young age decreases a woman’s lifetime breast cancer risk, the first five years following a pregnancy at any age are associated with increased breast cancer risk, a risk that continues to increase with age. Given societal trends to postpone child-birth later into women’s lives, the incidence of malignant post-partum breast cancer is expected to increase. Currently, breast cancers diagnosed 2-5 years post-partum account for nearly 25% of all pre-menopausal breast cancers. Importantly, breast cancers diagnosed 2-5 years post-partum are diagnosed more frequently as metastatic disease and correlate with decreased disease-free survival (DFS) versus breast cancers occurring in other young pre-menopausal women. In contrast, many studies show that breast cancers diagnosed during pregnancy do not correlate with decreased DFS, although there remains some debate about this conclusion. The observation that post-partum breast cancers correlate with reduced DFS suggests that events occurring in the post-partum breast augment the malignant severity of tumors existing therein. This hypothesis is supported by studies in which breast cancer cells transplanted into involuting mouse mammary fatpads grow and invade more rapidly than cells transplanted into mammary glands of nulliparous mice, demonstrating a unique mammary micro-environment during involution versus other reproductive stages. We aimed to study how the changing landscape of the post-partum breast accelerates cancer progression.

Although the molecular mechanisms underlying the malignant severity of post-partum breast cancers (ppBCs) are unclear, they relate to stromal wound healing events during post-partum involution, a dynamic process characterized by widespread cell death in milk-producing mammary epithelial cells (MECs). Using both spontaneous and allografted mammary tumors in fully immune-competent mice, we discovered that post-partum involution increased mammary tumor metastasis 10-fold. Widespread cell death occurred not only in MECs, but also in tumor epithelium. Dying tumor cells were cleared through MerTK-dependent efferocytosis, which robustly induced transcription of wound healing cytokines interleukins (IL)-4, -10, and -13, and transforming growth factor (TGF)-beta. Genetic models of MerTK ablation and pharmacologic MerTK inhibition impaired efferocytosis in post-partum tumors, reduced M2-like macrophages without altering total macrophage levels, decreased TGF-beta expression and reduced post-partum tumor metastasis to levels seen in nulliparous mice. TGF-beta blockade reduced post-partum tumor metastasis. These data suggest that widespread cell death during post-partum involution triggers efferocytosis-induced wound healing cytokines in the tumor microenvironment that promote metastatic tumor progression.
**Title:** Cell autonomous and non-cell-autonomous activities of heat shock factor 1 support tumor initiation, progression and metastasis

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**Body:** For tumors to expand, invade and metastasize, the recruitment and reprogramming of non-malignant stromal cells is required. Yet surprisingly little is known about the factors that drive the transcriptional reprogramming of stromal cells within tumors. That such reprogramming must occur is clear from evidence that normal fibroblasts usually constitute a tumor-restrictive environment. The goal of our study was to characterize stromal factors that actively support or enable malignancy. We were guided by the rationale that these would not be classical oncogenes, as non-malignant stromal cells are relatively stable genetically. Instead, we hypothesized that tumors might hijack normal physiological pathways and programs in the stroma, subverting them to enable neoplastic growth and metastatic dissemination.

Our work shows that Heat-shock Factor 1 (HSF1), an evolutionarily conserved transcriptional master regulator, plays a crucial role in this process. Across a broad range of human cancers, HSF1 is activated not only in the malignant cells themselves, but in cancer-associated fibroblasts (CAFs). HSF1 drives a transcriptional program in CAFs that complements, yet is completely different from, the program it drives in adjacent cancer cells. This CAF program is uniquely structured to support the malignant potential of cancer cells in a non-cell-autonomous way and is mediated by two central stromal signaling molecules–TGFβ and stromal-derived factor 1 (SDF1). In two independent cohorts of early stage breast patients, and an additional cohort of lung cancer patients, high stromal HSF1 activation is strongly associated with poor patient outcome. Moreover, stromal HSF1 activation was a more robust prognostic indicator of outcome than HSF1 in the cancer cells. The complementary but distinct roles of HSF1 in cancer and stromal cells within tumors have both diagnostic and therapeutic implications. From a diagnostic perspective, assessing HSF1 in both stromal and cancer cells might guide treatment choices in early stage breast cancers thereby avoiding overtreatment and its associated morbidities. From a therapeutic perspective, the dependence of even the most robust cancers on supporting stromal cells, and the relative genetic stability of the stroma, make HSF1 an attractive target for intervention in both breast cancer cells and stroma. As we and others have suggested, the nearly unthwartable ability of advanced breast cancers to evolve resistance to virtually every available therapy makes it attractive to target normal biological networks that have been co-opted to support malignancy, rather than relying solely on the targeting of mutated malignant drivers.
Title: Estrogen signaling through astrocytes promotes migration and invasion of ER-negative brain metastatic breast cancer cells

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Body: Approximately 16% of patients with breast cancer develop symptomatic brain metastases (BMs) and the majority [~80%] will die subsequently. Symptomatic BMs are more prevalent in breast tumors overexpressing Human Epidermal Growth Factor Receptors (HER1, EGFR) or 2 (HER2) and triple-negative (TN) tumors (lacking estrogen receptor (ER), progesterone receptor (PR) and HER2). Although the majority of breast cancer cells colonizing the brain lack ER, central nervous system (CNS) metastases are more frequent in pre-menopausal women with high levels of circulating estrogens. Since astrocytes are ER+ cells that surround and infiltrate brain metastases, we hypothesize that estrogens act in a paracrine manner on reactive astrocytes to promote BMs.

Results: To determine the effect of estrogen in brain colonization, a brain-seeking sub-line of human MDA-MB-231 TN breast carcinoma cells (231Br) cells were injected intracardially in ovariectomized female nude mice supplemented with placebo (n=5) or 1mg 17-βestradiol (E2) pellets (n=5), and metastases were detected by gadolinium-enhanced magnetic resonance imaging (MRI) 5 weeks later. 100% (5/5) of mice from E2 group showed large and multiple BM when compared to 3/5 (60%) of placebo mice. Further, E2-treated mice showed decreased survival (41.6%, n=12) by 3 weeks following IC injection of 231Br cells compared to E2-depleted mice (84%, n=12) (P=0.03). Since astrocytes are the most abundant brain cell type expressing ERs, we focused on their interaction with 231BR cells. Using global gene expression arrays and RT-PCR we found that E2 upregulated Egf and Tgf-a in primary mouse astrocytes and human astrocytes (derived from neural stem cells), and that anti-estrogens blocked this effect. Concentrated conditioned media (CM) from E2-treated astrocytes increased P-EGFR levels in 231Br cells compared to vehicle (EtOH), or E2+ICI astrocytic CM. Moreover, CM from E2-treated astrocytes significantly increased migration and invasion of 231Br cells as compared to EtOH-treated CM, and ERs inhibitors abolish this effect. Treatment of 231Br cells with lapatinib abolished EGFR activation in response to E2-astrocytic CM, and decreased migration and invasion of 231BR cells. These data suggest that EGFR-ligands upregulated by E2 in astrocytes activated brain metastatic cells-EGFR resulting in increased migration and invasion. To identify the mechanisms of increased migration and invasion we performed global gene expression profiling of 231Br cells co-cultured with E2- or EtOH-stimulated-astrocytes. Co-culture with E2-treated astrocytes significantly increased expression of metastatic mediators Matrix-metalloproteinase-9 (MMP9) and S100 Calcium-binding protein A4 (S100A4) in 231Br cells, and treatment of astrocytes with ER inhibitors abolished this effect. Furthermore, MMP9 or S100A4 knockdown in 231Br cells significantly decreased migration and/or invasion in response to E2-astrocytic CM, suggesting that MMP9 and S100A4 are functional mediators of the paracrine effect of E2. Conclusion: These studies suggest that EGFR activation by astrocytic ligands is at least one mechanism by which E2 contributes to the promotion of BM of ER-negative breast cancer cells and suggests a novel role for ER in breast cancer BM.
Title: Contributions of STAT3 to pro-tumorigenic alterations within the microenvironment during mammary tumorigenesis

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Body: Interactions between tumor cells and their surrounding microenvironment are critical for the development and progression of breast cancer. Specifically, oncogenic alterations such as aberrant activation of growth factor receptors in epithelial cells lead to a pro-tumorigenic inflammatory response characterized by increased production of inflammatory factors and increased macrophage infiltration. Our studies focus on identifying novel mechanisms through which tumor-derived cytokines act in an autocrine manner to regulate tumor cells and in a paracrine manner to regulate infiltrating inflammatory cells. In recent studies, we have shown that following activation of the fibroblast growth factor receptor 1 (FGFR1) oncogene, mammary epithelial cells produce high levels of interleukin 6 (IL-6) family members. These cytokines activate signal transducer and activator of transcription 3 (STAT3) in both epithelial cells and macrophages. STAT3 is constitutively activated in up to 70% of primary breast tumors, and has been shown to enhance proliferation and angiogenesis as well as aid in immune suppression. Using a novel mouse model of FGFR1-driven tumor growth, we demonstrated that inhibition of STAT3 led to decreased tumor growth. Histological examination revealed high levels of STAT3 activation in both tumor cells and macrophages. Thus, further studies focused on identifying mechanisms through which STAT3 activation in different cellular compartments contributes to mammary tumor growth. Initial studies focused on the consequences of STAT3 activation in epithelial/tumor cells. We found that epithelial cell STAT3 activation resulted in accumulation of the extracellular matrix component hyaluronan, which contributed to epithelial cell proliferation, survival and migration. Meanwhile, STAT3 activation in macrophages led to expression changes within a variety of molecules which are known to enhance tumor cell survival, tumor invasion and regulation of the anti-tumor immune response. Thus, it becomes evident that systemic inhibition of STAT3 function can potentially inhibit tumor growth and progression by acting on both tumor cells and on the surrounding stroma. As STAT3 is being considered as a viable target for breast cancer therapy, understanding the mechanisms through which STAT3 promotes tumor growth and progression will be critical for developing optimal therapeutic strategies.
CD146 positive and negative stroma direct breast tumor estrogen receptor levels, therapeutic response and metastatic potential

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Background: Cellular heterogeneity within all breast cancer subtypes remains a major cause of treatment failure and development of metastatic disease. Currently, both preclinical studies and drug development efforts focus almost exclusively on the epithelial component of breast cancers. Despite strong preclinical data novel therapies often fail in clinical testing. We propose that this failure is, in part, due to taking tumor cells out of their context and using pre-clinical models that fail to capture the complexity of human disease. We hypothesize that tumors hijack normal components of the tissue microenvironment and use it to their advantage. Here we demonstrate that similar to the normal hematopoietic niche, two major subtypes of breast cancer stroma can be defined by CD146 expression. We further show that the ratio of the stromal subtypes alters the response to therapy and increases the metastatic potential of breast cancer cells (BCC).

Results: Tumor associated stroma, from all breast cancer subtypes, contains a mixture of CD146+ and CD146- fibroblasts. We isolated and derived pure human CD146+ and CD146- clonal lines. Both subtypes expressed markers of activated fibroblasts and clustered by gene expression profiling with normal stromal cell lines HS27A (CD146+) or HS5 (CD146-) according to CD146 expression. Although both stromal subtypes were derived from tumor associated tissue, CD146+ breast cancer stroma clustered with normal breast associated stroma and correlated with good clinical outcome (Finak et. al. Nature Med 2008). CD146- stroma clustered with breast cancer associated stroma and predicted worse outcome.

Using cell line and patient-derived xenograft models of estrogen receptor (ER) positive breast cancer, we demonstrated that CD146+ compared to CD146- stroma supported significantly higher ER expression in BCCs. BCC co-cultured with CD146+ stroma responded more robustly to estrogen treatment and anti-endocrine therapy with tamoxifen.

Next we used expression profiling data to predict stromal influence on treatment response. CD146+ stroma expressed 3-fold more TGFβ than CD146- stroma. Inhibiting TGFβ decreased proliferation 2-fold in BCCs grown in media conditioned by CD146+ stroma, but not by CD146- stroma. Conversely, HBEGF expression was 3-fold higher in CD146- stroma compared to CD146+ stroma. Inhibiting EGFR decreased proliferation 1.5-fold in BCCs grown in conditioned by CD146- stroma, but not by CD146+ stroma. In addition, intracardiac injection of stroma resulted in distant metastases in our primary orthotopic PDX model of breast cancer.

Lastly we confirmed our preclinical observation using a small set of clinical samples. Patients with high CD146+ to CD146- stromal ratio had better clinical outcomes than patients with high CD146- to CD146+ stromal ratio.

Conclusion: We conclude that stromal subtypes defined by CD146 expression direct the heterogeneity of ER expression, response to therapy and the metastatic potential of breast cancer cells.
Title: Breast cancer tissue context determines whether inflammation with bacterial/psoriasin signature is pro- or anti-carcinogenesis

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Body: Objective: It is often neglected that breast tissue microbiota and systematic bacterial infection actively and potently influence the development of breast cancer. With reports of both pro-cancer and anti-cancer roles of bacterial inflammation, the actual effect of bacterial inflammation to breast cancer and its mechanism remain unclear and are of our interests.

Rationale: Different from other human tissues, breast tissue microbiota is majorly Gram-negative at both normal and pathological statuses. We discovered that bacterial inflammation in breast tissue has a signature of Psoriasin (S100A7) impact which resembles psoriasis of the skin. However, this Psoriasin mediated inflammation can either promote or inhibit breast cancer development. We hypothesize that different tissue context determines the differential effects of bacterial/Psoriasin inflammation on breast cancer development.

Results: We first verified the presence of commensal Gram-negative microbiota (and LPS) in breast tissues under normal and pathological circumstances using mouse models. We observed that in both normal and cancerous breast tissues, bacterial factors (such as LPS) found in breast triggered secretion of Psoriasin by mammary adenocytes and Psoriasin mediated inflammation. This type of inflammation is featured by macrophage recruitment and ductal infiltration induced by Psoriasin. However, in normal tissues, macrophages matured into M1 type (iNOS positive); whereas in cancerous tissues, macrophages matured into M2 type (Arginase-1 positive). In normal tissues, Psoriasin and M1 caused an unresolved accumulation of inflammation in mammary ducts without lactation, which might lead to accelerated cancer initiation. In tumors, Psoriasin and M2 increased tumor growth and metastasis in immune-competent mammary epithelial specific expression mouse models. This was further confirmed in human patient samples and large cohort bioinformatic analysis. Using human breast cancer cell lines and nude mice, we elucidated that inflammation related Psoriasin upregulation and secretion in cancer cells had differential effects on tumor growth depending on its tissue source. Psoriasin increased cell proliferation and tumor growth in basal-like cancer cells; whereas it decreased cell proliferation and tumor growth in non-basal-like cancer cells. And this difference was mediated through differential regulation of the NF-κB/miR-29b/p53 pathway. in vitro molecular study and in vivo models verified the important role of miR-29b switch in the differential effects of Psoriasin in different tissue types of breast cancer cells.

Conclusion: Bacterial/Psoriasin inflammation differentially influences breast cancer development depending on the tissue context, and it involves the effects on macrophages and cancer cells. Our work provides novel potentials to improving prognosis and targeting inflammation to treat breast cancer in personalized medicine.
EphA4-deleted microenvironment regulates cancer development of isografted 4T1 murine breast cancer cells via reduction of an IGF1 signal

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Background:
EphA4 (the erythropoietin-producing hepatocellular receptor A4) belongs to a large family of receptor tyrosine kinases that play critical roles in cancer progression. We have previously reported that the absence of EphA4 expression decreases the amount of IGF1 in the circulation and locally in tissues, which contributes to the short stature (Cell Reports 2012). It is known that 4T1 murine breast cancer is a good model of human metastatic breast cancer. 4T1 cells are also known to produce a large amount of granulocyte colony-stimulating factor which can cause extramedullary hematopoiesis associated with a poor recipient prognosis.

Aim:
To investigate whether EphA4-deleted microenvironment affects growth of primary tumors, tumor metastasis, and extramedullary hematopoiesis via a novel EphA4-mediated IGF1 synthesis pathway.

Methods:
We isografted 10⁵ mouse breast cancer cells (4T1) into the left inguinal mammary fatpad of both EphA4-knockout (KO) and control wild type (WT) female littermate mice. The parameters evaluated in vivo were growth of the primary tumors, distribution of metastatic foci, number of peripheral blood leukocytes (PBL), and splenomegaly. To examine the effect of IGF1 treatment on these parameters, recombinant human IGF1 (5 mg/kg body weight (BW)/day) was subcutaneously injected into the EphA4-KO mice for 9 weeks starting 4 weeks before grafting 4T1 cells. And the corresponding WT control mice were treated with saline alone for the same period. We evaluated the extent of metastasis by counting the number of metastatic foci larger than 1 mm in diameter in the lung, heart, liver, kidney, adrenal glands, lymph nodes, peritoneum, pleura and ovary.

Results:
In the absence of IGF1 injection, both the weight of primary tumors and the number of metastatic tumor foci were significantly reduced in EphA4-KO mice as compared with those in control WT littermate mice (n=6 of each genotype, t-test P<0.005). Splenomegaly (n=6 of each genotype, t-test P<0.001) and the number of PBL (n=6 of each genotype, t-test P<0.005) were also markedly decreased in EphA4-KO mice. When treated with IGF1, EphA4-KO mice showed significant weight gain of the primary tumors to almost the level of WT mice, had a greatly increased number of metastatic tumors, and showed an enhanced PBL number to the level of WT mice. However, IGF1 treatment could not enhance splenomegaly.

Conclusions:
EphA4 expression in the tumor microenvironment plays an important role in tumor growth, metastasis, leukemoid reaction and splenomegaly. Deletion of EphA4 in the microenvironment delays primary tumor growth and metastasis and reduces leukemoid reaction mainly by regulating the amount of IGF1 in the circulation and tissues. However, splenomegaly might not solely be mediated by an IGF1 signal. Our findings may prove a new therapeutic target for breast cancer.
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Title: Mesenchymal stem cells and macrophage interactions promote inflammatory breast cancer cell invasion and self renewal

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Body: Background: Inflammatory breast cancer (IBC) is responsible for 10% of breast cancer deaths. The hallmarks of IBC are skin involvement and a high propensity to metastasize. Our lab has shown previously, "normal" breast tissue from women with an IBC diagnosis had significantly greater macrophage infiltration and increased cells with stem cell markers compared to non IBC "normal" breast tissue. These changes were present prior to diagnosis in two patients where pre-IBC biopsies were available. Therefore, we hypothesized changes in the normal breast microenvironment prior to tumor formation contributes to the IBC phenotype.

Methods: To study our hypothesis we used a co-culture system to measure the interactions between normal macrophages (Raw 264.7 cell line), bone-marrow derived mesenchymal stem cells (MSCs), and IBC cells (SUM 149, IBC3, and KPL4). Conditioned media (CM) from MSC culture was added to macrophages overnight. The macrophages were subsequently analyzed for their surface markers and cytokine production. Reciprocally, MSCs were "educated" by macrophages by adding CM from polarized M2 macrophages to the MSC culture media at a 1:1 ratio. MSCs and cancer cells were co-cultured in trans-wells (Boyden chambers) for 24 hours. Migration and invasion in vitro was determined by adding MSCs or IBC cells to the insert of the trans-well and cultured in combination with either polarized macrophages or macrophage educated MSCs for 24 hours. After co-culture, IBC cells were analyzed for their ability to form mammospheres.

Results: Mouse macrophages polarized (using IL-4) into a M2 phenotype (Tumor associated macrophage) secreted 3 fold more IL-6 compared to unstimulated or M1 polarized macrophages. The addition of MSC CM to the macrophage culture for 24 hours polarized the macrophages into a similar M2 phenotype described above with 4-fold increase in IL-6 secretion compared to unstimulated macrophages (P<0.01). When M2 macrophages at the bottom of the co-culture system were co-cultured with MSC, the number of MSCs migrating increased 2 fold with the addition of M2 macrophages compared to media alone, or unstimulated macrophages (P<0.05). IBC cells showed a 2 fold enhancement of invasion towards M2 educated MSCs compared to either uneducated MSCs, or media alone (P<0.05). IBC cells co cultured with M2 educated MSCs grew 3 fold more mammospheres compared to IBC cells grown with uneducated MSCs (P<0.01). IBC cells added to the bottom of the co-culture system also enhanced migration of MSCs and did so to a greater extent than non-IBC cells (P<0.01). Lastly, the addition of IL-6 neutralizing antibody inhibited IBC induced MSC migration.

Conclusions: Herein we demonstrate reciprocal tumor interactions between normal cells in the IBC microenvironment. MSC and macrophages can influence each other to increase the tumor promoting influence of each on IBC cells. Our results suggest IL-6 a mediator of these tumor promoting influences and is important for the IBC induced migration of MSCs. Currently we are investigating the in vivo interactions between macrophages and MSCs in an orthotopic IBC mouse model.
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Title: Slit2 inhibits breast cancer growth and metastasis by modulating tumor microenvironment

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Body: Distant metastasis of breast cancer to vital organs such as lung is a major cause of breast cancer related death. Recent insights suggest that tumor microenvironment (stroma) plays a key role in enhancing the metastatic potential of breast cancer cells. Primarily discovered as neuronal guidance cue, Slit2 has recently been emerged as an important tumor suppressor gene. Slit2 is reported to be genetically mutated and highly methylated in various cancers including breast cancer. Recent studies have also correlated higher Slit2 expression with better overall survival of breast cancer patients. However, its effect on lung specific metastasis and tumor microenvironment is not studied yet. To analyze the effect of rSlit2 on breast cancer growth and metastasis, we used MVT-1 breast cancer cells in orthotopic syngenic preclinical mouse model. Intra-peritoneally injected rSlit2 significantly inhibited MVT-1 implanted tumor growth compared to control group. Next, we analyzed the lungs for metastatic nodules and interestingly, rSlit2 significantly inhibited lung metastasis. Further analysis revealed that rSlit2 treated tumors had significantly less number of CD11b+/Gr1+ and CD11b+/F4/80+ myeloid cells compared to control. Furthermore, we analyzed whether recruited macrophages were of immunosuppressive M2 phenotype and it was found that rSlit2 treated tumors had significantly reduced number of CD11b+/F4/80+/CD206+ cells. Immunohistochemical analysis (IHC) also confirmed reduced number of M2 macrophages present in rSlit2 treated tumors. We also validated these findings in human breast cancer tissue microarray and inverse correlation was observed between Slit2 expression and invasive/metastatic breast cancer. To explore the Slit2 mediated molecular mechanisms on macrophages; we used RAW264.7 cells induced by MVT1 cells conditioned media (CM) and recombinant TGF-β1. Interestingly, rSlit2 successfully inhibited expression of M2 macrophage activation marker (Arginase1, IRAM-M and IL-10) induced by MVT-1 CM and TGF-β1. To further confirm the role of Slit2 in M2 activation, we analyzed cytokine profiling of RAW264.7 cells and found that TGF- β1 enhanced M2 related cytokines such as IL-10, CXCL13 and CCL17 and these effects were inhibited by rSlit2. We further observed that rSlit2 inhibit TGF-β1 induced downstream signaling, which results in reduced β-catenin expression and translocation to the nucleus in RAW 264.7 cells. IHC analysis also showed reduced β-catenin expression in rSlit2 treated tumor sections. Taken together, these results suggest that rSlit2 inhibits tumor growth and metastasis via inhibiting recruitment of myeloid/macrophages and alternative activation of M2 macrophages in preclinical mouse model. The results from this study suggest that rSlit2 could be used as a potential therapeutic agent to target breast cancer metastasis.
Comparing tissue compositions of within-individual mammographically high and low dense breast tissue

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Purpose
Mammographic density (MD) refers to the extent of radio-opaque breast tissue on a mammogram. Adjusted for a women’s age and body mass index (BMI), it is a strong and independent risk factor for breast cancer (BC). The presentation of mammographically dense breast is not uncommon in the normal female population, and patients’ awareness of its association with BC risk has been growing following mandatory reporting of MD by physicians in several states of America. Women with breasts that have over 75% dense tissue are 4 to 6 times more likely to develop BC than women with breasts that have less than 10% dense tissue. However, the biological basis of how high MD raises BC risk remains elusive. We aimed to examine the histological and molecular differences between high and low dense breast tissues of healthy women, using specimens accrued from prophylactic mastectomy procedures.

Method
48 women between 2008 and 2013 underwent prophylactic mastectomy at St Vincent’s Hospital and Peter MacCallum Cancer Centre due to a high BC risk profile. Of these, 41 were eligible for analyses. Tissue slice resected from the mastectomy specimen was X-rayed, and high (HD) and low dense (LD) regions were dissected based on the radiological appearance. The histological composition, immunohistochemistry and proliferation status were assessed on matched high and low MD tissue of the same breast. Signed rank test and paired t test were used for quantitative analyses of potential differences between HD and LD tissue.

Result
HD tissue demonstrated a significantly greater proportion of stroma (p<0.0001) and epithelium (p<0.0001), and less amount of fat (p<0.0001) than LD tissue (n=41 women). Epithelium from HD region also demonstrated epithelial-mesenchymal transition (EMT) plasticity, which was evident as the co-expression of cytokeratin (CK)-19 and vimentin in the glandular area. There was no significant difference with regards to oestrogen receptor (ER) (p= 0.2772), progesterone receptor (PR) (p= 0.9910), and Ki-67 (p= 0.6028) expression between HD and LD tissue.

Conclusion and Significance
We found that increased stroma and epithelium proportions contribute to the dense appearance on mammogram. Moreover, dense tissue did not demonstrate differed hormonal receptor expression or proliferation status from non-dense tissue, but showed a preponderance of EMT in the form of co-localisation by both CK-19 and vimentin in some of the epithelial cells. Our study is the first to report EMT phenomenon in benign mammary tissue, and suggests that investigations of the stromal micro-environment, and their interactions with epithelium may be key to improving our understanding on MD-mediated BC risk.
Title: Both metabolic syndrome and statin use are more common in women with breast inflammation

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Body: Background: Metabolic syndrome is associated with increased breast cancer (BC) risk. After menopause, obesity is associated with increased risk of hormone receptor (HR)-positive breast cancer. We demonstrated an obesity → inflammation → aromatase axis in breast white adipose tissue (WAT) where inflammation is defined by the presence of crown-like structures (CLS) consisting of a dead or dying adipocyte encircled by macrophages. CLS in the breast (CLS-B) are associated with elevated body mass index (BMI), postmenopausal status, increased adipocyte size, and increased tissue levels of proinflammatory mediators and aromatase. As obesity is a component of the metabolic syndrome and is associated with inflammation, we compared levels of relevant circulating factors in women with and without breast WAT inflammation.

Methods: We prospectively collected paired WAT and fasting serum and plasma from women undergoing mastectomy at MSKCC. WAT inflammation was detected by CD68 immunohistochemistry to identify CLS-B by light microscopy. Plasma levels of glucose, insulin, hsCRP, leptin, adiponectin, and IL-6 were measured by ELISA. Serum levels of total cholesterol, triglycerides (TG), HDL and LDL cholesterol were determined. Insulin resistance (IR) was assessed using fasting plasma glucose and insulin levels via the updated Homeostasis Model Assessment (HOMA2-IR). Associations between CLS-B and clinicopathologic features, including medication usage, were analyzed by logistic regression and Fisher’s exact test. Differences in serum/plasma endpoints between subjects with and without CLS-B were evaluated using Student t-test and nonparametric Wilcoxon rank-sum test.

Results: From 11/2011 – 3/2013 we accrued 100 patients (pts); median age 47 years (range 27 – 70). Overall, CLS-B were found in 52/100 (52%) pts: 18/19 (95%) obese pts, 17/33 (52%) overweight pts, and 17/48 (35%) normal BMI pts. A clinical diagnosis of dyslipidemia was present in 14/52 (27%) pts with CLS-B and 1/48 (2%) pts without CLS-B (P<0.001). CLS-B were found in 10/11 (91%) statin users, but in only 42/89 (47%) non-users (P=0.008). Fasting glucose, insulin, LDL, TG, leptin, hsCRP, and IL-6 levels were higher in pts with CLS-B (Table). HOMA2-IR was greater in pts with CLS-B (mean 0.63 ±0.34) versus those without CLS-B (mean 0.46 ±0.23; P=0.006).

<table>
<thead>
<tr>
<th>Fasting level, mean (SD)</th>
<th>No CLS-B, N=48</th>
<th>CLS-B +, N=52</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose, mg/dL</td>
<td>73.2 (8.0)</td>
<td>84.3 (37.6)</td>
<td>0.04</td>
</tr>
<tr>
<td>Insulin, mU/L</td>
<td>4.3 (2.1)</td>
<td>5.6 (2.9)</td>
<td>0.01</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dL</td>
<td>105.9 (31.0)</td>
<td>119.4 (32.4)</td>
<td>0.04</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dL</td>
<td>71.9 (15.8)</td>
<td>62.1 (16.1)</td>
<td>0.003</td>
</tr>
<tr>
<td>Triglycerides, mg/dL</td>
<td>69.2 (26.3)</td>
<td>104.9 (50.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Leptin, pg/mL</td>
<td>12.0 (10.1)</td>
<td>22.6 (19.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Adiponectin, ng/mL</td>
<td>13.7 (5.2)</td>
<td>10.4 (5.4)</td>
<td>0.002</td>
</tr>
<tr>
<td>hsCRP, ng/mL</td>
<td>1.0 (1.4)</td>
<td>2.3 (2.7)</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Conclusions: Breast WAT inflammation, which we have previously linked to increased aromatase activity, is associated with biochemical changes that occur in the metabolic syndrome, a risk factor for BC. Statin use is more common in patients with breast WAT inflammation and metabolic syndrome. Clinically, statin use may be a surrogate identifier of a population that is at increased baseline risk of BC. These findings may account for the variability in results of prior studies examining statin use and breast cancer risk due to elevated risk in users compared to non-users.
2014 San Antonio Breast Cancer Symposium

Publication Number: P4-04-13
Average Grade: 6.50

Title: Cancer-derived miR-105 promotes tumor invasion and destroys the natural barriers against metastasis

Shizhen Emily Wang1, Weiying Zhou1, Miranda Y Fong1, Yongfen Min2 and Pengnian Charles Lin2. 1City of Hope Beckman Research Institute and Medical Center, Duarte, CA and 2National Cancer Institute, Frederick, MD.

Body: Cancer-secreted miRNAs are emerging mediators of cancer–host crosstalk. In this study, we set out to identify cancer-derived miRNAs that participate in cancer metastasis by adapting the niche cells. We show that miR-105, which is characteristically expressed and secreted by metastatic breast cancer cells, is a potent regulator of migration through targeting the tight junction protein ZO-1. In epithelial and endothelial monolayers, exosome-mediated transfer of cancer-secreted miR-105 efficiently destroys tight junctions and the integrity of these natural barriers against metastasis. Overexpression of miR-105 in non-metastatic cancer cells induces metastasis and vascular permeability in distant organs, whereas intervention of miR-105 in highly metastatic tumors alleviates this effect. By testing clinical specimens from breast cancer patients, we further show that miR-105 can be detected in the circulation at the pre-metastatic stage, and its levels in the blood and tumor are associated with ZO-1 expression and metastatic progression in early-stage breast cancer. Our results demonstrate for the first time the dual roles of miR-105 in inducing the migratory potential of cancer cells as well as destroying the epithelial and endothelial barriers in the cancer niche by targeting the cellular tight junctions. In breast cancer patients, increased levels of miR-105 in the circulation can be detected at the pre-metastatic stage and predict the occurrence of metastasis. Anti-miR-105 treatment suppresses metastasis and abolishes the systemic effect of tumor-derived miR-105 on niche adaptation. Therefore, these pre-clinical observations strongly suggest clinical applications of miR-105 as a predictive or early-diagnostic blood-borne marker as well as a therapeutic target for breast cancer metastasis.
**Title:** High mammographic density is associated with deposition of organised fibrillar collagen and increased stiffness in periductal breast stroma

Ashu Gandhi¹, Cliona C Kirwan², James C McConnell³, Oliver V O'Connell⁴, Michael J Sherratt³ and Charles H Streuli⁴.

¹Manchester Academic Health Sciences Centre, University Hospital of South Manchester, University of Manchester, Manchester, Greater Manchester, United Kingdom; ²Institute of Cancer Sciences, University Hospital of South Manchester, University of Manchester, Manchester, Greater Manchester, United Kingdom; ³Centre for Tissue Injury & Repair, Faculty of Medical and Health Sciences, University of Manchester, Manchester, Greater Manchester, United Kingdom and ⁴University of Manchester, Faculty of Life Sciences, Manchester, Greater Manchester, United Kingdom.

**Body: Introduction**
High mammographic density (MD) in women is strongly associated with breast cancer risk. However the structural and compositional differences between dense and non-dense breast tissues are not well defined. We determined the relationship between MD, collagen deposition and fibril alignment, and tissue micro-stiffness, in similarly aged individuals without adjacent cancer.

**Methods**
Fresh tissue samples were collected from post-menopausal women undergoing breast screening. Collagen deposition and fibril organisation were analysed using light microscopy of wax-sections stained with H&E, Trichrome or Picrosirius Red (with polarising light), and quantified using ImageJ. Local tissue stiffness was measured using atomic force microscopy (AFM) of hydrated tissue. For AFM, 3 x 25 µm² areas of each sample were indented at 400 equally spaced points with a 1 µm diameter spherical probe at a loading rate of 1 Hz (Reduced Modulus; YM).

**Results**
Volumetric MD (Volpara™) was determined in 22 women (54-66y) undergoing risk-reducing surgery or mastectomy. Localised regions of elevated density, determined from digital mammograms, were isolated from patients of low and high overall MD, using a new collaborative workflow linking radiologist, surgeon, pathologist, and tissue biobank.

All elevated-density regions contained considerable amounts of stromal connective tissue. However, there were significant differences in these regions from women with low vs high overall MD. Picrosirius Red staining of the localised areas of density revealed that the percentage organised fibrillar collagen content, particularly in the periductal breast stroma, strongly correlated with overall MD.

AFM showed that the localised micro-stiffness of dense areas increased significantly in the breast stroma of patients with high overall MD (Volpara score > 15) compared with those of low overall MD (Volpara score < 5).

**Conclusions**
High MD is a significant risk factor for breast cancer, yet its molecular determinants in the normal, non-cancerous breast are poorly defined. We have shown that high MD is associated with more organised fibrillar collagen, leading to increased stiffness of the periductal breast stroma. Women with low and high MD all have regions with localised density, which contain both stromal connective tissue and epithelial ducts/lobules. However, our results show that these localised areas have differences in collagen organisation and tissue micro-mechanics. We now hypothesise that in the connective tissue of women with high MD, altered synthesis, deposition and turnover of stromal proteins alters the local biomechanical properties within the breast, providing a stiffer microenvironment, and contributing to cancer onset.
Title: Hierarchy of breast cancer associated fibroblasts communicate with cancer cells via microRNAs to drive breast cancer progression

Sanket H Shah¹, Phil Miller¹, Zheng Ao¹, Emilio Issa¹, Katherine Drews Elger¹, Ram Datar¹, Marc Lippman¹ and Dorrya El-Ashry¹. ¹University of Miami, Miami, FL.

Body: Background: Increasing evidence has demonstrated that stromal cells play a pivotal role to promote breast cancer progression and metastasis. Breast cancer stroma is comprised mainly of Cancer Associated Fibroblasts (CAFs). Upon interaction with tumor cells, CAFs promote tumor progression by providing paracrine oncogenic signals mediated by activation of various pathways including developmental pathways, integrin signaling, and the MAPK pathway in tumor cells. CAFs have also been shown to promote the survival of CTCs and help them in metastasis at distant sites. Using breast cancer patient tumor datasets, we have previously identified a microRNA signature reflective of hyperactive MAPK signaling and that is significantly associated with reduced recurrence-free and overall survival. We have established 3 primary breast CAF lines, one from a Luminal A breast cancer, one from an ER-/Her2 amplified cancer, and one from a triple negative cancer, along with several primary tumor-derived dissociated tumor (DT) culture models that are tumorigenic in vivo and vary in metastatic ability. The CAFs express several hMAPK-microRNAs preferentially compared to the DTs. In addition to the paracrine interaction of stromal and tumor cells mediated by chemokines or hormones, miRNA cross talk between stromal and tumor cells can also occur.

Results: To further investigate the connection between our miRNA signature and stroma, we analyzed the TCGA and METABRIC breast cancer datasets and found that the hMAPK-miRNA identifies tumors that with significiant stromal cell infiltrate. To investigate the role of specific expression of hMAPK-miRNAs in the CAFs, CM was isolated from CAFs from “aggressive” tumors, from the “indolent” tumor, and from normal human mammary fibroblasts (HMFs) and analyzed for exosome and microRNA secretion. CAFs from the aggressive tumors secrete more exosomes and more hMAPK-microRNAs into the CM than do HMFs or CAFs from the indolent tumor. Importantly, conditioned media (CM) from the “aggressive” CAFs activate MAPK and repress ER protein, mRNA and ER 3’UTR-reporter activity in ER+ MCF-7 breast cancer cells, while HMFs and “indolent” CAFs did not. Exosomes from the “aggressive” CAFs were responsible for the ER repression. To determine if the secreted miRNA differences exhibited by the CAFs could be seen in patients, serum from breast cancer patients with metastatic breast cancer and patients without metastases was analyzed for microRNA expression. Differentially expressed circulating hMAPK-miRNAs were identified in serum from metastatic breast cancer patients compared with patients without metastases. Further analyses of the serum for CTCs and CAFs show that serum samples from metastatic patients had a significantly higher number of CTCs with CAFs compared to serum from patients without metastases.

Conclusions: Collectively these data suggest that different CAF populations have distinct abilities to influence the phenotype and behavior of associated cancer cells and that CAF secreted hMAPK-miRNAs may play important roles in breast cancer progression. They further suggest that these CAF secreted miRNAs can be found in patient serum along with circulating CAFs.
Title: Obesity-associated systemic interleukin-6 promotes pre-adipocyte aromatase expression via increased breast cancer cell prostaglandin E2 production

Laura W Bowers¹, Andrew J Brenner², Stephen D Hursting³, Rajeshwar R Tekmal² and Linda A deGraffenried¹. ¹University of Texas, Austin, TX; ²University of Texas Health Science Center, San Antonio, TX and ³University of North Carolina, Chapel Hill, NC.

Body: Obesity is associated with a worse breast cancer prognosis, particularly in estrogen receptor alpha (ERα) positive, postmenopausal patients. Resistance to aromatase inhibitor treatment may be one mechanism mediating this effect, as obese patients have a lower response rate to this class of drugs and higher breast tissue aromatase expression. We hypothesized that obesity-associated circulating factors promote breast cancer cell cyclooxygenase-2 (COX-2) expression and prostaglandin E2 (PGE2) production, resulting in an elevation in pre-adipocyte aromatase expression that enhances breast cancer cell estrogen receptor activity and proliferation.

Methods: We utilized an in vitro model of the obese patient’s tumor microenvironment in which cultured MCF-7 breast cancer cells and pre-adipocytes were exposed to pooled serum from obese (OB; BMI ≥30.0 kg/m²) or normal weight (N; BMI 18.5-24.9 kg/m²) postmenopausal women.

Results: Exposure to OB versus N patient sera significantly increased MCF-7 cell COX-2 expression and PGE2 production. This was coupled with 89% greater pre-adipocyte aromatase expression following culture in conditioned media (CM) from MCF-7 cells exposed to OB versus N patient sera, a difference nullified by treatment of the MCF-7 cells with the COX-2 inhibitor celecoxib during CM generation. Previous analysis of the sera revealed significantly higher interleukin 6 (IL-6) concentrations in the OB versus N patient samples. Depletion of IL-6 from the sera resulted in neutralization of the difference between OB and N CM-stimulated aromatase expression. N CM generated with the addition of IL-6 treatment at the time of N patient sera exposure also induced pre-adipocyte aromatase expression that was statistically equivalent to the OB CM condition. Finally, CM from pre-adipocyte/MCF-7 cell co-cultures exposed to OB patient sera stimulated greater MCF-7 and T47D breast cancer cell ERα activity and proliferation in comparison to N.

Conclusions: This study indicates that obesity-associated systemic IL-6 indirectly enhances pre-adipocyte aromatase expression via increased breast cancer cell PGE2 production, thereby promoting greater cancer cell ERα activity and proliferation. Investigation regarding the efficacy of a COX-2 inhibitor/aromatase inhibitor combination therapy in the obese postmenopausal patient population is warranted.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P4-04-17  
**Average Grade:** 4.40

**Title:** Tumor-infiltrating neutrophils in breast cancer subtypes: A retrospective cohort study

Enrique Soto-Perez-de-Celis¹, Mariana A Tenorio-Serralta¹, Yanin Chavarri-Guerra¹, Eucario Leon-Rodriguez¹ and Armando Gamboa-Domínguez¹. ¹Instituto Nacional de Ciencias Médicas y Nutrición "Salvador Zubirán", Mexico City, DF, Mexico.

**Body:**

**Background:** Tumor infiltrating neutrophils (TINs) are related to aggressiveness and poor prognosis in several human cancers. Despite evidence pointing towards the pivotal role of inflammatory cells in the initiation, progression and response to treatment of breast cancer (BC), the relevance of TINs in BC has not been systematically investigated. We sought to determine if TINs were present in BC and to compare their presence among different subtypes. We hypothesized that the presence of TINs would correlate with more aggressive histologic subtypes, particularly triple negative (TN) tumors.

**Methods:** This retrospective cohort study analyzed patients with BC treated at our institution from 2006 to 2012. Women with treatment-naïve stage I-III BC and with available medical records were included. Those with metastatic disease and/or lacking material for pathologic review were excluded from analysis. Tumors were divided into three subtypes: luminal type (LT) (hormone-receptor[HR]-positive, HER2-negative); HER2-positive and TN. Clinical and pathological characteristics were recorded. The primary outcome was the amount of TINs, defined as neutrophils in direct contact with tumor cells, within each tumor. Hematoxylin & eosin stained sections were examined by a dedicated breast pathologist blinded to tumor subtype, and the number of TINs per 10 high power fields (HPF, 40x) was recorded. To evaluate the capacity of TIN measurements in predicting HER2+ or TN tumors and to identify an optimal cut-off value for TIN positivity, ROC curve analysis was performed. Fisher’s exact test was used to test for independence between qualitative variables, and logistic regression models were used for quantitative variables and multivariate analysis.

**Results:** 133 patients were assessed for inclusion. 28 were excluded because of missing material, incorrect staging or erroneous pathological diagnosis and 105 were analyzed. 72 tumors (69%) were classified as LT, 15 (14%) as HER2+ and 18 (17%) as TN. The mean TIN count was 1.82 x 10 HPF (0-28). ROC analysis determined a cut-off value for positivity of >1 TIN x 10 HPF (AUC 0.85; 95% CI 0.76-0.93, p<0.001). Tumors with >1 TIN x 10 HPF were considered TIN-positive. 27% of all tumors were TIN+ (n=28). 16 of 18 TN (88%), 8 of 15 HER2+ (53%) and 4 of 68 LT tumors (5%) were TIN+ (p<0.001). 79% of HR-ve tumors (19 of 24) were TIN+, in contrast with 11% of HR+ve (9 of 81) (p<0.001). HER2 expression (p=0.023); tumor grade (p<0.001) and Ki67 (p<0.001) were also associated with TIN positivity. Age, menopausal status and T and N stage were not significant for the presence of TINs. On multivariate analysis, only Ki67 (OR 1.05, 95% CI 1.01-1.1, p=0.008) and HR negativity (OR 10.6; 95% CI 2.5-45.8, p=0.002) were associated with a higher likelihood of TIN positivity.

**Conclusions:** Although TINs are uncommon in BC, they are present in most TN and in half of HER2+ tumors. In fact, the absence of HR expression was the strongest predictor for TIN positivity. These results raise the question as to whether TINs, as part of the tumor microenvironment, have a role in the aggressiveness and progression of TN tumors and warrant further investigation of TINs in this BC subtype, particularly in relation with response to treatment and prognosis.
Title: Recruitment of tumor associated macrophages in patients with breast cancer is not host dependent but tumor grade dependent

Ann Smeets¹, Giuseppe Floris¹, Kathleen Lambein², Sigrid Hatse¹, Annouschka Laenen³, Ines Nevelsteen¹, Hans Wildiers¹ and Marie-Rose Christiaens¹. ¹University Hospitals, Leuven, Belgium; ²Ghent University Hospital, Ghent, Belgium and ³KU Leuven, Leuven, Belgium.

Body: Background:
It has been shown that tumor related inflammation plays a crucial role in breast cancer progression. However, it remains unclear whether the density of immune cells in the tumor micro-environment is determined by tumour or by host characteristics. To answer this question, we measured tumor associated macrophage (TAM) density in 52 patients with a synchronous bilateral breast cancer with different lymph node status on both sites.

Materials and methods:
TAMs were identified by CD68 and CD163 antibodies. Tumour stromal macrophages were counted using a semi-quantitative method.

<table>
<thead>
<tr>
<th>Grade</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>no TSM</td>
</tr>
<tr>
<td>I</td>
<td>scanty TSM</td>
</tr>
<tr>
<td>II</td>
<td>small foci of TSM</td>
</tr>
<tr>
<td>III</td>
<td>large foci of TSM</td>
</tr>
<tr>
<td>IV</td>
<td>diffuse dense infiltrate of TSM</td>
</tr>
</tbody>
</table>

TSM = tumor stromal macrophages

Macrophage count was scored by two independent pathologists who were blinded from the clinicopathological data during the assessment.

To evaluate the relative impact on TAM density exerted by the tumor itself and by the host, we used the likelihood ratio tests for the variance components related to host and tumor in determination of CD68 and CD163 levels.

Results:
In 60% of the tumors, the scores for CD68 and CD163 of both pathologists were identical resulting in a moderate concordance according to the weighted kappa coefficient test (0.534; CI 0.418-0.649, p<0.0001).

The TAM density strongly correlated with the number of mitosis and secondly with tumor grade. The results for CD68 and CD163 were similar. The association of clinicopathological variables with CD63 are shown.

Association of clinicopathological variables with CD163

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>Score (No%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0</td>
<td>I</td>
</tr>
<tr>
<td>Histological subtype</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDC</td>
<td>148</td>
<td>3(2%)</td>
<td>64 (43%)</td>
</tr>
<tr>
<td>ILC</td>
<td>31</td>
<td>0 (0%)</td>
<td>21 (68%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>8</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Mitosis
For both CD 68 and CD163, the tumor introduces important clustering or correlation, whereas the host does not introduce any additional correlation. TAM recruitment is therefore primarily determined by tumor-related characteristics.
**Title:** Tumor infiltrating lymphocytes in inflammatory breast cancer

Cecile Colpaert¹, Melike Marsan², Peter Vermeulen¹, Luc Dirix², Steven Van Laere² and Inflammatory Breast Cancer International Consortium³. ¹Oncology Centre, GZA Hospitals, Iridium Cancer Net, Antwerp, Belgium; ²Translational Cancer Research Unit, Oncology Centre, GZA Hospitals, Iridium Cancer Net, Antwerp, Belgium and ³Inflammatory Breast Cancer International Consortium.

**Body:** Inflammatory breast cancer (IBC) is a rare and aggressive form of breast cancer with poor prognosis. Multimodal treatment including neo-adjuvant chemotherapy is the treatment of choice for IBC patients, with pathological complete response (pCR) being one of the most important prognostic factors.

Tumor infiltrating lymphocytes (TILs) are an independent predictor of response to neo-adjuvant chemotherapy in breast cancer (Denkert C. et al., 2010). In a search for multigene predictors of pCR in IBC using DNA micro-arrays, a 107 gene signature was found that distinguished between responders and non-responders. This signature was enriched for immunity-related genes showing that in IBC, as in non-IBC, response to neo-adjuvant chemotherapy is associated with immunity related processes (Bertucci F. et al., 2014).

Standard H&E stained sections of formalin-fixed paraffin-embedded pre-treatment tumor tissue of 77 IBC patients were used to evaluate the presence of TILs according to the Denkert method: mononuclear cell infiltrates were considered to be intratumoral TILs when making direct contact with the tumor cells, while stromal TILs (strTILs) are mononuclear cells being present in the tumor stroma without making direct contact with tumor cells.

Intratumoral TILs were observed in 10.4% (8/77) of the patients. Stromal TILs (strTILs) were present in all tumors: in 35% (27/77) strTILs occupied less than 5% of the tumor stroma, in 62% (48/77) strTILs occupied between 5 and 50% of the tumor stroma and 2.6% (2/77) were "lymphocyte predominant breast cancers (LPBC)" with strTILs occupying more than 50% of the tumor stroma. One LPBC was HER2 positive, the other one was triple negative. There was a significant association of strTILs with response to neo-adjuvant chemotherapy: pCR was obtained in 13% of the patients with strTILs <5%, in 20% of the patients with strTILs between 5 and 50% and in both patients with LPBC (Chi² p=0.018).

Tertiary lymphoid structures - lymphoid follicles with germinal centres, known to be associated with better clinical outcomes (Gu-Trantien C. et al., 2013) - were detected in 2.6% of the patients (2/77); both patients had a disease free survival of more than 3 years.

This study shows that IBC tumors do not contain more TILs than non-IBC tumors and that, as in non-IBC, there is a positive association of stromal TILs with pCR in IBC. Further validation of these results and further study of lymphocyte subsets in IBC is warranted.
Title: Host response to microbes found in the tumor microenvironment and healthy adjacent tissue

Andrew W Chung¹, Marian Navarrete¹, Peter A Sieling¹ and Delphine J Lee¹. ¹Dirks/Dougherty Laboratory for Cancer Research, John Wayne Cancer Institute at Providence Saint John's Health Center, Santa Monica, CA.

Body: Next generation sequencing methods have defined the bacterial microbiome in human breast milk and breast tissue, but the role of bacteria or their components in breast cancer has not been well defined. There is growing evidence in humans and animals that alterations of the gut microbiome may contribute to host susceptibility to cancer. However, the presence of bacteria at the site of the tumor microenvironment may also contribute to carcinogenesis (e.g. by promoting chronic inflammation) or immune surveillance (e.g. by stimulating antitumor pathways). Previously, we found that bacterial load in breast cancer tissue is inversely correlated to the progression of disease and that antimicrobial gene expression was comparatively higher in breast tissue from healthy subjects. Our investigation of the microbiome of breast tissue showed that overall microbiome signatures in breast cancer patients are similar when comparing their healthy adjacent tissue vs. the tumor tissue. However, our published preliminary studies did identify some differences in the abundance of specific bacteria between the two tissues. Here we investigated how those bacteria in healthy adjacent vs. tumor tissue may differentially stimulate cells found in breast tissue including breast ductal epithelium, adipocytes, and immune cells. Our preliminary data suggest that bacteria found in healthy adjacent vs. tumor tissue stimulate differential cytokine responses that may shape the local tumor microenvironment and beyond and may also contribute to local/regional immune surveillance.
Title: Epithelial and stromal components are equivalently affected in breast fibroepithelial progression. A gene expression analysis of phyllodes and fibroadenomas tumors

Angela F Logullo¹, Mabel Planila³, Elisa Napolitano², Fernando A Soares² and Dirce Carraro². ¹Universidade Federal de São Paulo, São Paulo, Brazil; ²A.C.Camargo Cancer Center, São Paulo, Brazil and ³Universidad de Concepción, Chile, Conception, Chile.

Body: Rationale: Phyllodes tumours (PTs) account for 0.5 to 1% of all breast lesions and, analogous to fibroadenomas (FA), are considered true biphasic neoplasms. However, unlike in FA, their stromal element is more cellular and may outgrow the epithelium, and PT may recur locally and can undergo malignant progression to sarcoma and metastasize. Recent evidence emphasize that epithelium and stroma interactions are critical for tumour development and progression, but molecular alterations in both, epithelial and stromal cells, in the arising of PT onset is not well characterized. Thus, in order to contribute for the identification of the molecular events involved in PT tumorigenesis, we performed gene expression analysis of both types of cells captured from PT and FA.

Methods: Epithelial (EpC) and stromal cells (StC) from 13 PT and 3 FAs were separately submitted to laser capture microdissection (LCM) by CapSure HS™ Arcturus®. Procedures were performed in 10 min to prevent RNA degradation. RNA was extracted using the Pico Pure RNA Isolation Kit™ Arcturus® and was twice amplified the first as described in Castro et al., 2008 with adaptation and the second using low input Quick-Amp Labeling kit* and hybridized to the microarray slide SurePrint G3 Hmm 60K*. Signals were captured by Scanner Agilent Bundle model B* and Agilent Feature Extraction (v 10.7.1) program* and data was analyzed by genespring GX12.1 software* (*Agilent Technologies, USA). A Fold Change ≥2,0 and p≤0,05 were considered significant.

RESULTS: Flag quality filter determined 8.067 coding probes. Differentially expressed genes (DEG) between PT and FA were assessed with data from EpC and StC captured cells separately. A total of 3386 DEG were identified in EpC, and 1910 were up regulated in PT. SC samples revealed 2.619 probes with significant difference among them 1.616 up regulated in PT. Hierarchical clustering based on the DEG from EpC and StC, separately, could not discriminate between TP and FA samples. When low grade PT (n=7) were compared to high grade PT (n=6) the universe of DEG was smaller: EpC derived samples revealed 30 probes with only 14 up regulated in high grade PTs, and StC samples revealed 6 up regulated genes within only 10 probes with DEG in PT. Conclusion: EpC are genetically altered as much as StC in breast fibroepitelial lesions carcinogenesis. Low grade and high grade PTs are similar in RNA and gene expression level. DEGs identified are potential diagnostic targets. Moreover, EpC and StC components seem to share main affected gene pathways, suggesting a common carcinogenesis process.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P4-04-24  
**Average Grade:** 5.80  

**Title:** Metastatic lymph node fibroblasts maintain similar profile of stromal biomarkers registered in primary breast carcinomas  

Fiorita GL Mundim¹, Fatima S Pasini², Suely Nonogaki³, Fernando A Soares³, M Mitzi Brentani²,³ and Angela F Logullo¹. ¹Escola Paulista de Medicina EPM-UNIFESP, São Paulo, SP, Brazil; ²Faculdade de Medicina da Universidade de São Paulo, São Paulo, SP, Brazil and ³Hospital A.C. Camargo, São Paulo, SP, Brazil.

**Body:** Background: Lymph node metastasis is the predominant dissemination route of breast carcinoma (BC) and remains as an important prognostic indicator of this disease. Several studies demonstrated a similar gene profiling or gene/biological markers assessed by immunohistochemistry in neoplastic epithelial cells at both sites. Recent evidence pointed that cancer associated fibroblasts (CAFs) play a critical role in breast cancer progression and migration. The evidence of CAFs in close association to epithelial tumor cells within lymph node metastasis raised the possibility of a common source of CAFs in these two lesions. However, little is known about the differentially expressed genes or proteins between fibroblasts of the primary tumor and its nodal metastasis. The contribution of CAFs in lymph node colonization is still unknown.

Objective: Our aim was to compare the profile of biomarkers between fibroblasts in lymph nodes and those found in the primaries. We compared differences in frequency of activated CAFs through the expression of α-SMA and S100A4 in 43 matched pairs of invasive breast carcinomas and respective macro-metastatic lymph nodes arrayed in TMAs. We also investigated a panel of immunohistochemical biomarkers associated to TGFβ1 (that induces α-SMA and myofibroblast differentiation in CAFs) including CXCR4, pAKT, pm-TOR and c-myc. In addition the frequency of all these markers were assessed in samples of matched disease free lymph nodes obtained from the same patients (which contain fibroblastic reticular cells, limited to the exterior capsule).

Results: CAFs characterization confirmed the positive expression of α-SMA (approximately 50%) and S100A4 (100%) on fibroblasts of both sites in contrast to uninvolved lymph nodes, which were uniformly negative. As expected, CAFs were ER, PR, Her-2, CD9 and p53 negative at both sites. As Ki67 labeling rate was always lower than 10% in fibroblasts they were considered negative. Comparison between fibroblasts from the stromal component of the primaries and respective lymph nodes indicated that the frequency of TGFβ1, CXCR4 and pAKT positivity was similar whereas a significant lower rate of c-myc and pm-TOR frequency was observed in the metastatic lymph nodal fibroblasts. All biological markers were negative in normal lymph nodes highlighting their association with activated fibroblasts. None of the markers showed association with presence/absence of lymph node metastasis or with other clinic-pathological parameters. Primary carcinomas lacked any potential associations among the evaluated biological markers in fibroblast cells, however, in the stromal tissue of lymph nodes, significant relationships between TGFβ1 and CXCR4, pAKT and c-myc, were observed.

Conclusion: Our results indicated the presence of activated fibroblasts expressing α-SMA and S100A4 in fibroblasts of breast cancer primaries as well in their matched metastatic lymph nodes. High concordance for the expression of the analyzed biomarkers in matched pairs of breast cancer primaries and metastatic lymph nodes suggested the maintenance of a similar supportive microenvironment in metastatic sites. Financial Support: FAPESP and CNPq.
Title: Naked mole rat hyaluronan synthase 2 displays similar effects as human hyaluronan synthase 2 and promotes tumor
growth in a mouse xenograft model

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Diego, CA.

Body: Accumulation of hyaluronan (HA) is correlated with poor prognosis in many human cancer including breast cancer and
pancreatic cancer. Hyaluronan synthase 2 (HAS2) is one of the three HA synthase genes (HAS1-3) found in mammals.
Up-regulation of the HAS2 gene has been observed in breast cancer patients; and HAS2 expression correlates with the incidence
of metastasis and lower rate of overall patient survival (Okuda et al 2012). Interestingly, a recent study suggested that the naked
mole rat (nmr) HAS2 enzyme may function differently than the HAS2 proteins from mice and humans, and its expression had a
tumor suppressor effect (Tian et al 2013). The nmrHAS2 protein sequence was distinct from other mammals in the cytoplasmic
loop where the active site resides, which led to the hypothesis that nmrHAS2 may contribute to the production of extremely high
molecular weight HA species. Furthermore, nmrHAS2 is required for the resistance of nmr skin fibroblasts to malignant
transformation. In this study, we examined whether the nmrHAS2 gene can also render human cells resistant to tumor
progression. We have previously observed that HA level in the pancreatic cancer model AsPC1 can be modulated by the
over-expression of the human HAS3 protein (Osgood et al 2014). To compare and contrast the effect of nmrHAS2 and huHAS2
on HA production and tumor growth, nmrHAS2 and huHAS2 AsPC1 models were engineered using a recombinant lentivirus
encoding the nmrHAS2 and huHAS2 genes respectively. Over-expression of nmrHAS2 and huHAS2 both significantly increased
the HA level in AsPC1 cells. Both nmrHAS2 and huHAS2 promoted the growth of AsPC1 in an intramuscular xenograft tumor
model. Ex vivo analysis of tumor xenografts showed that nmrHAS2 and huHAS2 AsPC1 tumors contain elevated levels of HA,
and the size range of the HA in the nmrHAS2 tumors is similar to that in the huHAS2 AsPC1 tumors. Therefore, nmrHAS2
functions similarly as huHAS2 gene and promotes tumor growth in the human pancreatic tumor model AsPC1.

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Title: Down-regulation of p16INK4a inhibits miR-146b-5p and modulates IL-6 in breast stromal fibroblasts

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Body: Breast cancer is a major health problem that threatens millions of women’s lives each year worldwide. Cancer-associated fibroblasts (CAFs), which constitute the major component of the tumor stroma, have been reported to actively contribute to tumor cells proliferation and invasion. Recently, we have shown down-regulation of the tumor suppressor p16INK4a protein in breast cancer-associated fibroblasts. Moreover, p16INK4a deficiency led to the activation of the stromal fibroblasts, which express/secrete elevated levels of IL-6, a major player in breast carcinogenesis. We have shown here that p16INK4a negatively regulates the IL-6 expression and secretion in breast stromal fibroblasts. Furthermore, we have shown that IL-6 is playing a major role in mediating the paracrine pro-carcinogenic effect of p16-deficient fibroblasts. We have also shown that p16INK4a inhibits the IL-6 expression in a miRNA-146b-5p-dependent manner. Importantly, we present clear evidence that miR-146b-5p inhibition activates breast stromal fibroblast. Indeed, miR-146b-5p inhibition increased the migration/invasion abilities of breast stromal fibroblasts, and the paracrine effect of these cells on the migration/invasion of breast cancer cells. Furthermore, miR-146b-5p-deficient stromal fibroblasts triggered epithelial to mesenchymal transition in breast cancer cells in a paracrine manner. In addition, we have shown that miR-146b-5p is down-regulated in CAFs as compared to their adjacent counterpart fibroblasts. These results indicate that p16INK4a negatively regulates IL-6 through the activation of miR-146b-5p, which plays a major role in repressing breast stromal fibroblasts and inhibiting their pro-carcinogenic effects. This indicates that miR-146b-5p has cell-non-autonomous tumor suppressor function. Therefore, this miRNA could be of great therapeutic value.
Title: Chemokines released by breast cancer-associated fibroblasts induce epithelial to mesenchymal transition in MCF7 breast cancer cells

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Body: Purpose: The tumour microenvironment plays a critical role in tumour progression, with cancer-associated fibroblasts (CAFs) in particular being involved in this process (1). Treating breast cancer cells with conditioned media from CAFs has been shown to induce epithelial-mesenchymal transition (EMT) of the breast cancer cells compared to conditioned media from normal breast fibroblasts (NBFs) (2). This study aims to identify factors released by CAFs which induce EMT in breast cancer cells.

Methodology: Primary cultures of six pairs of CAFs and NBFs were established from breast cancer patient samples. Serum-free conditioned media from these cells were collected for chemokine array analysis. MCF7 breast cancer cells were treated with recombinant chemokines and their corresponding receptor antagonists. Vimentin levels, as determined by immunoblot, and cell invasiveness, as measured by transwell assay, were used as indicators of EMT.

Results: Chemokine array showed that CAFs secreted more CXCL1, CXCL8, CXCL12 and CCL2 compared to matched NBFs. Recombinant CXCL8, CXCL12 and CCL2, but not CXCL1, were able to induce vimentin and to increase invasiveness in MCF7 cells. Antagonists for receptors of CXCL8, CXCL12 and CCL2 counteracted the effect of EMT induction on MCF7 cells by recombinant chemokines.

Conclusion: CXCL8, CXCL12 and CCL2, which are secreted at higher levels by CAFs compared to NBFs, induced EMT and increased invasiveness in MCF7 cells. These results suggest that therapeutic targeting of these chemokines or their receptors may be inhibitory to metastasis in some breast cancers.

References:
Title: Genetic analysis of the role of Brca1 in suppression of basal-like breast cancer

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Body: BRCA1 mutation carriers are predisposed to developing basal-like breast cancers with high metastasis and poor prognosis. However, the mechanism underlying the function of BRCA1 in suppressing basal-like breast cancer remains unclear. We previously reported that deletion of p18Ink4c (p18), an inhibitor of CDK4 and CDK6, functionally inactivates the RB pathway, stimulates mammary luminal stem cell proliferation, and leads to spontaneous luminal tumor development whereas germline mutation of Brca1 alters the fate of luminal cells and causes luminal-to-basal mammary tumor transformation. We report here that disruption of Brca1 by both germline and epithelium-specific mutation of Brca1 in p18 deficient mice activates epithelial-to-mesenchymal transition (EMT) and induces basal-like breast tumors. Introduction of BRCA1 suppresses expression of EMT-inducing transcription factors (EMT-TFs) and inhibits EMT in mammary tumor cells. Knockdown of BRCA1 in human luminal cancer cells activates EMT and increases tumor formation potential. Consistently, EMT features are inversely correlated with BRCA1 in human breast cancers. These findings provide genetic evidence suggesting that BRCA1 suppresses EMT and inhibits basal-like tumor development.
Title: Overexpression of the chemokine receptor CCR2 in the breast epithelium is associated with progression of DCIS

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Body: Ductal carcinoma in situ (DCIS) is the most common type of non-invasive breast cancer diagnosed in women and an immediate precursor to invasive ductal carcinoma (IDC). It is largely unclear why some cases of DCIS progress to IDC, while others remain non-invasive. Current cyto- and histopathological approaches do not accurately predict disease progression, resulting in patients being under-treated or over-treated for DCIS. Our long-term goals are to identify key factors that lead to IDC that will enable the development of a molecular based approach to predict the risk of IDC, and a more tailored approach to treat DCIS. The CCL2/CCR2 chemokine pathway is best known for regulating macrophage recruitment to late stage breast tumors. In recent studies, we found that CCR2 and its binding ligand CCL2 were overexpressed in breast ductal carcinomas, correlating with tumor grade and poor patient prognosis. To further investigate the significance of CCR2 and CCL2, we performed flow cytometry analysis of breast epithelial cell lines, and found that increased CCR2 overexpression but not CCL2, was associated with invasive potential of the cell lines. CCL2 was found to be increased in stromal cells, particularly in cancer associated fibroblasts. Using a mammary intra-ductal injection model, we further examined for expression patterns of CCR2 and CCL2 in DCIS lesions derived from DCIS.com and Sum225 cells. Increased CCR2 was detected in DCIS.com lesions, which progressed to IDC within 10 weeks of injection, while CCR2 expression was found to be lowly expressed in non-invasive Sum225 lesions. CCL2 was detected in the stroma of both DCIS.com and Sum225 lesions. Overexpression of CCR2 in cultured non-invasive 67NR mammary carcinoma cells enhanced cell survival and increased wound closure. CCL2 treatment further enhanced cell survival and wound closure of CCR2 overexpressing 67NR cells. Knockdown of CCR2 in DCIS.com cells in 3D Matrigel: collagen cultures inhibited cellular invasion into the matrix. These studies indicate that CCR2 overexpression in ductal carcinoma cells, enhances intracellular signaling, and contributes to development of IDC. These data suggest that targeting the CCR2 pathway may be promising strategy for prevention or treatment of IDC.
Cytoplasmic PELP1 promotes breast cancer initiation via NF-κB signaling

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Progress in breast cancer prevention is limited by an inability to reliably predict which women will develop breast cancer and which high-risk women will respond to chemoprevention therapies. Thus, there is a critical need to determine the molecular mechanisms that promote breast cancer initiation in order to 1) identify biological markers of disease susceptibility and 2) to develop novel targeted chemoprevention agents. A promising target for preventing breast cancer initiation is proline, glutamic acid, and leucine rich protein 1 (PELP1). PELP1 was first identified as an estrogen receptor (ER) co-activator in ER+ breast cancer cell lines. Subsequent studies found that PELP1 functions in ER+ and ER- breast cancer cell lines and is overexpressed in 80% of invasive breast cancers. Interestingly, while PELP1 is primarily localized to the nucleus in the normal breast epithelial cells, in about 40% of invasive breast tumors a significant amount of PELP1 is localized in the cytoplasm. Interestingly, in a mammary gland specific transgenic mouse model, PELP1-cyto expression induced mammary gland hyperplasia. We therefore analyzed breast needle aspirates from asymptomatic high-risk women, and found cytoplasmic PELP1 in 4/11 (36%) samples. These findings suggest that altered localization of PELP1 may be an early event in breast cancer initiation.

The objective of this research project is to identify the molecular mechanisms associated with PELP1-induced breast cancer initiation. We performed global gene expression analyses of our in vitro human mammary epithelial cell (HMEC) models to identify potential downstream pathways regulated by PELP1-cyto. Interestingly, genes regulated by PELP1-cyto differ greatly from those regulated by nuclear PELP1. Our results showed that PELP1-cyto induces expression of cell survival genes, and inflammatory cytokines and chemokines, and activates the NF-κB signaling pathway. Thus, we hypothesize that cytoplasmic PELP1 induces activation of inflammatory cytokines and chemokines via NF-κB, which promotes tumor initiation and a pro-tumorigenic microenvironment.

In support of our hypothesis we have found that PELP1-cyto expression promotes phosphorylation of the non-canonical IKK, TBK1, and the NF-κB subunit p65. Additionally, we found that conditioned medium from PELP1-cyto cells, induced expression of IL-1β, TNFα, and IL-8 in THP-1 cells that had been differentiated into macrophages, suggesting that PELP1-cyto HMECs release cytokines that promote the activation of macrophages. The goal of future studies is to validate these findings in vitro and in vivo and identify the cytokines and/or chemokines induced by PELP1-cyto that promote breast cancer initiation and activation of tumor associated macrophages.
Title: MECP2 is a frequently amplified oncogene and potential therapeutic target in TNBC

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Body: Background: To identify new oncogenes that drive cancer development, we conducted an unbiased genome-scale screen for genes that can substitute for activated RAS in oncogenic transformation. We focused attention on one of the new potential oncogenes identified in this screen, Methyl CpG Binding Protein 2 (MECP2), which has no previously described role in malignancy and is amplified across ~20% of all human cancers, and ~30% of Triple-Negative Breast Cancer (TNBC). MECP2 is an X-linked gene known to bind methylated cytosines, and can act as a transcriptional repressor in this context. Recent studies show that it also acts as a transcriptional activator, likely through binding to another epigenetic modification of DNA, 5-hydroxymethylcytosine (5hmC).

Results: MECP2 is as potent as activated RAS in conferring anchorage independent growth upon primary human mammary epithelial cells (hMECs) previously transduced with the SV40 early region and hTERT (N−RAS hMECs). MECP2 partially rescues the growth inhibition of RAS-addicted human cancer cell lines after the shRNA-mediated suppression of RAS. MECP2 expresses two spliced isoforms; experiments showed the short isoform activates the MAPK pathway, while the long isoform activates the PI3K pathway. Neither isoform alone can cause growth of N−RAS hMECs as a xenograft in nude mice; together, they can. The transformation and growth factor pathway induction activities of MECP2 absolutely require its DNA-binding activity. A number of TNBC cell lines have amplified, overexpressed MECP2, and of the first 13 TNBC patient-derived xenografts examined, 4 have MECP2 overexpression. Several TNBC cell lines that have amplified, overexpressed MECP2 show significant growth inhibition after shRNA-mediated downregulation of MECP2 (MECP2 addiction), while a breast cancer cell line without MECP2 overexpression showed no such inhibition. N−RAS hMECs transformed with MECP2 are an order of magnitude more sensitive to either of the DNA methylation inhibitors 5-azacytidine or decitabine than isogenic cells transformed by activated RAS, or isogenic cells without an additional transforming gene. Further, we find that combined treatment with the DNA methylation inhibitor 5-azacytidine and the HDAC inhibitor Trichostatin A is synergistic in our hMEC experimental system.

Conclusion: MECP2 is a commonly amplified and overexpressed oncogene whose two splicing isoforms together recapitulate the major oncogenic functions of activated RAS. Because MECP2 requires DNA binding to methylated or hydroxymethylated cytosines for its tumor-promoting activities, DNA methylation inhibition with FDA-approved drugs may be therapeutic for tumors overexpressing MECP2.
Title: A novel TP53-dependent role for TLR4 in driving breast cancer

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Body: Breast cancer is a leading cause of cancer-related death. Toll-like receptor (TLR)-4 is an important mediator of cytokine secretion that is expressed in breast cancer cells, and is frequently mutated in a subset of breast tumors associated with high mutation load and poor patient survival. We show here that TLR4 plays an important role in driving breast cancer growth in a TP53 context-dependent manner. Using siRNA mediated knockdown, pharmacological inhibition and hyperactivation of TLR4 we demonstrate that TLR4 inhibits growth in breast cancer cells with wildtype TP53 by inducing G0/G1 arrest and decreasing mitotic entry. Conversely, in breast cancer cells with mutant TP53, TLR4 acts as an oncogene and drives cell growth by inducing mitosis. Furthermore, we identify the underlying mechanism for this dual TP53-dependent effect of TLR4 on breast cancer cell growth: differential interferon gamma (IFN-γ) secretion by tumor cells into their microenvironment. Hyperactivated TLR4 signaling in TP53 wildtype breast cancer cells results in increased IFN-γ secretion, identified by an unbiased cytokine array and validated by ELISA, thus inhibiting growth in an autocrine/paracrine fashion. Moreover, secreted IFN-γ is both necessary and sufficient for TLR4-induced growth inhibition in TP53 wildtype breast cancer cells. Finally, the dual TP53-dependent effect of TLR4 extrapolates to several other cancer types in silico, attaching potentially global significance to these results. Taken together, the data presented in this paper strongly suggest a novel role for TLR4 as a suppressor of cell growth in TP53 wildtype tumors, and identify differential IFN-γ secretion as the underlying mechanism. The results of this study are also translationally relevant, delineating the TP53 mutant breast cancer subset as a group that can benefit from pharmacological TLR4 inhibition. Most importantly, these results indicate a need for studying the effect of drivers of cancer growth pleiotropically rather than as isolated events.
Title: Elevated frequency of a deleterious deep intronic BRCA2 splice variant in non-Caucasian Americans

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Body: Introduction: In 2012, Anczukow et al (Clin Cancer Res 2012;18:4903-9) described a deep intronic BRCA2 variant between exons 12 and 13 that was discovered because a cell line from a patient with hereditary breast and ovarian cancer (HBOC) produced an aberrant mRNA. The variant (c.6937 +594 T>G; rs191253965), which further improved splice site sequence to consensus, was also identified in 8 additional families. Segregation analysis of 13 affected relatives from 6 families revealed that all affected family members had the c.6937 + 594 T>G variant, providing strong evidence that it is deleterious. All resided in France, but ethnicity was not specified. This variant was also seen in 5 individuals in the 1000 genomes project. Since this is a deep intronic variant, standard BRCA2 sequencing tests that determine only a limited number of bases into each intron would not detect it. For these reasons, we included a bait tile for the intron harboring this variant in the design of our hybrid capture-based next generation sequencing (NGS) test for comprehensive BRCA1 and BRCA2 analysis. Here we investigate the frequency of this variant in samples submitted for comprehensive BRCA1 and BRCA2 testing, and assessed carrier frequency in samples submitted for non-BRCA–related genetic testing.

Methods: We reviewed the de-identified results of the first 2300 clinical samples submitted to our reference laboratory for comprehensive BRCA1 and BRCA2 testing to assess the frequency of the c.6937 +594 T>G variant.

Results: This deep intronic variant was observed in 12 of the initial 2300 patient samples. All 12 individuals were affected with HBOC and were Hispanic. To estimate the population frequencies of this variant in various ethnic groups, we anonymized samples submitted for Cystic Fibrosis Carrier Detection and subjected them to a single-site assay for this variant. In all, 344 patients self-identified as Caucasian, 355 as Hispanic, 140 as African American, and 73 as Asian. The frequency of c.6937 +594 T>G heterozygotes was 0.30% (1 individual) in Caucasians, 1.1% (4) in Hispanics, 0.8% (1) in African Americans, and 1.4% (1) in Asians.

Conclusions: In an initial series of comprehensive BRCA analyses, we discovered that a deep intronic variant known to cause aberrant splicing and to segregate with HBOC is present at elevated frequencies in non-Caucasian Americans. We have already observed this variant in 12 Hispanic HBOC patients. Physicians could consider testing for this variant in women of non-Caucasian ancestry with HBOC who have had negative results on BRCA assays that do not test for c.6937 +594 T>G.
Title: Functional genomics of TP53 mutations and its impact in breast cancer progression

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Body: Somatic TP53 mutations are prevalent in basal-like breast cancer (BLBC) tumors. Patients with BLBC tumors have fewer treatment options and respond poorly to current therapies. The majority of TP53 point mutations occurs in the DNA binding domain and can be categorized as either DNA contact or structural mutations. TP53 mutation results in a dominant negative phenotype with neomorphic activity. We predicted that different p53 mutations may lead to different phenotypic characteristics. To investigate this, we generated MCF10A stable transduced cell lines over-expressing the ten most frequent TP53 point mutations associated with breast cancer located in the DNA binding domain of TP53. Ectopic expression of TP53 in these stable cells has been confirmed by qRT-PCR and immunoblot. To assess the impact of mutation on carcinogenesis, we developed a series of high-throughput quantitative assays that measure several hallmarks of cancer, including proliferation, escape from apoptosis, epithelial to mesenchymal transition (EMT), cell migration and invasion, anoikis and morphology in 3D. We observed that one DNA contact mutation with the substitution of a positively charged amino acid with hydrophobic side chains such as R248W, and two structural mutants Y234C and H179R are resistant to apoptosis in presence of doxorubicin, are the most invasive displaying a mesenchymal phenotype characterized by the presence of disrupted B-catenin and E-cadherin staining, with reported worst clinical outcome, suggesting that these are the most aggressive phenotypes. Interestingly, the DNA contact mutants (R248Q, R273H, R248W, and R273C) had a growth advantage in absence of growth factors while structural mutants (R175H, H179R, Y220C, Y234C and Y163C) were more resistant to apoptosis after the cells were challenged with doxorubicin. G245S is comparable to the MCF10Ap53wt and is less proliferative, sensitive to apoptosis, and neither migratory nor invasive. In comparison, R248W which is one of the most aggressive mutants, together with R273C, and H179R resist anoikis; but Y234C, requires matrix for attachment in order to be invasive. In conjunction, these results confirmed our hypothesis that different TP53 point mutants have distinct phenotypes and functional effects on hallmarks of cancer due to distinct underlying cellular programs.
Title: Cellular localization dictates the role for c-Abl in breast cancer

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Body: c-Abl is a ubiquitously expressed non-receptor tyrosine kinase that regulates numerous aspects of normal mammary epithelial cell (MEC) physiology. However, the exact role of c-Abl during mammary tumorigenesis remains controversial. Clinical trials utilizing the c-Abl antagonist, imatinib mesylate, for the treatment of metastatic breast cancer (BC) patients failed due to disease progression. Additionally, our previous studies overexpressing a constitutively active c-Abl mutant in aggressive murine triple negative breast cancer (TNBC) cells abrogated tumorigenesis when injected into syngeneic mice. Through data mining we found c-Abl expression to be down-regulated across BC subtypes and this correlated with a significantly worse relapse free survival rate for patients of triple negative and luminal A BC subtypes. Furthermore, through in silico analyses we determined that BC patients with high c-Abl expression responded to docetaxel treatment, whereas their low c-Abl expressing counterparts did not. Taken together these findings support a tumor suppressive role for c-Abl in BC and that its targeted activation could inhibit BC tumorigenesis as well as sensitize BC patients to docetaxel treatment. We show here that shRNA mediated depletion of c-Abl elicited docetaxel resistance in malignant MECs through three dimensional (3-D) culture and clonogenic assays on plastic. Furthermore, we show through data mining that c-Abl expression is down-regulated in response to docetaxel treatment in a cohort of BC patients. This finding was recapitulated in malignant MECs through which docetaxel treatment selected for cells that retained lower c-Abl expression highlighting the importance of c-Abl in this cell death pathway. Targeted activation of c-Abl utilizing a novel small molecule activator, DPH, presents a novel therapeutic strategy for sensitizing BC patients to docetaxel treatment. However, we show DPH-mediated c-Abl activation to inhibit the proliferation of luminal A MCF7 BC cells, but enhance proliferation of the TNBC MDA-MB-231-L2 cell line in vitro. The cellular localization of c-Abl is crucial for its tumor suppressive functions. In Philadelphia chromosome positive chronic myelogenous leukemia (CML) the constitutively active BCR-Abl gene fusion has a strict cytoplasmic localization, however when nuclear expression of BCR-Abl is enforced CML cells undergo apoptosis. Cellular fractionation of normal murine MECs cells indicate that c-Abl expression is detected both in cytoplasmic and nuclear compartments before and with greater amounts detected in the nucleus upon activation. A similar trend is seen in the luminal A MCF7 BC cell line, however c-Abl is predominantly expressed in the cytoplasm basally in the TNBC MDA-MB-231-L2 cell line and retains a strict cytoplasmic localization upon activation with DPH. These findings suggest a similar mechanism as CML of cytoplasmic isolation as a means of circumventing the tumor suppressive role of c-Abl across different BC subtypes. Data from ongoing immunohistochemical analyses of a cohort of BC patient tissue microarrays will inform whether nuclear versus cytoplasmic c-Abl will correlate with available patient outcome data to determine if cellular localization dictates a tumor suppressive or tumor promoting role for c-Abl.
Title: Matrin 3: A novel micro-tubule associated RNA binding protein that acts as a potent tumor suppressor

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Body: Microtubules are highly dynamic components of the cytoskeleton that play an important role in a wide range of cellular processes including cell division, cell motility and intracellular transport. Increasing evidence suggests that alterations in microtubule dynamics are critical for cancer growth and metastasis. Microtubule associated proteins (MAPs) regulate microtubule dynamics and consequently can affect sensitivity of cancer cells to microtubule targeting drugs. We discovered a novel microtubule associated protein "Matrin 3 (MATR3)" that is known to bind to RNA and play a critical role in RNA transport and RNA stabilization. Immunofluorescence analyses revealed that although MATR3 is predominantly a nuclear protein, it translocates to the cytoplasm and interacts with microtubules when breast cancer cells are treated with paclitaxel. We show that MATR3 associates with stabilized microtubules and it mediates microtubule polymerization in a taxol-dependent manner. Interestingly, our results reveal that MATR3 acts as an effective tumor suppressor as it inhibits breast cancer colony formation, migration and invasion of breast cancer cells in addition to suppressing breast tumor growth in vivo. Analysis of breast cancer samples showed a significantly lower expression of MATR3 when compared to normal adjacent tissues. Our results indicate the possibility that MATR3 mediates its tumor suppressor function by binding and regulating proteins that are known to affect microtubule dynamics. We believe that nuclear-cytoplasmic shuttling of MATR3 is critical for stability of key proteins that might regulate paclitaxel-dependent microtubule dynamics and subsequently cellular effects in cancer cells.
**Title:** PRKCQ, a novel protein kinase C preferentially expressed in triple negative breast cancer, drives oncogenic growth, survival and migration

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**Body:** Background/Rationale: PRKCQ, a member of the novel protein kinase C family, was identified in a functional genomic screen for regulators of anoikis resistance/anchorage-independent survival (Irie et al., 2010). Interestingly, it is preferentially expressed in the triple negative/basal subtype of breast tumors compared to Luminal or Her2+ tumors. We sought to determine the functional role of PRKCQ in triple negative breast cancer and evaluate PRKCQ as a candidate therapeutic target for this subtype. **Materials and Methods.** Consistent with its expression in triple negative patient breast tumors, PRKCQ is also highly expressed in several triple negative breast cancer cell lines. We utilized both gain- and loss-of-function approaches in these cell lines to determine the requirement for PRKCQ in oncogenic growth and survival using in vitro and in vivo models. **Results.** Isoform-specific downregulation of PRKCQ using shRNA vectors severely impaired growth of triple negative breast cancer cells in 2D monolayer and 3D Matrigel cultures. In 3D cultures, PRKCQ downregulation not only inhibited growth, but impaired the formation of invasive branching. PRKCQ downregulation also inhibited the growth of MDA231 primary tumor xenografts. All of these data support a requirement for PRKCQ expression in triple negative breast cancer cell growth. To determine if PRKCQ is sufficient to drive oncogenic phenotypes, we overexpressed PRKCQ in a non-transformed immortalized breast epithelial cell line, MCF-10A. PRKCQ expression conferred EGF-independent growth, migration and anoikis resistance. In PRKCQ-expressing MCF-10A cells, EGFR phosphorylation and activation were preserved in the absence of exogenous EGF ligand addition. We are currently elucidating the mechanisms responsible for PRKCQ-mediated, sustained activation of EGFR. **Conclusions.** PRKCQ is critically required for growth of triple negative breast cancer cells. Increased expression of PRKCQ is sufficient to drive oncogenic, growth factor-independent growth, survival and migration. Interestingly, PRKCQ could play a dual role in the development of triple negative breast cancer, as it may also function in the immune microenvironment to support the growth of these tumors. Therefore, PRKCQ is an attractive candidate therapeutic target for patients with triple negative breast cancer.
Title: CENPU acts as a new proto-oncogene to regulate tumorigenesis and cancer metastasis in breast cancer

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Body: Background: Centromere protein (CENPU) gene locus is at chromosome 4q35.1, encoding a protein with some classic functional domains; recently some research groups proved CENPU protein is a constitutive kinetochore component and regulates cell-cycle status by recruitment of function-specific proteins. Homologous gene in murine hematopoiesis system represents early erythroblasts-specific expression. Our previous research had found that about 25% of transgenic mice amplified CENPU would suffer breast cancer in body surface which induce us to hypothesize that CENPU gene and its protein played an important role in breast cancer occurrence and development.

Methods: CENPU protein expression was examined in normal mammary tissue, DCIS tissue, primary invasive breast cancer and different human breast cancer cell lines. Cell type and epitope-dependent subcellular-specific CENPU staining pattern in normal mammary gland epithelium and cancer biopsies were correlated to molecular and clinical parameters. A CENPU-specific small inhibitory RNA (siRNA) was introduced into MDA-MB-231 human breast cancer cell lines to investigate its effect on cancer cell growth, migration and invasion. Distribution of cell cycles were examined with flow cytometry. Test the tumorigenicities of si-control and si-CENPU cells by soft agar colony formation assay. Migration was observed by wound healing and transwell migration assays. The expression of related pathway proteins were determined by Western blotting analysis.

Results: Compared with normal mammary and DCIS tissues, CENPU protein was highest expression in primary invasive breast cancer tissue (P<0.001). Then we compared CENPU expression lever between no metastasis and metastasis patients during three year, and found that CENPU expression in recurrence group is higher than no-metastasis group (P<0.01). CENPU protein expression of different human breast cancer cell lines were detected, which found the CENPU in triple negative breast cancer cell line, MDA-MB-231, was the highest expression. Knockdown of CENPU by specific siRNA in MDA-MB-231 reduced the ability of colony formation and induced the G2/M phase arrest. Moreover, the migration rate of si-CENPU MDA-MB-231 cancer cells was significantly reduced as compared with si-controls (P<0.01). With the knockdown of CENPU, the E-cadherin expression was up-regulated and the N-cadherin, AKT1 and NF-$\kappa$B were down-regulated.

Conclusion: We propose that CENPU functions as a novel proto-oncogene to regulate oncogenesis and metastasis. In further, in vivo study is needed to evaluate the biologaical importance of CENPU in breast cancer.
Body: Deletions of chromosome 10q23, including the PTEN (phosphatase and tensin homolog) locus, are known to occur in breast cancer, but systematic analyses of its clinical relevance are lacking. We thus analyzed a tissue microarray (TMA) with 2,197 breast cancers by fluorescence in-situ hybridization (FISH) using a PTEN-specific probe, and found deletions in 19% of no special type, 9% of lobular, 46% of medullary and 4% of tubular cancers. 98.7% of deletions were heterozygous and 1.3% were homozygous. PTEN deletion was significantly linked to advanced tumor stage (p=0.0054), high tumor grade (p<0.0001), high tumor cell proliferation (Ki67 Labeling Index; p<0.0001), and shortened overall survival (p=0.0090). PTEN deletions were inversely associated with features of luminal type breast cancers (ER/PR positivity, CCND1 amplification). PTEN deletions were strongly linked to amplification of genes involved in the PTEN/AKT pathway such as MYC (p=0.0430) and HER2 (p=0.0065). Remarkably the combined analysis of MYC, HER2, and PTEN aberrations suggested that aberrations of multiple PTEN/AKT pathway genes have a strong additive effect on breast cancer prognosis. Cancers with two or three of these aberrations behaved significantly worse than cancers with none or one of these changes (p<0.0001). The particularly poor prognosis of patients with HER2 amplification and PTEN deletions challenges the concept of PTEN deletions interfering with trastuzumab therapy. In conclusion, PTEN deletion occurs in a relevant fraction of breast cancers, and is linked to aggressive tumors. Reduced PTEN function cooperates with MYC and HER2 activation in conferring aggressive phenotype to cancer cells.
Title: The PI3KCA mutations are frequent and remain along with recurrence of liver metastases from breast cancer in women who underwent up to 3 liver resections

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Body: The Liver resection associated with anticancer and hormonal treatments for metastatic breast cancer results in improved patient survival. However, many patients have tumor recurrence. The frequently observed mutations in PIK3CA that have been associated with resistance to chemotherapy and to anti-HER2 or anti-estrogen therapies treatment, could be involved in the recurrence of liver metastases (and targeted in the future). Therefore, we have analyzed the incidence of the two major 'hot spot' mutations in the helical and catalytic domains of PI3KCA in the liver metastases from breast cancer (LMBC) isolated in a selected cohort of 20 women who underwent at least 2 liver resections with curative intent. Twelve of them had a third hepatectomy. The first hepatectomy was between 1 to 6 yrs after the diagnosis of primary tumors. A total of 73 LMBC were analyzed.

The characteristics of the LMBC at the first hepatectomy: the median age was 52 yrs (range: 30 to 64). Five patients had a solitary tumor whereas the others had up to 5 nodules. The tumor stages varied from stage IA to IIIA and were ductal tumors for 80% of them. Hormonal status varied from the negativity for the receptors to estrogen (ER), progesterone (PR) and HER2 (n=4), to the clearcut positivity ER (n=2), PR (n=3), HER2 (n=4). Out of 20 patients, 6 had LMBC carrying PI3KCA mutations (1 H1047R, 6 E545K). When LMBC were multinodular, all the nodules except one harbored the PI3KCA mutation.

The characteristics of the recurrent LMBC: out of the 6 LMBC with PI3KCA mutations, 4 recurred and underwent a third hepatectomy. One patient whose LMBC did not harbor any PI3KCA mutations at the first liver resection had mutant PI3KCA in the LMBC from the second to the fourth liver resection. All the nodules of the 5 women harbored the same PI3KCA mutation. In parallel, 7 women underwent a third liver resection with no PI3KCA mutations in any of the nodules analyzed. There was no relationship between the recurrence of LMBC with PI3KCA mutations and the surgical margins. The frequency of women whose LMBC carry PI3KCA mutations increased at the third hepatectomy.

In conclusion, PI3KCA mutations are frequently observed in LMBC in all the nodules and persist along with the recurrence. However, PI3KCA could constitute a new target in the neoadjuvant setting before hepatectomy.
Examining the role of Nm23-H1 in the metastatic profile of triple negative breast cancer (TNBC)

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**Body:** Background: Triple negative breast cancer makes up 10-20% of all mammary tumors. It is so named because it exhibits low or no expression of both the estrogen receptor (ER) and progesterone receptor (PR), and has low or no expression of the human epidermal growth factor (HER2) receptor. With no current molecular targets identified, treatment options for TNBCs are limited to conventional chemotherapy, which has been shown to be only moderately effective. Furthermore, diagnosis is correlated with high rates of relapse and metastatic disease, low survival rate five years past diagnosis, and overall poor prognosis.

Nm23-H1 is the most well characterized in a class of metastasis suppressor genes that have received increased attention as a potential therapeutic target in breast cancers. Nm23-H1 mRNA is expressed at relatively low levels in highly metastatic tumor cells compared to normal and non-neoplastic tissues. Low expression levels correlate with increased metastasis and poor clinical prognosis in several type cancers, including breast cancer. Nm23-H1 inhibits a class of Rho small GTPases, including Rac1, Rho and cdc42, which mediate cell-to-cell adhesion and cytoskeletal reorganization, which ultimately modulates the metastatic profile, i.e. metastasis, cellular motility, and invasion.

Methods: Western blotting was used to examine the basal level of expression in a panel of mesenchymal and epithelial TNBC cell lines. Transient siRNA was used to silence Nm23-H1 in MDA MB 231 cells to determine the effects on downstream Nm23-H1 targets IQGAP2, Rac1, Rho and Cdc42. The effects of Nm23-H1 inhibition on the invasive potential of TNBC cells were assessed using Matrigel chamber assays. Spheroid migration assays were employed to assess the effects of Nm23-H1 inhibition on the migratory potential of TNBC cells.

Results: Our studies indicate that Nm23-H1 protein is expressed in all TNBC cell lines tested but to varying degrees, with mesenchymal cells expressing higher levels compared to epithelial cells. Immunoblotting showed that silencing Nm23-H1 resulted in an increase in phosphorylated Rac and Rac and the cytoskeletal intermediate filament vimentin. Silencing also decreased the invasion and migration of TNBC cells compared to control cells.

Conclusion: These results suggest a paradoxical role of the metastasis suppressor protein Nm23-H1 as an oncogene in TNBC cells. Nm23-H1 plays a vital role in the promotion of TNBC cellular migration, invasion, and the expression of proteins associated with motility and invasion. These data strongly suggest an oncogenic potential of Nm23-H1 in a subset of TNBCs and warrants further investigation of this protein a potential molecular marker for metastatic TNBCs.
Title: Epiregulin-induced matrix metalloproteinase-1 contributes to early stages of breast tumorigenesis

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Body: The earliest stages of malignant transformation require the acquisition of multiple phenotypes, such as proliferation, survival and migration, which contribute to tumor growth and progression. Identifying the key cellular and molecular factors that drive the formation of early breast cancer lesions will ultimately result in the development of preventive approaches for patients that are at high risk for developing invasive breast cancer. The epidermal growth factor (EGF) family of ligands has been implicated in promoting breast cancer growth and progression. The contributions of EGF family ligands and their receptors to breast cancer are complex, and the specific mechanisms through which different ligands regulate different stages of breast tumor initiation and growth are not well-defined. These studies focus on the EGF family member epiregulin (EREG) as a mediator of early stages of breast tumorigenesis. In comparison with other EGF family members, EREG was found to be highly expressed in MCF10DCIS.com cells compared with the non-transformed MCF10A cells. Increased EREG expression was also found in significantly more patient samples of human ductal carcinoma in situ (DCIS) than in samples of normal epithelium (64.5% vs. 29.4%, p<0.05). Therefore, further studies were performed to identify mechanisms through which EREG promotes the formation of DCIS lesions.

Experimental studies demonstrated that treatment of MCF10A cells with exogenous EREG enhanced multiple tumorigenic phenotypes, including proliferation, migration and survival. Examination of EREG-induced signaling pathways demonstrated that EREG promotes survival of MCF10A cells primarily through activation of the signal transducer and activator of transcription 5 (STAT5) pathway. Analysis of potential STAT5 target genes revealed that the EREG-STAT5 pathway induces expression of matrix metalloproteinase-1 (MMP-1), a novel STAT5 target gene that was subsequently found to promote survival in response to EREG treatment. To assess the consequences of loss of EREG expression on tumor formation, EREG was knocked down in MCF10DCIS.com cells using shRNA strategies. Loss of EREG led to decreased tumor growth in vivo, which corresponded with decreased cell survival and decreased MMP-1 expression. To determine the relevance of these findings in human tumors, samples of DCIS were analyzed for both EREG and MMP-1 expression. MMP-1 was significantly induced in DCIS lesions in comparison with normal breast epithelium, and EREG and MMP-1 were significantly correlated in a subset of DCIS samples (p=0.011). Together, these studies suggest that EREG contributes to early stages of breast tumorigenesis through regulation of MMP-1. Based on these studies, we propose that EREG contributes to the formation of preneoplastic lesions in a subset of breast cancers and further studies are focused on characterizing the mechanisms through which the EREG-MMP-1 pathway contributes to tumor initiation and growth. Understanding these mechanisms will ultimately lead to the identification of biomarkers of aggressive disease and novel targets for therapeutic strategies for breast cancer patients.
Title: Insulin receptor targeting in breast cancer through yeast surface display

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Body: Selective estrogen receptor modulators (SERMs) such as tamoxifen have been a conventional therapy for breast cancer patients expressing estrogen receptor, representing the most common breast cancer type. The development of secondary resistance to SERM is an unsolved clinical dilemma, leading to the exploration of more targeted therapies. The insulin-like growth factor (IGF) system is well documented and is implicated as a contributor to endocrine resistance. Unfortunately, antibodies directed against type I IGF receptor (IGF1R) failed to demonstrate efficacy in endocrine resistant breast cancer. We have previously shown that tamoxifen resistant (TamR) breast cancer cells lose IGF1R yet retain expression of insulin receptor (InsR), a closely related receptor to IGF1R. Thus, we hypothesized that InsR may serve as a compensatory pathway to the loss of IGF1R in TamR cells and InsR may be a target in the therapy of endocrine resistant breast cancer. Indeed, our preliminary results show that TamR breast cancer cells were more sensitive to insulin stimulation. As compared to the parental cells, TamR cells showed stronger PI3K/AKT and MAPK/ERK activation, greater MTT proliferation growth and anchorage-independent growth upon insulin treatment. Knocking down InsR in TamR cells with shRNA was able to attenuate their sensitivity towards insulin-mediated PI3K/MAPK activation and growth, suggesting InsR targeting may be necessary in endocrine resistant breast cancer. To develop new agents to target InsR, we used yeast surface display to develop an InsR-selective protein scaffold – the 10th type III domain of human fibronectin (Fn3). This technique has arisen as an alternative method for the development of specific binders of cell surface proteins. Compared to antibodies, the Fn3 proteins are smaller (~10kDa), thermally more stable, lack disulfide bonds and have the ability to bind a large variety of proteins. Using error-prone polymerase mutagenesis, we identified improved Fn3 proteins with increased InsR affinity, specificity, and stability. Our current results show that InsR is an important target in endocrine resistant breast cancer cells. Development of engineered Fn3 peptides with increased InsR binding specificity and affinity for InsR compared to IGF1R could be used to disrupt signaling through this pathway. Engineered Fn3 will further be evaluated as a potential imaging and therapeutic tool.
Title: Targeting the insulin receptor with a small peptide (S961) in cancer

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Body: The insulin-like growth factor (IGF)/insulin receptors are members of the superfamily of growth factor receptor tyrosine kinases. The insulin and type I IGF-I receptors (IGF-1R) have very similar structure and share high homology (~84%) in the kinase domain. Their dimeric structure allows for the formation of either holo or hybrid receptors. Thus, targeting of any individual receptor has been a challenge. We have previously shown that the insulin receptor is expressed in tamoxifen resistant breast cancer cell lines. Therefore, specific targeting might be a useful strategy to block this pathway. The antagonist S961 is a single chain peptide that binds insulin receptor and has been reported to have partial antagonist activity. The affinity of S961 for the insulin receptor is comparable to that of insulin and the selectivity of the insulin receptor versus IGF1 is higher than that of insulin itself (Sturis et al). To determine if S961 has activity in breast cancer cells, we tested its ability to disrupt insulin and IGF-I signaling and growth in a panel of cancer cell lines (MCF-7L, MCF-7L TamR, MDA-231, and HepG2 cells). MCF-7L cells express high levels of IGF1, while HepG2 express mostly insulin receptor. Pre-incubation with S961 significantly suppressed p-Akt and p-MAPK after insulin stimulation in the HepG2, but not in MCF-7L. In contrast, insulin receptor stimulation in MCF-7L TamR cells, which do not express IGF1R, was inhibited by S961. Even a 1000-fold increase in S961 was unable to suppress insulin receptor activation in cells expressing both insulin receptor and IGF1R. S961 inhibits insulin-stimulate cell cycle progression in MCF7L TamR cells. In these TamR cells, S961 also suppresses colony formation in anchorage independent growth assays. These data suggest S961 is an effective inhibitor of insulin receptor activation but only when little IGF1R is expressed. Thus, insulin receptor targeting might be useful in the management of endocrine resistant breast cancer.
Title: Targeting the PI3K-AKT-mTOR and RAF-MEK-ERK pathways in HER2 amplified breast cancer models

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Body: Background: The PI3K-AKT-mTOR and the RAF-MEK-ERK signaling pathways are critical for normal physiology and also commonly dysregulated in several cancers including breast cancer. Inhibition of one pathway can still result in the maintenance of signaling and tumor progression via the other reciprocal pathway. Here, we hypothesize that due to the existence of such "escape" mechanisms a dual targeting of these pathways may lead to superior therapeutic activity in HER2+ breast tumors. Methodology: This study evaluated the combination of a pan-PI3K inhibitor GDC-0941 and a MEK1/2 inhibitor GDC-0973 in different HER2+ breast cancer cell lines. HER2+/trastuzumab (T)-sensitive (BT474), HER2+/trastuzumab-resistant (BT474HerR) and HER2+/PIK3CA mutated (HCC1954) cells were used for this study. Growth inhibition/survival, apoptosis, signal transduction and antitumor efficacy were examined using 3D-ON-TOP assays, annexinV staining and mouse xenografts respectively, in T-sensitive, T-resistant and HER2+/PIK3CA mutated breast cancer cell lines. Results: 1) treatment with GDC-0941 caused dose dependent inhibition of phosphorylation of AKT (S473, T308), P70S6K, S6RP, and 4EBP1 (T37/46) and the combination of GDC-0941 plus GDC-0973 more effectively blocked p-S6RP and p-4EBP1, especially in BT474HerR and HCC1954 cells, 2) GDC-0973 inhibited activation of ERK and this inhibition was sustained when combined with GDC-0941, 3) all cell lines showed an increase in annexin V positivity (index of apoptosis) following the treatment of GDC-0941 alone and a combination of GDC-0941 plus GDC-0973 more effectively induced apoptosis in HER2+ cell lines, 4) similar to anti-proliferative and pro-apoptotic signals, a combination of GDC-0941 plus GDC-0973 more effectively abrogated anchorage-independent colony formation in 3D-ON-TOP assay and 5) xenograft data exhibited that the combination of T plus GDC-0941 has an enhanced anti-tumor effect in T-sensitive (80%), T-resistant (82%) and PIK3CA mutated models (58%), and most importantly, tumor regression was more pronounced in all three models when mice were treated with T plus GDC-0941 plus GDC-0973. Conclusions: Our findings suggest that simultaneous administration of MEK1/2 inhibitor may enhance the antitumor activity of PI3K targeted drug and may delay the appearance of resistance of HER2-targeted therapy in HER2 amplified breast cancer.
Prognostic value and clinicobiological associations of the EGFR / PI3K pathway in 204 consecutive localized sporadic triple negative breast cancers

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Background: Triple negative breast cancer (TNBC) is characterized by the lack of expression of hormone receptors and HER-2 antigen. This lack of drugs targets prompted the search for additional targeted therapies. The transmembrane EGFR receptor, also known as HER1, and the phosphatidylinositol 3 phosphate kinase (PI3K) appears frequently dysfunctional in TNBC. It thus appears important to simultaneously evaluate these biomarkers of interest in order to better characterize this population of tumors and the opportunity of dedicated biomarkers-oriented targeting in a European population of TNBC patients.

Material and methods: A total of 1,695 consecutive patients with breast cancer referred to the Val d’Aurelle Cancer Institute between 2002 and 2010 were prospectively entered into the database of a tumour DNA and cytosol biobank. 204 cases with a localized TNBC were selected for the present study. PIK3CA PCR amplification and high-resolution melting (HRM) analysis designed to span exon 9 and 20 mutations were performed on a Rotor-Gene 6000™ using the LightCycler 480 HRM PCR Master Mix™ kit. Bidirectional sequencing was performed by using PCR primers and an Applied Biosystems 3730xl DNA Analyzer. Regarding HER1 CNV determination, qPCR reactions were performed on an ABI Prism 7700 sequence detection apparatus. HER1 levels were normalized to ACBT and GAPDH expression. Measurements were performed in duplicate. The data were expressed as the HER1/ACBT or HER1/GAPDH relative copy number ratio. EGFR cystosol quantification was performed with EGFR Elisa Kit (Calbiochem). Proteins were quantified with the pierce method. Results were expressed in nmol/mg of protein.

Results: Regarding the PIK3CA gene, we identified 14 (6.9%) mutations in exon 9 and 17 (8.3%) mutations in exon 20. Exon 9 mutations were associated with SBR grade I-II (p=0.04) and exon 20 mutations were associated with size 3-4 (p=0.03). Overall mutations were only associated with SBR I-II (p=0.05). HER1 gene was deleted in 11 cases (5.3), normal in 154 cases (75.9%), amplified in 18 cases (8.9%) and presented a polysomy in 20 cases (9.9%). HER1 amplification was clearly associated with an increased EGFR protein content (p=0.03), while polysomic cases presented an EGFR content non-significantly different of the normal HER1 cases. After a median follow-up of 5.6yrs, the 5-year Disease Free Survival (DFS) rate was 76.1% (95% CI [69.2; 81.6]) and the 5-year Overall Survival (OS) rate was 81.3% (95% CI [74.8; 86.3]). PI3KCA exon 9 mutations were a significant pejorative prognostic factor in univariate and multivariate analysis, while a high (upper quartile) EGFR protein content was associated with a better prognosis, in uni- and multivariate analysis.

Conclusions: In this large cohort of localized TNBC, 15.2% of patients presented mutations in both exon 9 or 20 of PI3KCA, and 8.9% of the tumors presented HER1 amplification. Our study shows the negative prognosis value of exon 9 PI3KCA mutations. We confirm then the clinical importance to detect PI3KCA mutations in TNBC. In addition, a high EGFR tumoral content was associated with a better prognosis. The exact mechanism of this association warrant further studies.
Title: Growth hormone induction of oncogenic signaling promotes survival of endocrine resistant breast cancer cells

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Body: Targeting endocrine resistant breast cancer cells has been successfully accomplished by the use of mTORC1 inhibitors in combination with either aromatase inhibitors or tamoxifen. While there was preclinical data suggesting that targeting of the type I IGF receptor (IGF1R) might also be a target for endocrine resistant cells, clinical trials using IGF1R monoclonal antibodies showed no advantage and even suggested potential harm for the combination. IGF1R inhibition results in the disruption of an endocrine feedback loop and results in elevation of the pituitary growth factor growth hormone (GH). GH is involved in normal human growth, development, metabolism, and longevity. Growth hormone released from the pituitary stimulates production of IGF-I from the liver. The GH/IGF-1 axis is known to promote mammary gland hyperplasia and breast cancer carcinogenesis. Previous studies have shown that GH promotes carcinogenesis independently of insulin-like growth factor. The role of GH in specific subtypes of breast carcinoma remains to be defined. Our laboratory has found that estrogen receptor positive breast cancer cell lines up regulated oncogenic STAT5, Akt, and MAPK signaling pathways in response to GH. Tamoxifen resistant (TamR) and long-term estrogen deprived (LTED) breast cancer cell lines derived from MCF-7 and T47D cells demonstrated increased oncogenic signaling in response to GH compared to the corresponding parental cell lines from which they were derived. In contrast, GH stimulation of a triple negative breast cancer cell line (MDA-MB-231) failed to induce signaling via these oncogenic pathways. Although GH treatment of parental, TamR, and LTED breast cancer cells had minimal effect on cell proliferation, cell cycle progression, or migration; GH signaling protected cells from cytotoxic chemotherapy induced apoptosis. This data shows that GH signaling is found in endocrine sensitive and resistant cells. Furthermore, GH may function in protecting cells from cell death induced by either endocrine or cytotoxic drugs. GH receptor blockade may be a valid treatment option for endocrine resistant breast cancer.
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Title: Expression of growth hormone releasing hormone receptor (GHRH-R) in primary and metastatic mammary carcinomas

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Body: In addition to its nominative function as a neurohormone acting on the pituitary, Growth Hormone Releasing Hormone (GHRH) has been shown to modify the growth behavior of numerous cancers, including breast. GHRH is produced by tumor cells, acts in an autocrine/paracrine manner, and requires the presence of GHRH receptor (GHRH-R) on the tumor cells to exert its effects. As this work has been done predominantly on tumor cell lines and xenografts, we set out to examine the clinical analog.

Matched primary and metachronous metastases from 50 breast cancers were included in this study of GHRH-R in breast cancer. Immunohistochemistry for GHRH-R (AbCam) was performed on paraffin sections and the staining results were assessed semi-quantitatively from 0 (negative) to 3+ (strongly positive). A section from normal pituitary was used as positive control. Forty-three of the primary breast cancers (86%) that ultimately relapsed or metastasized showed moderate to strong immunohistochemical expression of GHRH-R. Tumors from the metastatic foci also showed strong immunoreactivity in 78%, 71%, and 44% of liver, brain, and bone foci, respectively. The lower intensity of staining in bone samples may be due to the effect of the decalcification process routinely performed before staining. Whenever present, the non-neoplastic glands adjacent to breast tumors showed either negative or 1+ reaction for GHRH-R. We conclude that the great majority of mammary carcinomas at primary and metastatic sites express GHRH-R. This finding could potentially serve as a basis for therapeutic approaches using peptide receptor antagonists. By receptor blockade using GHRH receptor antagonists, with minimal pharmacologic side-effects, we have been able to control the growth in a number of tumor cell lines (HCC1806, MDAMB468, MDAMB435S, MCF7, T47D, HCC1937, BT474, and MX1s). Based on the presence of GHRH receptors in these clinical tumors, we conclude that clinical trials evaluating GHRH receptor antagonists are indicated.
**Title:** Calorie restriction normalizes global microRNA expression by preventing the loss of dicer expression during mammary tumorigenesis

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**Body:** MicroRNA (miRNAs) are small, post-transcriptional regulators that play an integral role in maintenance of cellular functions and whose dyregulation has been shown to promote many types of cancer, including breast cancer. Global downregulation of miRNAs has emerged as a common theme in human breast tumors and has been shown to contribute to oncogenesis. One of the primary mechanisms through which miRNAs are globally dysregulated is downregulation of enzymes involved in miRNA biogenesis. We aim to establish how calorie restriction (CR), which potently inhibits breast cancer progression, regulates global miRNA expression and miRNA biogenesis enzyme functionality in mammary tumors. To address these questions, 100 female Sprague Dawley rats were administered either dimethylbenz(a)anthracene (DMBA) or vehicle control at 50 days of age, then randomized to receive either control (AIN-76A) diet ad-libitum (n=40) or a 30% CR diet regimen (n=60). Resultant mammary tumors were allowed to develop for 12 weeks. Calorie restriction significantly increased survival to study endpoint relative to control diet (75% vs 35%, respectively) (p=0.0047). Furthermore, of the animals that developed tumors, CR significantly decreased median tumor area by 56% compared to control diet (109.4 mm² vs 250.9 mm², respectively) (p=0.0286). Global miRNA expression was analyzed through miRNA-specific sequencing. Calorie restriction had a broad effect on miRNA expression, illustrated by the fact that of all the miRNAs with a greater than two-fold expression difference between CR and control, 80% are overexpressed in CR tumors compared to control tumors. These results can be explained by the additional finding that CR was able to prevent the loss of Dicer expression, a key miRNA biogenesis enzyme, observed in control-fed mammary tumor tissue compared to normal tissue. This important finding suggests that global miRNA normalization through the retention of Dicer expression during cancer progression could be a contributing mechanism to CR’s anticancer effects. We plan to further these investigations by exogenously manipulating Dicer expression in Rama25 cells, which were originally derived from a DMBA-induced mammary tumor from a Sprague Dawley rat, and analyzing the resultant tumorigenic potential in vitro and in vivo. The results obtained will provide insights into the mechanisms of breast cancer progression and how CR inhibits progression through microRNA modulation.
Title: A large integrated-gene profiling analysis identifies prognostic microRNAs and correlated DNA repair genes in estrogen receptor positive and negative breast cancers

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Body: Background: MicroRNAs (miRNAs) are small non-coding RNAs involved in the pathogenesis of breast cancer (BC). Estrogen receptor (ER) is one of the most important factors influencing BC outcome. In this study we aim to assess the prognostic value in a cross-project analysis for all known miRNAs in ER-positive (ER+) and ER-negative (ER-) BC separately.

Materials and Methods: We assessed the prognostic value of miRNA expression in three independent BC datasets: TCGA (n=782), Metabric [M] (n=1,293) and GSE40267 (n=181) including 472, 853, and 475 miRNAs, respectively. Overall survival (OS) data was available for all patients. Statistical analysis was performed with R software. Survival analysis was performed using Cox proportional hazards regression in each dataset separately. To define an mRNA expression fingerprint for each miRNA, correlation between miRNA and mRNA expressions was computed using Spearman rank correlation in the Metabric dataset. Subsequently, specific metagenes were established using significant correlated mRNAs ("Metabric mRNA", M-mRNA) for each miRNA. Metagenes were also used in the Cox regression analysis. The entire analysis was performed for each miRNA in ER-positive and ER-negative BCs separately.

Results: In the ER+ BC, 283 miRNAs reached statistical significance in at least one dataset. The best performing and significant miRNAs in all datasets were miR-195 (M: HR=1.73, p=3.5E-06; TCGA: HR=2.17, p=3.5E-06, M-mRNA: HR=0.54, p=2.0E-10), miR-199b (M: HR=1.74, p=3.8E-06; TCGA: HR=0.46, p=0.028, M-mRNA: HR=0.56, p=2.8E-09), and miR-210 (M: HR=0.7, p=0.003; TCGA: HR=0.45, p=0.014, Metabric mRNA: HR=1.34, p=0.002). These 3 miRNAs retained their prognostic significance also in a multivariate analysis paired with HER2 status, lymph node status, size, grade and MKI67 expression. In the ER- group 73 significant miRNAs were identified. The best performing miRNAs in at least two datasets were miR-155 (GSE40267: HR=2.21, p=0.034, M: HR=1.67, p=0.008) and miR-381 (GSE40267: HR=0.41, p=0.026; M: HR=0.67, p=0.038). However, the miRNAs in ER- BC in a multivariate analysis were not independent from tumor size. The metagenes identified were significantly enriched in DNA repair genes (e.g. AURKB, TEK) and exhibited an inverse correlation to survival as compared to miRNA expression.

Conclusions: This is the first large-scale miRNA expression analysis demonstrating different prognostic miRNAs in ER+ and ER-BCs. Correlated miRNA-metagenes are involved in the regulation of DNA repair and genomic stability.
MicroRNAs correlating with outcome in patients treated with first-line bevacizumab for metastatic breast cancer

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Body: Background: Biomarkers predicting response to bevacizumab containing therapy in breast cancer are of urgent need. MiRNAs, small non-coding RNAs, can be involved in tumor evolution including regulation of angiogenesis and development of treatment resistance. Therefore, miRNAs can provide both prognostic and predictive information in different cancer entities.

Patients and methods: A genome-wide miRNA profiling using high-throughput TaqMan® Array Human MicroRNA Cards enabling quantification of 754 unique human miRNAs was performed. Formalin-fixed paraffin-embedded specimens from 60 patients treated with bevacizumab as first-line therapy at our institution were analyzed. Based on the median overall progression-free survival (PFS) patients were divided into a responder (G1) and a non-responder group (G2). Differentially expressed miRNAs were selected considering a more than two-fold change and a false discovery rate (FDR) < 10% as significant. Further interesting miRNAs were selected by a multivariate logistic regression approach using LASSO (Least Absolute Selection and Shrinkage Operator) regularization.

Results: Overall median PFS was 9.3 months with a median PFS of 17.5 and 5.0 months in G1 and G2, respectively. Eight miRNAs (miR-19b-3p, miR-21-5p, miR-9-5p, miR-590-5p, miR-106b-5p, miR-20a-5p) were significantly differentially expressed between these groups (FDR < 10%). Their expression levels were all negatively associated with G1. Additionally, four miRNAs (miR-210-3p, miR-224-5p, miR-155-5p, miR-28-5p) might be interesting predictive biomarkers as they were included in a logistic regression classification model providing an optimal separation between responder and non-responder group (evident from the area under curve [AUC] and 100 x 5-fold cross validation). Currently these data is being validated in a patient cohort not treated with bevacizumab in order to detect the predictive value of these miRNAs for a bevacizumab-based treatment. These validated results will be presented at the meeting.

Conclusion: Twelve miRNAs in breast cancer tissue could be identified showing promising predictive value for bevacizumab-based therapy. Although these data need to be confirmed, differences in miRNA expression could help identifying patients with greater benefit from anti-VEGF agents.
Title: Immunohistochemical determination of *arylamine N-acetyltransferase 1 (NAT1)* as the target of miR-1290 and prognostic biomarker of breast cancer

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**Body: Introduction:** There are large-scale molecular differences between estrogen receptor α (ERα)-positive breast cancer and ER-negative breast cancers. In ERα-positive breast cancer, recent analyses have shown that ERα-positives can be divided into two subtypes such as luminal A and luminal B. These subtypes differ in characteristics such as response to endocrine therapy and chemotherapy, and prognosis. In a previous study, we identified a microRNA, miR-1290, that was significantly down-regulated in luminal A tumors and its potential target *arylamine N-acetyltransferase 1 (NAT1)*. The aim of this study was to clarify whether NAT1 is a bona fide target of miR-1290, and to investigate the impact of NAT1 on breast cancer prognosis.

**Methods:** Luciferase reporter assay was employed to validate NAT1 as a putative miR-1290 target gene. Protein expressions of NAT1, ERα, progesterone receptor (PgR) and HER2 were analyzed in 394 breast cancer samples by immunohistochemistry.

**Results:** NAT1 was shown to be a direct target of miR-1290 by luciferase reporter assay. Expression levels of NAT1 were positively correlated with expression levels of ERα (*P* < 0.0001) and PgR (*P* < 0.0001), whereas expression levels of NAT1 were negatively correlated with both tumor grade and tumor size (*P* < 0.0001). Kaplan-Meier analysis showed that NAT1 presence was significantly associated with increased overall survival (OS) (*P* = 0.0416) in breast cancer patients (n=394). Similarly, significant associations of NAT1 presence were shown with disease-free survival (DFS) (*P* = 0.0048) and OS (*P* = 0.0055) in patients who received adjuvant endocrine therapy with tamoxifen (n = 176). Moreover, NAT1 presence was also significantly associated with increased DFS (*P* = 0.0025) and OS (*P* = 0.0007) in lymph node-positive breast cancer patients (n=147). Univariate and multivariate analyses showed significant associations between expression levels of NAT1 and DFS (*P* = 0.0005 and 0.019, respectively). **Conclusion:** We reported that miR-1290 directly targets NAT1 3'-UTR and NAT1 protein expression is correlated with improved OS of breast cancer. Moreover, NAT1 is a possible prognostic biomarker for lymph node-positive breast cancer. Thus, miR-1290 and its potential target NAT1 are associated with characteristics of breast cancer.
Title: Exosome-mediated trafficking of microRNAs by breast cancer cells

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Body: Introduction
Cellular communication in the primary tumour micro-environment is known to play a key role in tumour development and progression. Exosomes are membrane-derived nanovesicles that are actively secreted by cells. Exosomes have been implicated in cell-to-cell communication through the transfer of genetic material including messenger RNA (mRNA), and more recently microRNA (miRNA). miRNAs are small non-coding RNAs approximately 22 nucleotides in length. They play an important role in posttranscriptional regulation of gene expression. Recent reports suggest exosome-mediated trafficking of microRNAs between cells. The aim of this study was to identify the panel of exosome-encapsulated microRNAs secreted by breast cancer cells in vitro.

Methods
Four breast cancer cell lines, MDA-MB-231, BT-20, Sk-Br-3 and T47D were cultured in exosome-depleted media for 48 hours. Exosomes secreted by the cells were isolated through a process of differential centrifugation, microfiltration and ultracentrifugation. The presence of exosomes was first confirmed by Transmission Electron Microscopy (TEM) and Western Blot analysis. Global miRNA array analysis of RNA extracted from exosomes was performed to identify the panel of miRNAs secreted by these cells. miRNA targets of interest were further validated using relative quantification PCR (RQ-PCR). Transfer of Red fluorescent protein (RFP)-labelled exosomes between cell populations was visualized using Confocal microscopy.

Results
TEM analysis of secreted exosomes revealed vesicular bodies of 40-100nm in size. Immunoblotting confirmed the presence of the exosome-associated protein CD63. MicroRNA array analysis of exosome fractions targeting 2089 miRNAs, revealed secretion of between 324 and 394 miRNAs by the cell lines. The miRNAs appeared to cluster in a biologically relevant fashion. 282 miRNAs were common to exosomes from all 4 cell lines, while a small selection were specific to individual cell lines. Exosome-mediated trafficking of miRNA targets of interest including miR-451a and miR-744-5p was successfully validated using RQ-PCR. Further validated targets included miR-10b, miR-145, miR-492 and miR-498, all of which have established roles in regulation of proliferation and apoptosis in cancer. Confocal microscopy supported visualization of miRNA-enriched exosome release from donor cells and subsequent uptake of the RFP-labelled exosomes by recipient cells.

Conclusions
A distinct panel of miRNAs are actively and selectively packaged into exosomes and secreted by breast cancer cells. This transfer of functional miRNAs between cells may play an important role in intercellular communication in the primary tumour microenvironment. The data presented also has potentially important implications in the identification of a circulating miRNA signature for breast cancer detection.
Title: miR-9 expression, retinoids and their potential role in trastuzumab resistance

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Body: HER2 positive breast cancer accounts for approximately 20-25% of breast cancers. Trastuzumab, a humanised monoclonal antibody, is approved for the treatment of HER2 positive breast cancer. However, the majority of metastatic HER2 positive breast cancers progress on trastuzumab treatment due to either innate or acquired resistance. The aim of this study is to investigate the role of microRNAs (miRNAs) in a cell line model of acquired trastuzumab resistance.

The trastuzumab resistant cell line, SKBR3-T was established by continuous exposure to trastuzumab (10 µg/ml) for 6 months. miRNA extracted from SKBR3 and SKBR3-T was profiled using Taqman low density arrays (TLDA). Differentially regulated miRNAs were selected using >2-fold change and a P-value of <0.05. Individual quantitative RT-PCR (qRT-PCR) was performed to confirm miRNA alterations. Proliferation assays were performed using the acid phosphatase method. Taqman gene expression assays for retinoic acid receptor alpha (RARA) were performed using GAPDH as an endogenous control. Immunodetection of RARA was performed using α-tubulin as a control.

Six differentially regulated miRNAs were identified in the SKBR3-T cells. qRT-PCR assays confirmed that four were significantly altered in SKBR3-T compared to SKBR3 cells including miR-9 which was 2.2 fold up-regulated (p=0.04). Utilising miRWalk, we identified RARA as a target for miR-9. We confirmed that RARA mRNA and protein expression are reduced in SKBR3-T cells compared to SKBR3 cells. Treatment with all-trans retinoic acid (ATRA) (0.2 µM–0.025 µM) alone inhibited growth of the SKBR3 cell line in a dose dependent manner but not in the SKBR3-T cell line. Combined treatment with trastuzumab (10 µg/uL) and ATRA (0.2 µM) had a significantly greater growth inhibitory effect on the SKBR3 cell line (90.1±5.2 %) (p=0.0002) than either trastuzumab (39.1±4.0 %) (p=0.0004) or ATRA alone (57.9±4.2 %) (p=0.001). Interestingly, despite relative insensitivity to ATRA in the SKBR3-T cells (23.7±5.0%) (p=0.86), combined treatment with trastuzumab produced significant growth inhibition in the SKBR3-T cells (74.2±5.1%) compared to either trastuzumab (24.1±4.4%) (p=0.0001) or ATRA (23.7±5.0%) (p=0.86) alone.

miR-9 is up-regulated in the acquired trastuzumab resistant SKBR3-T cell line, RARA expression is downregulated, and SKBR3-T cells are resistant to ATRA treatment compared to SKBR3 cells. However, SKBR3-T cells are sensitive to combined treatment with trastuzumab and ATRA. Thus combined treatment with ATRA and trastuzumab may overcome acquired resistance to trastuzumab in HER2 positive breast cancer.
Title: A MAPK microRNA signature significantly associated with poor outcome and predictive of response to tamoxifen therapy in ER+ breast cancer

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Body: Background: Hyperactivation of ERK1/2 MAPK (hMAPK) leads to loss of estrogen receptor (ER) expression and poor outcome in breast cancer. Recent evidence suggests that microRNAs (miRNAs) play important regulatory roles and serve as biomarkers of disease. We previously reported a miRNA signature indicative of hyperactivation of MAPK signaling in breast cancer and its associations with pathological features of breast cancer and breast cancer clinical outcomes. This hMAPK-miRNA signature identified ER+ breast cancers with reduced recurrence-free and overall survival. Here we report on a leave-one-out analysis of this hMAPK-miRNA signature to narrow down and identify those miRNAs that may be critically important to poor clinical outcomes associated with hyperactive MAPK signaling among patients with ER+ breast cancer.

Methods: We performed a leave-one-out analysis of the full hMAPK-miRNA signature, wherein we determined the impact that removal of individual members of the signature had on the ability of the hMAPK-miRNA signature to predict poor disease survival in patients from the METABRIC breast cancer dataset with ER+ cancers. We identified a subsignature of 21 miRNAs that is very significantly associated with poor clinical outcome in patients with ER+ breast cancer from a large patient cohort, according to multivariate Cox proportional hazard analysis.

Summary of Results: Of 57 miRNAs in our original hMAPK-miRNA signature, 21 were retained following leave-one-out analysis. "High-hMAPK" status as described by this signature significantly correlated with adverse pathological characteristics of breast cancer, including ER-negativity, increased tumor grade, and enrichment for Basal and HER2 subtypes. Kaplan-Meier survival analysis of primary breast cancers from the METABRIC dataset indicate that the 21 miRNA subsignature improves upon the prognostic capability of the overall signature in this large patient cohort, and particularly in providing prognostic capability in breast cancers of the luminal-A and luminal-B molecular subtypes. Furthermore, multivariate Cox proportional hazards analysis suggests that classification of breast cancers as "high-hMAPK" based on this leave-one-out miRNA signature is an independent risk factor for poor disease outcome, and is a more significant indicator of poor disease status than hormone receptor status, tumor grade, molecular subtype, and HER2 status. Patients with ER-positive breast cancer who were treated with hormone therapy and classified as "high-hMAPK" by this 21 miRNA signature displayed significantly poorer disease survival compared to patients classified as "low hMAPK". Kaplan-Meier survival analysis and multivariate analysis of independent breast cancer cohorts with miRNA expression data confirm these observations.

Conclusions: We report a subset of 21 of the hMAPK-miRNAs that are important prognostic factors in ER-positive breast cancer, and that may have predictive value in estimating whether an ER-positive breast cancer may be resistant to hormone therapy. Additionally, these 21 miRNAs may be potential effectors of MAPK signaling, and could serve as novel biomarkers or therapeutic targets in breast cancer.
Title: Circulating miRNAs as surrogate markers for hormonosensitivity in patients with hormone receptor-positive metastatic breast cancer? A pilot study

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Body: Background: Clinicians need new predictive biomarkers of response to therapy hormonal in patients with hormone receptor-positive (HR+) breast cancer (BC). Tumor-associated miRNAs are interesting new markers. Several data indicate the extensive alterations in miRNAs regulation upon estrogen pathway and suggest the utility of considering miRNAs expression in the understanding of hormonal therapy efficacy.

Methods: We have conducted a bicentric, prospective clinical trial in patients who must received an anti-estrogen (Tamoxifen) or an aromatase inhibitor (+/- LHRH agonist) in first line for a HR+ metastatic BC. Plasma of patients were collected before the first administration of hormonal therapy (T=0) and after 1 (T=1), 3, 6 months and thereafter in case of objective response or/and progression disease. After extraction from plasma expression i) of 372 miRNAs was analyzed on microRNA Ready-to-Use PCR, Human panel I, V3 from Exiqonand ii) of a selection of candidate miRNAs described in the litterature to be associated with estrogen pathway and hormonotherapy response in tissues were analyzed using the BioMark™ 96.96 Dynamic Array (Fluidigm Corporation) with Exiqon primers. The primary end point of our study, was the feasibility of detection of circulating miRNAs as biomarkers in plasma of patients. Key secondary end points were to compare i) the concentration of determinated miRNAs according patients, under therapy hormonam and according the efficacy or not ii) the profile of miRNAs between patients at T=0 + T=1 month and in the same patient under treatment.

Results: From March 2012 to January 2014, 39 patients were enrolled (5 under tamoxifen and 34 under aromatase inhibitor). At first concentration of circulating miRNAs in pools of patient plasmas from T=0 and T=1 was compared by RTqPCR. miRNAs with a significative fold change ratio together with several miRNAs from the litterature were then further analyzed for individual patient plasmas at T=0 and T=1. Several miRNAs and notably miRNAs previously described in breast tumors to be associated with estrogen pathway and hormonotherapy response are well-detected in plasma and, some of them show difference between T=0 and T=1. After univariate and multivariate analysis, the plasma miRNAs significantly associated with a therapy response will be next examined at 3 and 6 months.

Conclusion: This pilot study provides that tumor-associated circulating miRNAs could be measured in the plasma of patients and that alterations in miRNAs concentration upon hormonal therapy could be observed. More results will be presented at the SABCS meeting.
Title: Breast cancer specific expression of miRNA deciphered using next generation sequencing of LCM procured cells

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Body: The objective of this study is to decipher miRNA expression profiles of laser capture microdissection (LCM)-procured carcinoma cells compared to those of intact serial sections of a breast cancer biopsy. Our hypothesis is that miRNA signatures discerned from specific carcinoma cell populations more precisely correlate with clinical behavior than that provided by conventional biomarkers of intact tissue biopsies. De-identified frozen biopsies of invasive ductal carcinomas of known grade and biomarker status containing 35-70% cancer were selected from an IRB-approved Biorepository (JLW). Serial tissue sections were stained with H & E and 12-15,000 carcinoma cells were collected from an adjacent section. RNA was extracted using PureLink RNA Mini Kit™ (Invitrogen), evaluated (Agilent Bioanalyzer) and sequenced for miRNA expression using the Ion Torrent System (Thermo Fisher). Total RNAs were enriched for small RNA species using mirVana miRNA™ kits (Thermo Fisher) and RNA libraries were constructed from 5 ng of enriched RNA using Ion Total RNA-Seq Kit v2. Barcodes were utilized to multiplex libraries for template preparation and sequencing on Proton PI™ chips as twelve-plex library pools. Each library was sequenced to an average of 30M reads on the Ion Proton™sequencer with the Ion PI™ chip. Sequence reads were aligned to miRNA precursor hairpins available from the miRBase (miRBase.org) miRNA repository. Aligned reads to each miRBase reference miRNA were then reported. Using the R statistical software package, DESeq (Bioconductor), counts for all libraries were normalized and relative expression was calculated. Mapping statistics (e.g., aligned reads (range 59-79%) and miRBase matches (range 69-85%)) were assessed for each library. Comparison of expressed miRNAs from intact tissue sections with those of cognate carcinoma cells procured by LCM revealed, in general, that smaller defined miRNA gene sets were expressed in isolated populations of carcinoma cells. miRNA expression patterns of experimental pairs (intact vs LCM-processed) using MA-plots were highly variable in carcinomas with different grades, suggesting a relationship to disease status. Gene frequency plots, comparing expression from intact tissue sections to that of LCM-processed cell population, revealed subsets of differently expressed miRNAs. To increase statistical power, a follow-up experiment was performed with triplicate libraries from 4 different representative carcinoma samples. In addition to miRNA sequencing, targeted RNA sequencing with an Ion AmpliSeq™ RNA panel was used to capture gene expression information from the12 additional samples. From these replicated libraries, we are able to combine mRNA and miRNA expression information to create an expected profile from these breast carcinoma tissue samples. Application of Next Generation Sequencing of miRNAs and Ion AmpliSeq™ RNA panels using LCM-processed cells and intact tissue provides an innovative approach for assessing differential expression of miRNA and mRNA levels involved in breast cancer behavior. Supported in part by a grant from the Phi Beta Psi Charity Trust (JLW & SAA) and a CTSP Award from the Commonwealth of Kentucky (JLW). For research use only.
Title: MiRNAs are important regulators of Pax-5 expression and function during breast cancer progression

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Body: Recent studies have enabled the identification of important factors regulating cancer progression, one of these being the Pax-5 gene. Pax-5, an essential developmental factor of B cells, is aberrantly expressed in various B cell cancer lesions and solid tumors such as breast carcinoma. Although Pax-5 downstream activity is relatively well characterized, the regulation of aberrant Pax-5 expression in a cancer specific context is poorly understood. To investigate the regulation of Pax-5 expression, we turned our attention to micro-RNAs (miRNAs). MiRNAs are highly conserved, small non-coding RNA molecules that regulate key biological processes. Extensive studies also show their deregulation in multiple cancer lesions. In this study, we aim to elucidate a causal link between differentially expressed miRNAs in cancer cells and their putative targeting of Pax-5-dependent cancer processes. With the help of biobank data and bioinformatics analyses, we observe that miRNAs 484 and 210 are aberrantly expressed in breast cancer cells and cross-reference with their predicted capacity to target the Pax-5 mRNA 3’ untranslated region (3’UTR). Using anti- or pre-miRNAs transfected into Pax-5 expressing breast cancer cell lines (MCF-7 and MB231), we demonstrate that miRNAs 484 and 210 are capable of regulating Pax-5 expression. In addition, miRNA-regulated Pax-5 expression resulted in a concomitant alteration in Pax-5-mediated phenotype and cancer processes. This is the first study demonstrating the regulation of Pax-5 expression and function by non-coding RNAs in cancer cells. We believe that the aberrant expression of Pax-5 in cancer cells is in part due to deregulated miRNA expression profiles. This study will bring insight in regards to cancer regulating processes associated with miRNA and Pax-5 deregulations and help us better understand aberrant Pax-5 expression levels within cancerous states. This study can therefore provide the eventual possibility of earlier, more efficient diagnostics as well as more targeted treatments for cancer patients.
Title: Single nucleotide polymorphism in miRNA binding domain of BRCA1 gene as risk factor in development of familial and early-onset breast cancer

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Body: Background:
A significant proportion of familial and early-onset breast cancers occur in individuals without coding mutations of BRCA1. MicroRNAs negatively regulate mRNA translation by binding to 3’-untranslated region (3’UTR) are implicated in cancer. In the present study, we aimed to determine genetic variation at 3’UTR of BRCA1 in familial and early-onset breast cancer patients with and without mutation in the coding regions of BRCA1 and identify specific 3’UTR variations possibility to be a risk factor for cancer formation.

Materials and Method:
The patients were between 21 and 65 years of age (median, 42.13±87 years). The study used case information from 100 patients with breast cancer who were treated in the Department of Breast Surgery at Uludag University in Bursa, Turkey. The study was approved by the local Ethics Committee (2012-5/11) and conformed to the ethical standards of the Helsinki Declaration. 3’UTR region of BRCA1 gene were screened in 100 breast cancer patients using heteroduplex analysis and followed by direct sequencing of detected variants. In order to analysis of the data, pearson chi-square test was performed using SPSS 21.00 software (SPSS, Chicago, IL, USA).

Results:
SNP: rs12516 (BRCA1) variation in the 3’UTR region was identified in 27% patients. This variation was also determined in 17% controls which had not family history with cancer. There was not found any significant association between patients and controls (p > 0.05). In comparation to variation in BRCA1 3’UTR and presence of BRCA1 mutation of patients, there was a statistically significant difference (p = 0.035). Additionally, in comparation to variation 3’UTR region of BRCA1 gene and clinical parameters of patients, there was no significant association (p > 0.05).

Conclusions:
This study reveals new information about 3’UTR region of BRCA1 polymorphism types and frequencies in the Turkish population. The 3’UTR variant of BRCA1 gene may have potential to be a genetic marker in patients with BRCA1 mutation of increased early-onset or familial breast cancer development risk. However, the significance of these polymorphisms in Turkish families with breast cancer is not clear, and learning this will require more detailed studies of large patient/control groups.
Title: Assessing the safety and feasibility of efficient hypothesis testing in patients with metastatic triple negative breast cancer

C Anthony Blau¹, Colin Pritchard¹, Michael O Dorschner¹, Sibel Blau², Brigham Mecham³, Elisabeth Mahen¹, VK Gadi¹, Wayne Monsky¹, Kimberly Burton¹, Arturo Ramirez⁴, Jackie Stilwell⁵, Eric Kladjian⁶, Carol Collins⁷, Jeannine S McCune¹, William S Noble¹, Julie Gralow¹, Frank Senecal⁸, Linda Dhaene², Nicole Kuderer¹, Jennifer Specht¹, Chaozhong Song¹, Carla Grandori⁷, Nathan Price⁶, Mary Goldman⁵, Aime Radenbaugh⁵, David Haussler⁵ and Jingchun Zhu⁵. ¹Center for Cancer Innovation, University of Washington, Seattle, WA; ²Northwest Medical Specialties, Puyallup and Tacoma, WA; ³Trialomics, Seattle, WA; ⁴Rarecyte, Seattle, WA; ⁵University of California, Santa Cruz, CA; ⁶Institute for Systems Biology, Seattle, WA and ⁷Fred Hutchinson Cancer Research Center, Seattle, WA.

Body: We hypothesize that new insights into how cancers progress and respond to treatment will come from clinical trials that i) extensively characterize the molecular features of a patient’s cancer; ii) use results to predict drug susceptibilities; iii) treat in accordance with these predictions; and iv) learn from individual patient outcomes to iterate and improve over time. To investigate the feasibility of this type of clinical study, we launched the “Intensive Trial of OMics in Cancer” (ITOMIC) for patients with metastatic triple negative breast cancer (TNBC) (Clinicaltrials.gov ID: NCT01957514). Eligible patients have metastatic TNBC, are platinum-naive, and are scheduled to receive Cisplatin. Biopsies are performed under carefully controlled conditions prior to Cisplatin – starting all subjects on a common treatment path, and uncoupling the time needed for specimen analysis from immediate therapy. Biopsies are repeated upon completion of Cisplatin and following subsequent therapies. A subset of specimens is chosen for whole Exome Sequencing, deep sequencing of a panel of cancer associated genes, and RNA-sequencing. De-identified results are placed on a web-based server for analysis and discussed at a meeting of the ITOMIC tumor board. A report describing results and potential therapies is provided to the subject’s oncologist. Treatment decisions are left to the discretion of the oncologist. If a decision is taken to pursue treatments identified in our report we offer assistance in accessing those treatments.

Ten patients have been screened and seven have enrolled. Subjects range in age from 40 to 77 years and all but one has received extensive prior treatment for metastatic TNBC. All seven underwent an initial set of biopsies, targeting between two and five metastatic sites. For most metastatic sites, multiple core needle passes are performed. All subjects tolerated the biopsies well without significant adverse events, and all started treatment with Cisplatin. Three subjects completed Cisplatin and underwent a second round of biopsies. Potential targets for therapy were identified in 5 of the first 6 subjects, and three subjects have received four predicted therapies: 1) a patient with somatic loss of BRCA1 and two linked FGFR2 activating mutations, who was treated first with Veliparib through a single-patient IND and then switched to Ponatinib which produced a partial response; 2) a patient with a novel missense ROS1 mutation treated with crizotinib; and 3) a patient with CYP3A4 copy gain treated with cyclophosphamide.

Conclusion: Our early experience indicates that this approach is feasible and may increase the efficiency of learning from patients with advanced cancer.
PAPAyA – The genome informatics framework for oncology applications and other clinical domains

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**Body:** Background: Genomics is expected to transform oncology clinical practice. However, converting genomic data into clinically actionable information is a daunting task. Genomic high-throughput technologies produce massive amounts of raw data, and its complexity presents a formidable challenge for clinical adoption. While algorithms exist to convert genomic data into meaningful biological information, they are geared towards the bioinformatics expert user, lack clinical annotation and interpretation, and are not addressing the needs of clinical experts. Furthermore, the main problem is that sequencing results should not be treated like a test as they are geared towards precision diagnostics which require patient clinical data.

Methods: We present PAPAyA, a genome informatics platform that provides an overall solution to the genomic data overload which includes analysis of whole transcriptome and exome data, secure storage and management of this data and the interactive presentation of patient genome information in a contextualized manner. It is a continuum of analytics and user experiences with deep understanding of the clinical questions and the workflow. PAPAyA is a framework for hosting multiple genome informatics applications that bring information that is Connected, Digital and in Real Time. Connected in many dimensions - across hospital systems, across time, across many hospitals and their affiliates. Digital means retrievable (without complicated SQL queries) not just stored bits across modalities (genomics is a single vertical that pulls other information towards precise treatment). Real time means up to date ACTGs get converted into actionable information fast, and get to the right clinical expert without manual actions and printing reports. The framework sits on top of a digital health platform that offers core capabilities such as: persisting data and provisioning service (elastic computing), security, auditing, business workflow engine, reports service, user management service, patient identity service, provider registry service.

At its core, PAPAyA provides full pipelines that analyze genomic high-throughput data, and further extract and prioritize clinically-meaningful information from the patient's genome. Furthermore, PAPAyA enables storage and secure management of this complex data which allows clinicians to query the data in a clinical action-oriented framework, as opposed to data aggregation and reporting framework.

Results: We demonstrate the usability of PAPAyA using public data from TCGA breast cancer cohort. We show the utility of executing in-silico assays applied on RNAseq data, such as ER/PR/Her2, differential expression of long-noncoding RNAs, gene fusions, subtyping, hypoxia index, and other known signatures from the biomedical literature. In parallel, we annotate full exomes in order to find clinically relevant information for variants of unknown significance as well as variants with known disease and response phenotype using biological, functional, and clinical annotation resources.

Conclusion: We have implemented an automated open learning system for processing genomic data to aid in clinical decision making. We already started the pilot phase and clinical evaluation with key academic collaborators.
Title: Demonstration of the evolutionary dynamics of the progression from breast hyperplasia to cancer using the duct epithelial agent based model (DEABM)

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Body: Introduction: Abnormal proliferation in the breast is clinically seen ranging from hyperplasia to atypia to in situ carcinomas (DCIS) to invasive cancers. Whether these states comprise points along an evolutionary continuum is a subject of debate, but has significant implications for prevention, prognosis, and treatment. Due to the length of time involved, tumor evolution is difficult to study in vivo and in vitro models. We hypothesize a continuum from hyperplasia to cancer can be demonstrated by examining patterns of accumulated mutations in tumors. We employ a previously developed computational model of ductal epithelial dynamics, the DEABM (1), to simulate and track accumulated mutations during the progression from a normal epithelial population through hyperplastic states to the development of invasive tumors

Methods: Simulation experiments were performed using an expanded DEABM, which incorporates the functions of luminal and myoepithelial cells, their progenitors and fibroblasts. DNA damage can potentially disrupt 12 functional pathways regulated by oncogenes and tumor suppressors implicated in breast cancer (BRCA1, P53, E-cadherin, RUNX3, Myc, ER, TGF-b, MMP-3, EGFR, HER-2 and Telomerase). The expanded DEABM allows for sequential tracking of genetic lesions in each cell. 3,000 simulations in both wild-type (WT) and BRCA1-mutated states were run over 40 years of simulated menses. Cell populations were characterized as normal, hyperplastic or malignant based on quantitative cell expansion from baseline, as well as by mutational profiles, sequential order of mutations and ER status.

Results: Tumor profiles approximated epidemiologic rates of cancer incidence and ER/HER2 status. 2.6 % of WT developed malignancy vs. 45.9% of BRCA1. WT tumors were 54% ER+/HER2-, 17% ER+/HER2+, 6% ER-/HER2+ and 22% ER-/HER2-. BRCA1 tumors were 29% ER+/HER2-, 7% ER+/HER2+, 13% ER-/HER2+ and 50% ER-/HER2-. Hyperplastic populations carried more mutations than non-hyperplastic populations (p>.01) and were more likely to carry mutations in telomerase, E-cadherin and genes related to ER expression (TGFB, RUNX3, p<.01), similar to the early mutations found in ER+ tumors (RUNX3, TGFB, telomerase p<.01). Early P53 mutations were common in all tumors (p<.01). ER- tumors were more likely to carry early mutations in BRCA1, MYC and genes associated with epithelial-mesenchymal transition (MMP-3, p<.01). ER- tumors carried significantly more mutations than ER+ (p<.01), corresponding to data on increased genomic instability in ER- and BRCA1-associated tumors.

Conclusion: The DEABM generates diverse tumors that express tumor markers consistent with epidemiologic data. The DEABM also generates non-invasive, hyperplastic populations, analogous to atypia and/or DCIS, via mutations to genes known to be present in hyperplastic lesions and early mutations in breast cancers. The results demonstrate that agent-based models are well-suited to studying tumor evolution through stages of carcinogenesis and have the potential to be used to develop prevention and treatment strategies.

Title: Impact of adjuvant chemotherapy on clinical and biological ageing in older breast cancer patients

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Body: Background. This prospective observational study aimed to evaluate the impact of adjuvant chemotherapy on clinical and biological markers of ageing and frailty.

Materials and methods. Eligible patients were females ≥70y with early invasive breast cancer for whom adjuvant chemotherapy (4 x docetaxel-cyclophosphamide +/- trastuzumab and Granulocyte-Colony Stimulating Factor) was planned (ChemoG). The control group consisted of breast cancer patients for whom adjuvant chemotherapy was not indicated (ControlG). Patients were enrolled after surgery, underwent blood sampling and received full geriatric assessment (GA) and Quality of Life, (QoL) evaluation at baseline, at 3 months (3m) and 1 year (1y). GA results were summarized in a single score, LOFS (Leuven Oncology Frailty Score), ranging from 10 (very fit) to 0 (very frail). Chemotherapy administration and toxicity were recorded. An extensive (ageing) biomarker analysis is being performed, but this abstract focuses on mean leukocyte telomere length (TL), and circulating inflammatory cytokines (IL-6, IL-10, IGF-1, TNF-alfa, MCP-1, and RANTES). The primary endpoint was to assess whether adjuvant chemotherapy induces accelerated telomere attrition at 1y. Secondary endpoints were the evolution of other ageing biomarkers, LOFS and QoL during chemotherapy; correlations between ageing biomarkers, chronological age and LOFS at inclusion; and the predictive value of ageing biomarkers for functional decline, QoL decline, and chemotherapy toxicity.

Results. 57 patients were included (ChemoG), and 53 (ControlG), with mean age at diagnosis of 73.8y and 76.8y, and mean LOFS of 7.5 (SD 2.3) and 6.8 (SD 1.6). TL was similar in both groups at baseline, and decreased at 3m (p 0.05) and 1y (p 0.0009) in ChemoG, with a similar evolution in ControlG indicating no difference in evolution between both groups (test for interaction p 0.88). RANTES showed a similar decline at 1y in both groups. The other 5 markers remained stable in ControlG while significantly changing in ChemoG (significant time interaction): IL-6 decreased at 3m in the ChemoG (p 0.01) and returned to baseline values at 1y; MCP-1 strongly decreased at 3m (p <0.0001) but increased above baseline value at 1y (p <0.0001); IGF-1 had a similar initial decline at 3m (p <0.0001) yet with only slight recovery at 1y (p 0.006). On the other hand, IL-10 increased at 3m (p 0.04) but decreased at 1y (p <0.0001), and TNF-alfa increased at 3m (p 0.001) and 1y (p <0.0001). LOFS declined in ChemoG at 3m (p 0.0007) but returned to baseline level at 1y (p 0.6) while remaining stable over time in ControlG. Global QoL decreased slightly at 3m in ChemoG and returned to baseline while remaining stable in ControlG. IL-6 correlated most strongly with chronological age (p 0.0008) and LOFS (p 0.03) while associations were less clear for the other biomarkers. In ChemoG, MCP-1 and RANTES were associated with functional decline (IADL ≥1 point decline at 1y) but no biomarkers were associated with QoL decline and grade II-III-IV toxicity.

Conclusions. TL does not evolve differently over time in patients treated with or without chemotherapy. Chemotherapy has measurable impact on clinical frailty and other ageing biomarkers at 3 months, but these effects disappear at 1y follow-up.
Title: Evaluation of the role of EBV in breast cancer

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Body: Background: A number of factors, both modifiable and non-modifiable, are associated with increased risk of developing breast cancer. Although these factors may help predict risk and prognosis, these known factors are imperfect and suggest that additional factors exist that promote the development and behavior of breast cancer. The Epstein-Barr virus (EBV), which is associated with tumor types such as Burkitt’s lymphoma and gastric cancer, may contribute to breast cancer development and/or progression, however, current research has led to mixed results, with some groups finding a positive correlation between EBV and breast cancer and others failing to find an association.

Methods: The Clinical Breast Care Project database was queried to identify patients with high-quality, research-grade frozen tumor specimens and serum samples available. Previous exposure to EBV was determined using the Epstein-Barr virus (EBNA) IgG Human ELISA Kit. EBV, B-cells and macrophages were detected by immunofluorescence (IF) in seropositive patients using probes for EBNA1, CD-68 and CD-20, respectively. EBV and B-cell status was scored as present or absent while macrophages were classified as sparse, focal (weak, moderate or strong expression) or disseminated (macrophages detected throughout the tumor and stroma).

Results: ELISA analysis on 211 serum samples found that 202 (96%) patients were seropositive for past EBV exposure. IF data has been generated for 31 tumors, from which six had no detectable B-cells. Of the remaining B-cell positive tumors, 32% (8/25) were positive for EBNA1 within the B-cells. Distribution of intrinsic subtypes was similar between both EBV+ and EBV- tumors, however, diagnosis with a late-stage tumor and lymph node metastasis was higher in EBV+ (50% stage III/IV, 88% lymph node positive) compared to EBV- tumors (24% stage III/IV, 47% lymph node positive). In addition, EBV+ tumors were more likely to have sparse/focal(weak) macrophage staining (63%) compared to EBV- tumors (47%).

Conclusions: These data demonstrate that latent EBV is present within B-cells in a significant number of invasive breast tumors. Decreased detection of macrophages within the tumor component supports the theory that expression of EBV proteins impairs the function and maturation of macrophages, thus impairing immune surveillance and creating a pro-tumorigenic environment. This altered immune response may contribute to the later stage and increased frequency of metastasis in patients with EBV positive B-cells within the tumor microenvironment.
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Title: Prognostic impact of discordance between different risk assessment tools in early breast cancer (recurrence score, central grade, Ki67): Early outcome analysis from the prospective phase III WSG-PlanB trial

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Body: Background: In early HR+, HER2-negative breast cancer (BC), the 2013 St. Gallen Consensus recommends adjuvant chemotherapy (CT) for patients with nodal involvement, Recurrence Score (RS) >25, grade G3, or high Ki67. However, risk assessment by these factors may be discordant; e.g., in PlanB, about 60% of centrally G3 tumors did not have RS >25. Here, we present first cumulative outcome data from PlanB for evaluation of different risk assessment tools.

Methods: The WSG-PlanB trial was designed to evaluate anthracyline-free adjuvant CT, 6 x TC vs. 4 x EC - 4 x Doc in HER2-negative BC. Since an early amendment (August, 2009), HR+ patients with 0-3 involved nodes and RS \leq 11 were selected to omit CT, receiving only adjuvant endocrine therapy. The primary trial endpoint is event-free survival (EFS, events: relapse, second malignancy, death); secondary endpoints include relapse-free (RFS) and overall survival (OS). Central grade and luminal B classification were centrally assessed by an independent trial pathologist.

Results: From April 2009 to December 2011, 3198 patients were recruited; of these, 2449 were randomized to chemotherapy. Median age was 56 years; 84.1% were HR+ by local pathology, 60.8% node-negative. The central tumor bank population reported here included 3071 cases. RS was available in 2566/2741 cases registered as HR+; of these, 18% had Recurrence Score of 0-11, 60.4% RS 12-25, and 11.6% RS >25. In 343 patients (14.1% of pN0-1 patients after amendment), CT was omitted based on RS \leq 11.

By central assessment, in HR+ disease, grade was distributed as follows: G1/G2/G3: 5.3%/62.6%/32.1%; only 43.5% of central G3 tumors were locally G3; overall concordance between central and local grade was 65.6%. 41.7% of (central) HR+ patients, had "luminal B" tumors (central Ki67 \geq 20% and/or PR \leq 20%).

After 35 months median follow-up, 131 events, including 103 relapses, have been documented; 3-year EFS and RFS in the no-chemotherapy group were 98.4% and 99.0%, respectively.

In the central HR+ population, EFS was substantially poorer in patients with RS >25 than in others (3y EFS: 92% vs. 98% in both RS 12-25 and RS 0-11; p<.001) (n.b.: all patients with RS \geq 12 received CT). RFS was lower in luminal B than in luminal A patients (3y RFS: 96% to 99%, p=.03); EFS did not differ significantly. Involved lymph nodes, Ki67, central grade, tumor size, and RS were univariate prognostic factors for EFS. In multivariate analysis (EFS) in central HR+ disease including these factors, tumor size (fractionally ranked), central G3 (vs. G1 or G2), lymph nodes (\geq 2 vs. <2), and RS (fractionally ranked) were all significant predictors for poor EFS.

Discussion: In spite of receiving no adjuvant CT, patients with RS 0-11 (HR+ HER2- pN0-1) had excellent 3y-EFS. The excellent outcome of patients with RS 12-25 receiving CT suggests potential CT overtreatment in a subgroup. The ongoing WSG-ADAPT trial addresses this issue. Early results of WSG-PlanB suggest that quality-assured pathology together with Oncotype DX® are essential in identifying high-risk patients to avoid undertreatment.
Title: Worse breast cancer prognosis in insulin treated diabetic patients - A population based registry study in Sweden

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Body: Background. Diabetes may be linked to incidence of different tumor diseases and prognosis through various mechanisms such as the disease itself, hyperglycemia, obesity and anti-diabetes therapy.

Material and methods. The study includes all women with BC diagnosed in Sweden between 2000 through 2008 (n=54406). The women had no previous cancer diagnosis during the period of 1958-1999. Dates of birth, BC diagnosis and TNM-stage were directly extracted from the cancer registry. The women's anti-diabetes therapy was gathered from the Swedish Prescribed Drug Registry. Information regarding the cause of death and date of death was obtained from the Cause of Death Registry and the Swedish Population Register up until the 31st of December 2012 and 31st of December 2013 respectively. Analyses have been restricted to patients receiving insulin therapy (n=2463) and their breast cancer prognosis has been calculated in comparison with breast cancer patients without diabetes. All analyses were adjusted for TNM-stage and age at diagnosis.

Results. Patients with insulin treated diabetes had a worse prognosis compared with other women with breast cancer (HR 1.7, 95%CI 1.5-2.0). The worse prognosis could be seen both for patients with ER+ and ER- tumors. The worst prognosis was seen for patients treated with NPH insulins (HR 2.8, 95% CI 2.4-3.3) while patients treated with long-acting insulin analogs had an intermediate prognosis (HR 1.6, 95% CI 1.2-2.2). Those women treated with NPH insulins and metformin had a slightly worse prognosis (HR 1.4, 95% CI 1.0-1.8). The results for breast cancer specific survival and total survival were similar.

Conclusion. Our results imply that insulin treated breast cancer patients have a worse survival compared with other women with breast cancer regardless of tumor stage. Metformin therapy may partially counteract the association.
Title: Androgen receptor expression is an independent marker of lower residual risk in the TACT2 trial (CRUK/05/019)

Jane Bayani¹, James Morden², Sunil Skaria⁶, Peter Bliss⁷, Robert Grieve⁸, Adrian Harnett⁹, Chris Bradley¹⁰, Diana Ritchie¹¹, Peter Barrett-Lee³, Peter Canney⁴, David Cameron⁵, Judith Bliss² and John Bartlett¹. ¹Transformative Pathology, Ontario Institute for Cancer Research, Toronto, Canada; ²Institute of Cancer Research Clinical Trials & Statistics Unit (ICR-CTSU), London, United Kingdom; ³Velindre NHS Trust Cancer Centre, Cardiff, United Kingdom; ⁴Beatson Oncology Centre, Glasgow, United Kingdom; ⁵Edinburgh Cancer Research Centre, University of Edinburgh and NHS Lothian, Edinburgh, United Kingdom; ⁶Mid Essex Hospital Services NHS Trust, Chelmsford; ⁷South Devon Healthcare NHS Foundation Trust, Torquay; ⁸University Hospitals Coventry and Warwickshire NHS Trust, Coventry; ⁹Norfolk & Norwich University Hospital, Norwich; ¹⁰Bradford Teaching Hospitals, NHS Trust, Bradford and ¹¹Beatson West of Scotland Cancer Centre, Glasgow.

Body: Introduction:
TACT2 (CRUK/05/019), a multicentre randomized phase III trial in patients with node +ve or high risk node -ve invasive EBC with E-CMF as control tested two hypotheses in a 2x2 factorial design, with previously presented results showing: i) no evidence of a benefit from accelerated 2-weekly epirubicin (aE) compared to standard 3-weekly epirubicin (E) (Cameron 2012); and ii) capecitabine (X) gives equivalent efficacy but preferential side-effect profile to CMF (Canney 2014).

Studies suggest that Androgen receptor (AR) expression may be associated with improved outcome in early breast cancer. We performed an analysis of AR expression in TACT2 patients to test the hypothesis that AR would represent an independent predictor of residual risk following adjuvant therapy.

Methods:
Tumour samples were collected prospectively from 3803/4391 TACT2 patients, Tissue micro-arrays were constructed as per published guidelines and central AR, ER, PgR, HER2, Ki67, CK5/6, EGFr and BCL2 staining performed and quantified by imaged analysis for ER, PgR, HER2, Ki67, CK5/6 and BCL2. EGFr and CK5/6 were dichotomised by light microscopy evaluation of cores. After planned biomarker analyses had been performed, a subset of TACT2 cases for whom remaining tissue was available were analysed for AR expression. Data for 1878 cases was available for this analysis (49% of those consenting to tissue collection). AR expression was dichotomised as AR-ve (<10%) vs AR+ve (>10%). Log-rank tests were used to explore the prognostic value of AR on time to recurrence (TTR). Cox-regression models were used to test the independent prognostic value of AR in the presence of tumour size, grade, nodal status, and biological subtypes. Marker by treatment interaction terms were included in models to test the predictive value of AR for both randomised treatment comparisons.

Results:
1398/1878 (74%) patients were classed as AR+ve and 480/1878 (26%) as AR-ve. The proportion of AR+ve patients differed significantly between biological subtypes (Luminal A 269/293 (92%); Luminal B 784/928 (84%); HER+ve 86/111 (77%); Basal-like 37/216 (17%); 5-marker-ve 37/93 (40%); \( \chi^2 p<0.001 \))

117/480 (24%) AR-ve patients had a TTR event compared with 183/1398 (13%) AR+ve patients (HR for AR-ve compared to AR+ve = 2.05 95%CI 1.63-2.59; \( p<0.001 \)). AR expression remained independently prognostic following adjustment for nodal status, grade, tumour size and biological subtype (HR1.68, 95%CI 1.26-2.25; \( p<0.001 \)). Exploratory analysis suggested the prognostic impact of AR might be predominantly in luminal B and 5-marker-ve breast cancers; however the interaction between subtype and AR was not statistically significant.

No differential treatment effect between AR subtypes was observed for either randomised treatment comparison (E/aE or CMF/X).

Conclusion:
AR is an independent prognostic marker for residual risk following chemotherapy in this large study which included patients with both luminal and non-luminal cancers. AR expression patterns differ between molecular subtypes of early breast cancer, with evidence suggesting the impact of AR on residual risk may also differ between subtypes. AR is currently being explored as another potential target for therapy so these data could have future clinical relevance.
**Title:** Intrinsic subtypes and BCL2 as predictive and prognostic biomarkers in the TACT2 trial (CRUK/05/019)

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**Body: Introduction:**
TACT2, a multicentre randomized phase III trial in patients with node +ve or high risk node-ve invasive EBC with E-CMF as control tested two hypotheses in a 2x2 factorial design, presented results showing: i) no evidence of benefit from accelerated 2-weekly epirubicin (aE) compared to standard 3-weekly epirubicin (E) (Cameron 2012); and ii) capecitabine (X) gives equivalent efficacy but preferential side-effect profile to CMF (Canney 2014).

Here we present prognostic and potential predictive value of translational biomarkers. We address two main hypotheses: i) aE is less effective in patients with luminal A than patients with other subtypes (Coates 2012) and ii) BCL2 is an independent prognosis marker (Callagy 2006). We also explore the relationship between CK5/6, EGFR and BCL2 and residual risk following chemotherapy.

**Methods:**
Tumour samples were collected prospectively from 3803 patients (86.6% of the 4391 TACT2 patients and 94.5% of those consenting). Tissue microarrays were constructed as per published guidelines and central ER, PgR, HER2, Ki67, CK5/6, EGFR and BCL2 staining performed and quantified by imaged analysis for ER, PgR, HER2, Ki67 and BCL2. EGFR and CK5/6 were dichotomised by light microscopy evaluation of cores. 94.5-97.6% of cases were stained and successfully analysed for individual biomarkers by IHC. Patients were categorised into 4 BC subtypes by central ER/PgR/HER2/Ki67 (Luminal A, B, HER2+ve, Triple-ve (TN)) with TN further divided into basal-like (CK5/6 or EGFR +ve) and 5-marker-ve (both CK5/6 and EGFR -ve).

Log-rank tests assessed prognostic effect of each marker individually and cancer subtypes on time to tumour recurrence (TTR). Cox-regression models tested independent prognostic value of BCL2 in the presence of tumour size, grade, nodal status, and biological subtype.

**Results:**
No evidence of a difference in the efficacy of aE compared with E between the 4 patient subtypes was observed (Luminal A: n=608, HR (for aE compared with E) 0.80 (95% CI 0.47-1.38); Luminal B: n=1804, HR=0.97 (95% CI 0.76-1.23); HER2+ve: n=219, HR=1.03 (95% CI 0.53-1.99); TN: n=638, HR=1.00 (95% CI 0.72-1.39); Test for heterogeneity p=0.84). When subdividing the TN group into basal-like and 5-marker-ve, HRs were 0.66 (95% CI 0.45-0.98) and HR 1.56 (95% CI 0.82-2.96) respectively, post-hoc analysis for heterogeneity between these 2 subtypes p=0.025. No differential effect between subtypes was observed for the comparison of CMF and X.

82/434 (18.9%) patients with low BCL2 expression (≤10%) had a TTR event compared with 444/3158 (14.1%) with high BCL2 (>10%), univariate HR 1.39, 95% CI 1.10-1.76, p=0.006. However this difference was no longer seen after adjustment for clinical factors and biological subtype (HR 1.17 95% CI 0.90-1.52, p=0.25).

**Conclusion:**
We found no statistical evidence that luminal A cancers are associated with reduced benefit from aE vs E. A hypothesis generating observation that benefit from aE vs E might be different between basal-like and 5-marker-ve cancers should be interpreted with caution due to the small numbers of cases and the retrospective nature of the analysis. In this study BCL2 did not provide independent prognostic information when corrected for conventional histopathological features.
**Title:** Does androgen receptor (AR) expression impact on residual risk? A TEAM pathology study

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**Body:** Introduction: Several studies have suggested that AR expression, particularly in luminal cancers following endocrine therapy, may be associated with improved outcome in early breast cancer. We performed an analysis of AR expression in the TEAM pathology cohort to test the hypothesis that AR would represent an independent predictor of residual risk following adjuvant endocrine therapy.

Methods:
Triplicate 0.6mm² TMA cores from the TEAM pathology cohort (N=4,598) were stained using AR clone ER179(2) on a Ventana automated staining platform and analysed by image analysis using the Ariol Image analysis platform. Continuous histoscores were generated as previously described (Bartlett et al, JCO 2011).

Results: AR histoscores were generated from 3866/4598 (84%) of available cases. Median AR histoscores were 227 (interquartile range 195-262). In a univariate Cox proportional hazard model with AR histoscore as a continuous variable, increased AR histoscores were significantly associated with a reduced hazard of distant disease relapse or death from breast cancer. The hazard ratio (HR) associated with a 50 unit increase in the histoscore was 0.88, 95% confidence interval (95%CI) 0.83-0.93, \( P < 0.0001 \). There was evidence that a log transformation of the histoscore resulted in a better fitting model (\( P < 0.0001 \)) resulting in the following model estimates HR= 0.93, 95% CI 0.89-0.98, \( P = 0.006 \). However, a multivariate model of AR histoscore including other known prognostic factors such as age, grade, tumour size, number of positive nodes, HER2 status, ER and PgR histoscores found AR histoscore was not independently prognostic for distant relapse or death (HR=1.00, 95% CI 0.94-1.06, \( P = 0.96 \)). There was no significant interaction between AR expression and type of endocrine treatment (Tamoxifen → exemestane versus exemestane alone) in either univariate (HR 1.003 95%CI 0.91-1.11, \( p=0.96 \)) or multivariate (HR 0.92, 95%CI 0.81-1.04, \( p=0.18 \)) analysis.

Logistic regression analysis was performed to investigate the association between AR histoscore (2 groups above and below the median) and the known prognostic factors mentioned above. Increased AR histoscore is associated with good risk factors; young age, low grade, small tumours, decreasing ki67 and increasing ER and PgR histoscores.

Conclusion: AR expression is common in luminal breast cancers. However, in this study AR histoscore does not add residual risk information beyond what can already be assessed using conventional prognostic factors. High AR expression is associated with good prognostic factors, including young age, low tumour grade, small tumour size, lower Ki67 and higher ER/PgR expression.
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Title: TLE3 is not a predictive biomarker for taxane sensitivity in the NCIC CTG MA.21 clinical trial

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Body: Background: TLE3, a nuclear transcriptional repressor downstream of the WNT signaling pathway, has been identified as a candidate predictive biomarker of taxane benefit in early breast cancer. However, robust clinical evidence is required before implementing novel diagnostic biomarkers. We tested the hypothesis that TLE3 predicts for benefit from taxane containing polychemotherapy in the NCIC CTG MA.21 clinical trial.

Methods: MA.21 accrued 2104 patients [701 each to cyclophosphamide, epirubicin, and 5 fluorouracil (CEF) and epirubicin and cyclophosphamide with filgrastim and epoetin alfa followed by paclitaxel (EC/T), 702 to doxorubicin and cyclophosphamide followed by paclitaxel (AC/T)] who were followed median 8 years by the final analysis. EC/T and CEF were not significantly different (p= 0.69) while AC/T was inferior to both EC/T and CEF (respectively, p=0.001 and p=0004). Tissue microarrays were constructed from 1097 of the 2104 patients. Patient characteristics were well balanced between those included in the TLE3 analysis and the full trial population. Up to four 0.6 mm tumor cores were stained for TLE3 expression by immunohistochemistry using a previously validated methodology. Continuous visual TLE3 score was the average % positive stain across all cores, while continuous automated score was sum of cells with positive stain/ total cells assessed in all cores. The primary objective used the EC/T (taxane-containing) and CEF (non-taxane; similar dose-density) arms for a test of predictive effect of TLE3 on relapse free survival. TLE3 was positive if >30% of cells stained, the established cut-point, with data available from at least 1 core/tumor. We also examined quartile cut-points, multivariate effects of TLE3, and compared AC/T and CEF.

Results: MA.21 patients had 83.2% TLE3+ tumors by visual score and 80.6% TLE3+ by automated image analysis greater than the predicted rate of TLE3 positivity (58.6%) based on prior series and adjusting for clinicopathological features. TLE3 expression was significantly positively associated with ER expression (91.2% of ER+ were TLE3+; p<0.0001). There was no evidence of a predictive effect of TLE3 expression with respect to taxane benefit using the established 30% cut-point, nor quartile cut-points. The treatment and TLE3 interaction term for the EC/T by CEF comparison was not significant (stratified p=0.68, for manual TLE3; p=0.44, for automated TLE3).

Conclusions: MA.21 patients had a much higher proportion of TLE3+ tumors than anticipated. Multiple assessments of TLE3 cut-points yielded no evidence that it was predictive of taxane benefit. In our trial TLE3 expression was not a biomarker for taxane benefit (EC/T vs CEF) when using either previously established or common quartile cut-offs for expression in breast cancer.
Comparison of multiparameter tests in the UK OPTIMA-Prelim trial

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Body: Introduction

All published adjuvant chemotherapy trials in breast cancer have made the assumption that the proportional benefits of chemotherapy apply uniformly across molecular subgroups. However, it can be argued that chemotherapy effectiveness for luminal A breast cancer is low in comparison to other subtypes irrespective of tumour stage. A logical extension of this argument is that novel multiparametric tests that use biological features of breast cancers to assess risk may also inform chemotherapy benefit in luminal cancers. The OPTIMA trial is a multi-centre, partially blinded, randomised clinical trial with a non-inferiority endpoint, and an adaptive design, to compare standard treatment (chemotherapy followed by endocrine therapy) with multi-parameter test-guided treatment allocation to either chemotherapy followed by endocrine therapy or endocrine therapy alone. OPTIMA-prelim aimed to compare the predicted risk stratification, sub-type classification and cost effectiveness of different multiparameter tests performed on the same patient population.

Methods

Over 20 months of recruitment 285 patients were randomised to OPTIMA-prelim. Tissue was collected centrally, ER and HER2 status confirmed and samples provided for testing with Oncotype DX™, Prosigna™ (PAM50), Mammaprint™, Mammatyper™, IHC4-AQUA and IHC4 using conventional biomarkers. Sub-type classification was provided by Blueprint™, Mammatyper™ and Prosigna™. Each test was performed at central diagnostic laboratories (OncotypeDx, Mmaaprint/Blueprint, Mammatyper) or in a central laboratory (Prosigna™/IHC4) strictly according to GLP practices.

Results

Samples from 181 patients randomised by January 2014 were tested and data analysed for this study. Patients were categorised as low/intermediate or high risk using predetermined cut-offs for each test. Oncotype DX predicted a proportion of low-risk tumours (79%; 95% CI 73-85%) similar to that predicted as either low or intermediate risk using Prosigna ROR_P (71%; 95% CI 64-78%) and IHC4 (69%; 95% CI 62-76%), whilst MammaPrint identified the fewest low-risk tumours (59%; 95% CI 52-66%). Strikingly, a comparison between tests showed modest agreement between tests when dichotomising results between high vs low/intermediate risk. Disagreement between different tests, in assigning individual tumours to risk categories, is not uncommon; for the four tests [Oncotype DX, MammaPrint, Prosigna ROR_P (low/int) and IHC4 (low/int)], only 71 (39%) tumours were classified as low/intermediate risk for all four tests and only 17 (9%) tumours were high risk for all four tests, 93 (52%) tumours were assigned to different risk categories by different tests. Similarly all three subtypes tests (Blueprint/Prosigna/Mammatyper) each assigned 59% of tumors to luminal A subtype but only 70% of these cases were classified as luminal A by all three assays.

Conclusion

Existing evidence on the comparative prognostic information provided by different tests suggests current multiparameter tests provide broadly equivalent risk information for the population of women with luminal breast cancers. However, for the individual patient, tests may provide differing risk categorisation or indeed subtype information.

Acknowledgement

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expressed therein are those of the authors and do not necessarily reflect those of the HTA programme, NIHR, NHS or Department of Health.
**Title:** Pathological assessment of discordant cases for molecular (BluePrint and MammaPrint) vs clinical subtypes for breast cancer, among 6,694 patients from the EORTC 10041/BIG 3-04 (MINDACT) trial

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**Body:**

**Background**

Biology has become the main driver of breast cancer (BC) therapy. Biological subtypes have been recommended as a guide for treatment selection. Molecularly identified subtypes can be determined by gene expression profiling. Alternatively, pathology can be used to define surrogates of these subtypes. These methodologies are not always concordant, which can lead to different systemic therapies. The purpose of this preplanned translational research is to investigate the concordance between molecular based BluePrint and MammaPrint breast cancer subtypes and pathological surrogates (based on ER, PgR, HER2 & Ki67) and to characterize the discordant cases.

**Methods**

MINDACT is an international, prospective, randomized, phase III trial investigating the clinical utility of MammaPrint in selecting patients with early BC for adjuvant chemotherapy (CT), which enrolled 6,694 patients. Molecular subtyping data were obtained by MammaPrint and BluePrint (Agendia, Amsterdam, the Netherlands) on frozen samples (n=6,694) classifying patients in the following subtypes: Luminal A (Luminal-type/MammaPrint Low Risk); Luminal B (Luminal-type/MammaPrint High Risk); HER2-type; and Basal-type. ER, PgR, HER2 and Ki67 protein status were centrally assessed on FFPE blocks by IHC and/or FISH in the European Institute of Oncology, Milan, Italy (n=5,740; 86%). Patients were also classified according to the St. 2013 Gallen recommendations [Goldhirsch et al. 2013], which recognizes an additional category (Luminal B-like HER2+).

**Results**

Ki67 is often used as biomarker to distinguish Luminal A from Luminal B subgroups. The concordance between MammaPrint and centrally assessed Ki67 in Luminal-type patients is 60%, with a κ score of 0.26 (95% CI 0.24–0.28) indicating that Ki67 and MammaPrint cannot reliably substitute for each other. When using a cut-point of 20% instead of 14% the concordance increased to 78%, with a κ score of 0.44 (95% CI 0.41–0.47).

There is a relatively large group of clinical HER2+ cases that are BluePrint Luminal-type (208 out of 541; 38%) indicating that tumor expression of the Luminal profile is dominant compared with expression of the HER2 profile. These patients have high IHC ER results and all except for 1 fall into the group that St Gallen separately defines as Luminal B-like HER2-positive.

These patients may have lower response to trastuzumab [von Minckwitz et al. JCO 2012].

98 out of 622 BluePrint Basal-type patients are clinical Luminal HER2-. 2/3 of these patients have low centrally assessed IHC PR expression and 1/3 have low centrally assessed ER expression (≥1% and <10%).

**Conclusions**

Marked differences are observed between BluePrint and MammaPrint (microarray based) breast cancer subtypes and centrally re-assessed pathological surrogates (based on ER, PR, HER2 & Ki67). The greatest discordance is seen in the sub-stratification of Luminal patients, and in the HR+/HER2+ patients. The observed subtype discrepancies may have an important impact on treatment decision making.
Title: Comparison of immunohistochemical residual risk panels to predict risk in early breast cancers treated with endocrine therapy

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Body: Background: We compare two residual risk models combining immunohistochemical (IHC) biomarkers, IHC4 and Mammostrat, in the Edinburgh Breast Conservation Series (BCS) and in the Tamoxifen versus Exemestane Adjuvant Multinational (TEAM) trial.

Materials and Methods: The primary cohorts comprised 831 and 2,513 estrogen receptor (ER)-positive patients who did not receive adjuvant chemotherapy from the Edinburgh BCS and TEAM cohorts respectively. We evaluated prognostic scores for distant recurrence-free survival (DRFS).

Results: Low scores for both IHC4 and Mammostrat are associated with better DRFS. In multivariate Cox regression analyses the addition of both scores to clinical factors provided independent information on residual risk (p<0.05). In the larger TEAM cohort, IHC4 was the stronger predictor of DRFS but additional information was gained from including the Mammostrat score for all ER-positive patients (p<0.001).

Conclusion: The results showed that the scores have different capabilities in predicting DRFS depending on the study and subgroup of patients. However, significant benefit in estimating residual recurrence risk after treatment was observed from a combined use of both marker panels. This provides support for investigating their combined use for risk stratification of ER-positive early breast cancer patients.
Body: Background: We investigate the impact of follow-up duration to determine whether two immunohistochemical prognostic panels, IHC 4 and Mammostrat, provide information on the risk of early or late distant recurrence using the Edinburgh Breast Conservation Series and the Tamoxifen versus Exemestane Adjuvant Multinational (TEAM) trial.

Methods: The multivariable fractional polynomial time (MFPT) algorithm was used to determine which variables had possible non-proportional effects. The performance of the scores was assessed at various lengths of follow-up and Cox regression modelling performed over the intervals 0-5 years and > 5 years.

Results: We observed a strong time-dependence of both the IHC4 and Mammostrat scores with their effects decreasing over time. In the first five years of follow-up only, the addition of both scores to clinical factors provided statistically significant information (p<0.05) with increases in $R^2$ between 5 and 6% and increases in D-statistic between 0.16 and 0.21.

Conclusion: Our analyses confirm that the IHC4 and Mammostrat scores are strong prognostic factors for time to distant recurrence but this is restricted to the first 5 years after diagnosis. This provides evidence for their combined use to predict early recurrence events in order to select those patients who may/will have benefit from adjuvant chemotherapy.
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Title: CADER prognostic gene signature for disease free survival in hormone receptor positive breast cancer: NCIC CTG MA.12 phase III placebo-controlled tamoxifen trial

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Body: Background: The copy number aberration derived endocrine response (CADER) gene signature was developed as a prognostic marker for hormone receptor positive breast cancer; it combines information derived from both gene expression and copy number aberration and was trained on samples from both the neoadjuvant and adjuvant settings (see separate CADER nanostring assay methodology abstract, Luo et al). We examined the effects of CADER on outcome in the MA.12 trial to evaluate the association of CADER and PAM 50 with relapse.

Methods: From 1993 to 2000, MA.12 accrued 672 women to a placebo-controlled pre-menopausal trial of adjuvant tamoxifen with locally assessed hormone receptor +/- tumors. The 9.7 years median follow-up was used here. A secondary endpoint was disease free survival (DFS), defined as time from randomization to the earliest date of recurrence or death; censoring was at the last date the patient was known to be alive. Stratification factors were type of chemotherapy (CMF vs CEF vs AC), hormone receptor status (ER and/or PgR positive vs ER and PgR negative), and nodal status (0 vs 1–3 vs 4–9 vs 10+). Exact Fisher tests were used to compare baseline characteristics of those assessed, or not, for CADER. We examined the effects of the CADER classifications (sensitive, indeterminate, resistant) for hormone receptor positive patients. The prognostic effects of CADER, and preexisting qPCR PAM50 ROR-S, and PAM50 intrinsic subtype (Chia et al, CCR 16, 4465, 2012) on DFS were examined with stratified multivariate Cox regression, adjusted for treatment and baseline patient characteristics. An interaction test with treatment was used to assess evidence for CADER as a predictive signature. Graphical depiction was with adjusted Cox survivor plots.

Results: CADER was assessed in 434 (65%) of the 672 patients: 213 patients on the tamoxifen arm; 221, on the placebo arm. Proportionately more patients profiled for CADER underwent CEF adjuvant chemotherapy (p=0.03) so we performed only stratified analyses. Of the 434 patients, 317 (73%) had hormone receptor breast tumors. The CADER classifications significantly impacted DFS (p=0.04): hazard ratio (HR) of indeterminate CADER to sensitive=2.29 (95% CI 0.93-5.62; p=0.07); HR of resistant to sensitive=3.72 (95% CI 1.33-10.42, p=0.01). Patients with low ROR-S had longer DFS (p=0.04), while PAM50 intrinsic subtype did not significantly impact DFS (p=0.51). CADER and ROR-S were not predictive factors.

Conclusions: In MA.12 hormone-receptor patients, both CADER and ROR-S had significant prognostic effects on disease free survival. Neither CADER nor PAM50 ROR were predictive in this placebo-controlled tamoxifen trial. We confirmed here the earlier clinical prognostic relevance of CADER that was seen during the signature’s development.
Title: MammaPrint accurately predicts long-term survival (25 years) and adjuvant tamoxifen therapy benefit in lymph node negative patients

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Body: Background: Adjuvant endocrine therapy is a standard of care for early stage estrogen receptor positive (ER+) patients, but analyses show the majority of benefit accrues after 10 years of treatment. Recent changes to the American Society of Clinical Oncology recommendations now include the option to extend tamoxifen treatment duration to 10 years. In contrast, screening trial data with 25 years of follow-up suggest that 50% of screen-detected cancers may never come to clinical attention (Miller et al BMJ 2014). Better tools are needed to identify patients who benefit from any, 5 or 10 years of adjuvant endocrine intervention. MammaPrint (MP) is an FDA-cleared gene expression signature that categorizes untreated patients as Low (LR) or High risk (HR) for distant recurrence (DR) at 10 years, independent of ER and HER2 status. Herein, we examined the performance of MP to predict long-term (25 years) survival benefit from adjuvant endocrine therapy in early stage breast cancer patients randomized after surgery to receive 2 or 5 years tamoxifen vs observation only (control arm).

Methods: The Stockholm Tamoxifen Trial (STO) is a uniquely annotated trial with long-term follow-up. 733 samples were made available from the 1774 lymph node negative breast cancer patients, age <71, with a tumor size <30 mm, enrolled between November 1976 and May 1990. This group was randomized to adjuvant tamoxifen versus no adjuvant therapy (surgery alone). Long-term breast cancer specific survival was determined according to MP status (LR or HR as categorized by Agendia; or UltraLow, an additional threshold proposed in 2011 as predicting exceptionally good distant disease free survival >10 years) for all patients as well as for each of the randomized treatment arms. MP status was successfully assessed for 656 patients; 3 patients were excluded due to missing randomization data. Multivariate proportional hazards analyses (Cox) was performed, with the multivariate model adjusted for ER, PR and HER2 status (original assessment), tumor grade and size, and treatment cohort.

Results: A statistically significant difference in survival by MP risk categories was seen for all patients (P−log rank=0.0013), as well as for the tamoxifen treatment arm (P−log rank=0.011) and the control arm (P−log rank=0.047). By multivariate proportional hazards (Cox) analyses and after adjustment, women with MP−HR had a statistically significant two−fold increased risk of dying from breast cancer by 25 years (Hazards ratio, HR = 1.68, CI 1.13−2.48), compared to women with MP−LR. In this STO trial, the UltraLow threshold (MP index of > 0.355) was associated with a statistically significant survival advantage across all patient groups with a 95% breast cancer specific survival at 15 years.

Conclusions: MammaPrint accurately predicts for a statistically significant long−term survival benefit in MP−LR patients across all STO patients and, specifically, for those who received adjuvant tamoxifen. Separation of ‘UltraLow’ risk patients from the MP-LR group may facilitate clinical decisions for the duration of hormonal therapy. Additional analyses are underway to evaluate the timing of risk with and without endocrine treatment stratified by MP risk categories.
Title: Validation of the preoperative endocrine prognostic index in the ACOSOG (Alliance) Z1031 neoadjuvant aromatase inhibitor trial

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Body: Background: The Preoperative Endocrine Prognostic Index (PEPI) is a method to predict outcome after neoadjuvant endocrine therapy that integrates Ki67, estrogen receptor (ER) analysis and pathological stage from the surgical specimen (Ellis, JNCI 100:1380, 2008). We sought to further develop the PEPI for use in clinical trials by: a) establishing an efficient SOP for Ki67 analysis, b) determining the effect of simplifying the score by removing the ER component (modified "mPEPI") and c) independent validation of mPEPI in the ACOSOG (Alliance) Z1031 neoadjuvant aromatase inhibitor trial (Ellis, M, JCO 29:234, 2011).

Methods: Ki-67 assay development focused on reproducing a 2.7% Ki-67 cut point (CP) required for PEPI. Ventana Image analysis (IA) to replace labor-intensive visual point counting (VPC) was assessed to increase scoring efficiency. Discordant scores led to a triage approach where cases with complex histological features that could not be resolved by IA were flagged for VPC. The Ki-67 scoring approach was preliminarily validated on T1/2 N0 cases from the P024 and POL trials (SABCS 2013, abstract P3-05-190). Models with and without ER suggested ER was dispensable. A locked SOP for mPEPI was subsequently applied to the Z1031A trial. The primary endpoint was time from the date of surgery to local, regional, or distant recurrence in the mPEPI-0 group (T1/2 N0, Ki67 <2.7%) versus the mPEPI>0 group.

Results. mPEPI by IA was evaluated on 202 of 377 (53%) patients enrolled into Z1031A (6% of IA results were triaged to VPC). All patients have been followed a minimum of 2 years (median: 5 years; max: 7 years). Only 10 patients in the mPEPI-0 group (22.7%) received adjuvant chemotherapy, versus 78 in the mPEPI>0 group (49.4%). Time to breast cancer recurrence was decreased among those with mPEPI>0 status relative to those with mPEPI-0 status (log rank p=0.012). Only one disease event among 44 (2%) cases with mPEPI-0 was observed versus 26 of 158 cases with mPEPI>0 (16.5%) Conclusions. mPEPI-0 status can identify patients at low risk of relapse after neoadjuvant endocrine therapy: therefore mPEPI-0 status has operational characteristics similar to pCR after chemotherapy for ER-negative disease. mPEPI is undergoing prospective validation in the Alliance ALTERNATE trial that will assess whether Fulvestrant increases the mPEPI-0 rate and also will prospectively determine whether patients with mPEPI-0 status can safely be managed without adjuvant chemotherapy treatment.
Title: Molecular response in breast cancer treated with neoadjuvant chemotherapy with and without bevacizumab: Results from NeoAva - a randomized phase II study

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Body: The NeoAva study is a phase II clinical trial of patients with HER2 negative primary tumors of ≥25 mm treated with neoadjuvant chemotherapy (4 x FEC100 + 12 weeks of taxane-based therapy) and randomized (1:1) to receive bevacizumab or no bevacizumab. Mammography, ultrasound and MR imaging were used for response evaluation, in addition to final pathology assessment.

Tumor response were evaluable in 131 patients; of which 66 received bevacizumab in addition to chemotherapy. Tumor material was obtained at screening, 12 weeks into treatment and at surgical removal of tumors at 25 weeks. mRNA expression profiling was performed on Agilent 8x60K platform and the tumors were classified into LuminalA, LuminalB, Her2-enriched, Basal and Normal-like subtypes using the PAM50 classifier. Ratio of the tumor size at final pathology assessment, and at inclusion (by radiology assessment) was calculated to obtain a continuous scale of response reflecting the percentage of tumor shrinkage in response to therapy. Genomic Grade Index (GGI scores) based on expression profiles of 97 genes (including cell-cycle and proliferation genes) were calculated.

There were no significant differences in the tumor size, lymph node, hormone receptor status or PAM50 subtypes between the treatment arms. pCR in breast and axilla were obtained in 14 (21.1%) patients in the chemo+bev arm, and in 7 (10.6%) patients in the chemo-only arm. Tumors that obtained pCR were in higher number ER negative and TP53 mutated and exhibited Basal-like phenotype. The overall pCR rates were higher in the ER negative tumors compared to ER positive tumors (39.1% (9 of 23) vs 11.1% (12 of 108)). However, addition of bevacizumab seemed to improve pCR in the ER positive patient group (9 vs 3) and not in ER negative patient group (5 vs 4).

On evaluating the continuous response variable, ER status, TP53 mutation status and PAM50 subtypes were significantly associated to response (p < 0.001). GGI scores were highly correlated to response (p< 0.001), i.e tumors with higher GGI scores showed better response. Importantly, when the chemo+bev and the chemo-only arms were evaluated separately, although similar trend of associations was observed in both arms, the associations were found to be enhanced in the chemo+bev arm.

Next, we evaluated a shift in PAM50 subtypes across the timepoints. A shift towards a better prognosis group, i.e Luminal A or Normal-like profile was observed in response to therapy. Distribution of Luminal A and Normal-like tumors at week 25, (and not at screening or week 12) was significantly different in the chemo+bev vs chemo-only group (p = 0.026, Fisher’s exact test). GGI scores regressed across timepoints reflecting the loss of aggressive and proliferating component of the tumors in response to therapy. GGI scores in the chemo+bev group became significantly lower (p < 0.01) already at week 12. This suggests that the removal of the proliferating component of the tumors by chemotherapy is accelerated and improved by addition of bevacizumab.

These results, with potentially important clinical relevance will be further investigated with respect to subtypes and the molecular changes induced by antiangiogenic therapy.
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Title: Risk stratification within luminal B breast cancer using a second generation prognostic RNA signature

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Body: Background: Indication to adjuvant chemotherapy in early stage ER-positive breast cancer is usually based on clinico-pathological parameters, and has been recently improved by the introduction of prognostic expression profiles. Clinical or molecular features are efficient in identifying low-risk (pT1, histological grade 1, pN0, low RS, Luminal A subtype) and high-risk patients (>pT1, grade 3, N2, high RS). However, a large group of patients with intermediate clinical or molecular characteristic (grade 2, intermediate RS, luminal B) fall into the category between distinctly low and distinctly high risk and receive no treatment guidance from current decision tools.

Methods: From a population of 1929 chemo-naïve, hormone treated, luminal B (Her-2 negative), pT1-pT3, pN0-N1a breast cancer patients diagnosed and treated at the European Institute of Oncology from 1997 to 2005, we selected a random subcohort of 555 cases, in a case-cohort design. All the patients with local or distant metastasis which were not already included were added to the subcohort, leading to a total of 704 patients (208 with local or distant recurrences and 496 random controls). Luminal B status was determined by the immunohistochemical analysis of ER, PgR, HER2 and Ki-67, according to the 2011 St. Gallen criteria. FFPE sections of the primary tumor were analyzed for the mRNA expression of 43 genes by multiplex quantitative PCR. A molecular score (MS) was calculated from the average expression of 23 cell cycle progression genes, the average expression of seven lymphocyte specific genes and the expression of PR and ABCC5, based on a model derived from an independent training cohort. A combined score of MS and the clinical variables of tumor size, grade and node status was modeled in the training cohort and applied to the Luminal B set. The association between MS and the risk of distant metastasis was evaluated in a weighted multivariable Cox regression model, adjusted for traditional clinical factors and Ki-67 labeling index (LI).

Results: 640 samples, including 102 distant metastasis, had full clinical and expression data. In the 500 samples from the subcohort, median Ki67 LI was 21% (IQR=11%, Q1=16%, Q3=27%). Either one unit increase of Ki-67 LI (HR 1.06, 95%CI (1.04-1.08), p<0.0001) and of MS (HR 3.4, 95%CI (2.5-4.6), p<0.0001) were highly significant predictors of distant recurrence in univariable analysis. In multivariable analysis, the MS provided independent significant prognostic information after adjustment for Ki-67 LI, tumor size, grade and node status (HR 4.3, 95%CI (2.5-7.3), p=<0.0001). Using the combined score of MS and clinical variables, 383 patients or 77% of the subcohort had an estimated 10 year risk of distant recurrence of ≤10%. Similar results were obtained when samples were re-defined according to 2013 St. Gallen guidelines.

Conclusions: The MS provides important prognostic discrimination beyond traditional clinico-pathological characteristics, including Ki-67 LI, in Luminal B breast cancer, and contributes in identifying a subset of patients which may be successfully treated with endocrine therapy only.
Title: Low serum adiponectin level is an independent risk factor of DCIS in postmenopausal women at increased risk of breast cancer

Aliana Guerrieri-Gonzaga¹, Debora Macis¹, Sara Gandini¹, Valentina Aristarco¹, Harriet Johansson¹, Giorgia Bollani¹, Teresa Roth¹, Maria-Teresa Sandri¹, Jill Knox², Jack Cuzick² and Bernardo Bonanni¹. ¹European Institute of Oncology, Milan, Italy and ²Queen Mary University of London, London, United Kingdom.

Body: Background: The assessment of breast cancer (BC) risk is a key step for an effective preventive treatment. Besides the established risk assessment models, validation of independent predictive factors such as circulating biomarkers would improve patient selection and treatment efficacy.

Obesity and metabolic imbalance play an important role in BC risk in menopausal women. The role of adipocytes in energy homeostasis is currently under investigation for their emerging relationship with BC. Adipokines (such as leptin and adiponectin) are linked to insulin sensitivity and have been related to BC risk and prognosis. Adiponectin, a peptide hormone secreted by the adipose tissue, has been inversely related to BC risk both in observational studies and in a phase II chemoprevention trial in premenopausal women at increased risk.

Aim: We measured baseline serum adiponectin and leptin levels as well as HOMA index, in 534 postmenopausal women enrolled in 16 Italian centers and randomized in one of the two international phase III trials for BC prevention -the IBIS-II(Prevention) and IBIS-II(DCIS) trials- to assess whether these biomarkers were different in the healthy women at increased risk for BC cohort compared to the DCIS cohort.

Methods: Healthy postmenopausal women (aged 40-70) at increased risk for BC (on an age-dependent risk model; n=186) or DCIS patients who underwent radical surgery in the previous 6 months (n=348) were eligible according to the two separate protocol entry criteria. At baseline, fasting blood was collected, processed and stored at -80°C till biomarkers measurement. Insulin and glucose levels were measured with the Architect system (Abbott Laboratories, Abbott Park, IL 60064 USA). Serum adiponectin and leptin levels were determined with Immunoassays by R&D (Minneapolis, USA).

Results: Participant characteristics and biomarker levels (median, IQ range) by disease status are reported below.

<table>
<thead>
<tr>
<th></th>
<th>Healthy (n=186)</th>
<th>DCIS (n=348)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at entry</td>
<td>59 (55, 63)</td>
<td>60 (56, 65)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2 (22.9, 28.4)</td>
<td>25.0 (22.4, 28.1)</td>
</tr>
<tr>
<td>Adiponectin (ng/mL)</td>
<td>13063 (10279, 18157)</td>
<td>11498 (7722, 16909)</td>
</tr>
<tr>
<td>Leptin (pg/mL)</td>
<td>16181 (9594, 26391)</td>
<td>17284 (9675, 26173)</td>
</tr>
<tr>
<td>L/A ratio</td>
<td>1.33 (0.60, 2.09)</td>
<td>1.46 (0.69, 3.19)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>89 (81, 97)</td>
<td>93 (86, 103)</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>6.6 (5.0, 9.6)</td>
<td>7.5 (5.4, 10.6)</td>
</tr>
<tr>
<td>HOMA index</td>
<td>1.39 (1.04, 2.02)</td>
<td>1.79 (1.18, 2.61)</td>
</tr>
</tbody>
</table>

Adiponectin was significantly negatively correlated with leptin, L/A ratio, HOMA index and BMI, while leptin was positively correlated with L-A ratio, BMI, HOMA.

Logistic regression has been used and Odds Ratios (ORs) have been calculated to assess the association of DCIS with biomarkers. DCIS patients were significantly more frequent in the lowest quartile of adiponectin compared to the highest quartile (60% vs 75%; OR=2.49; 95% CI, 1.39-4.44, p= 0.0003) adjusting for center, BMI, HOMA index and age.

Conclusions: Low serum adiponectin levels in postmenopausal women are more frequent in DCIS patients compared to healthy at
risk subjects independently of BMI, HOMA index and age and results are similar to premenopausal women. Future investigations in both trials will assess whether adiponectin is also associated with BC events. 

Acknowledgments: J. Forbes and T. Howell, Co-Chairmen, IBIS-II Steering Committee.
Title: Validation of a multi-marker test that predicts recurrence in patients diagnosed with ductal carcinoma in situ (DCIS) treated with breast-conserving surgery (BCS)

Steven P Linke¹, Troy M Bremer¹, Pat Whitworth⁸, Alfred Lui³, Jess Savala³, Yelina Noskina³, Todd Barry⁶, Stephen Lyle², Stephanie C Walters², Karl Simin², Wenjing Zhou⁴, Karin Jirström⁵, Rose-Marie Amini⁴ and Fredrik Wärnberg⁴. ¹Prelude Corporation, Laguna Hills, CA; ²University of Massachusetts Medical School, Worcester, MA; ³PersonalizeDx, Lake Forest, CA; ⁴Uppsala University, Uppsala, Sweden; ⁵Lund University, Lund, Sweden; ⁶Spectrum Pathology, Mission Viejo, CA and ⁷Nashville Breast Center, Nashville, TN.

Body: Background: DCIS is diagnosed in ∼54,000 women/year in the US. Identifying patients who are most likely to recur has been deemed one of the most important unmet needs in breast cancer treatment. The goal of this study was to develop and blindly validate a multi-marker risk stratification test in DCIS patients treated with BCS.

Material and Methods: A variety of clinical, pathological, immunohistochemical, and in situ hybridization data were derived from a set of patients diagnosed with DCIS (without evidence of invasive breast cancer) between 1986 and 2004 in Uppland and Västmanland, Sweden—the Uppsala University Hospital (UUH) patient set. Separate models to predict DCIS and invasive event risk were developed using statistical pattern recognition and modeling methods on UUH patients treated with BCS in the absence of adjuvant therapy (n=200). In addition, an "overall" risk model was created by combining the DCIS and invasive models. The risk models consist of algorithms that combine data on p16/INK4A, Ki-67, COX2, PR, HER2, FOXA1, SIAH2, CD31, patient age, necrosis, tumor size, palpability, and/or margin status, along with predefined thresholds that create low, intermediate, and high risk groups ("elevated" risk groups combine the intermediate and high risk groups).

The models were then tested blindly on a set of patients diagnosed with DCIS and treated with BCS from 1999 through July 2008 at the University of Massachusetts Memorial Hospital, Worcester, MA (UMass patient set). Molecular marker data was collected with CLIA-validated assays and Board-certified pathologists, and other data was collected from medical records. Testing was done according to a predefined statistical analysis plan in the UMass patients equivalent to those in the UUH patient training set—those with complete marker data and treated with BCS, and were either PR-negative or not treated with hormone therapy (n=155). Event rates were assessed for up to 10-year outcome using Kaplan-Meier survival analysis. Hazard ratios (HRs) were determined using Cox proportional hazards analysis.

Results: The 10-year overall event rate in the full population was 10% (5% invasive plus 5% DCIS). For the invasive risk model, the low (n=53) and elevated (n=102) risk groups had 0% and 8% 10-year invasive event rates, respectively. For the DCIS risk model, the low (n=97) and elevated (n=58) risk groups had 1% and 13% 10-year DCIS event rates, respectively. For the overall risk model (combined risk of DCIS or invasive events), the low (n=34), intermediate (n=51), and high (n=70) risk groups had 0%, 12%, and 15% overall 10-year event rates, respectively (HR=4.1/bin, p=0.0088). The overall risk model maintained significance when adjusted for nuclear grade, tumor size, patient age, necrosis, and margin status, while none of these clinicopathologic factors demonstrated significance in the presence of the model.

Discussion: This study indicates that the present approach to risk stratification modeling can accurately identify patients at risk for DCIS or invasive events after a primary DCIS diagnosis. The models presented here are the basis of a comprehensive multi-marker panel undergoing formal validation.
Title: FOXA1 and PR predict ipsilateral event risk and identify a group with strong radiation response in ductal carcinoma in situ (DCIS)

Steven P Linke¹, Troy M Bremer¹, Pat Whitworth⁷, Alfred Lui³, Jess Savala³, Yelina Noskina³, Todd Barry⁶, Stephen Lyle², Stephanie C Walters², Karl Simin², Wenjing Zhou⁴, Karin Jirström⁵, Rose-Marie Amini⁴ and Fredrik Wärnberg⁴. ¹Prelude Corporation, Laguna Hills, CA; ²University of Massachusetts Medical School, Worcester, MA; ³PersonalizeDx, Lake Forest, CA; ⁴Uppsala University, Uppsala, Sweden; ⁵Lund University, Lund, Sweden; ⁶Spectrum Pathology, Mission Viejo, CA and ⁷Nashville Breast Center, Nashville, TN.

Body: Background: Identification of biomarkers in DCIS is critical to help guide treatment decisions, particularly for patients receiving breast-conserving surgery (BCS). The goal of this study was to assess FOXA1 levels in the context of PR status in primary DCIS to predict ipsilateral invasive and DCIS events, given the established roles of these endocrine signaling factors in breast cancer.

Material and Methods: Patients included in this study (n=518) were women diagnosed with DCIS without evidence of invasive cancer treated with BCS, and for whom tumor tissue was evaluable for both PR and FOXA1. An Uppsala University Hospital (UUH) set was diagnosed in 1986-2004 (117 treated and 122 not treated with adjuvant radiation therapy [RT]); and a University of Massachusetts Memorial Hospital (UMass) set was diagnosed in 1999-2008 (195 treated and 84 not treated with RT). Tumors were immunohistochemically stained for PR and FOXA1 and scored by pathologists for percentage (0-100) and intensity (0-3), with the product being calculated for FOXA1 immunoscore (0-300). Patients were considered PR+ when ≥10% of cells stained positively, and immunoscore thresholds of 100 and 270 were used to separate patients into FOXA1 low, intermediate, and high groups. Event rates were assessed for 10-year outcome using Kaplan-Meier survival analysis. Hazard ratios (HR) were determined using Cox proportional hazards analysis.

Results: Neither FOXA1 nor PR were prognostic as independent factors for either invasive or DCIS event risk. However, in PR-patients, the invasive event rate decreased with increasing FOXA1 (24%, 4%, and 0%, respectively, in the FOXA1 low, intermediate, and high groups, HR=6.7/bin, p=0.0034), and, in PR+ patients, the invasive event rate increased with increasing FOXA1 (0%, 10%, and 13%, respectively, HR=2.95/bin, p=0.041). In contrast, the DCIS event rate increased in PR- patients with increasing FOXA1 (3%, 12%, and 26%, respectively, HR=2.7/bin, p=0.020), and the DCIS event rate was lower in PR+ patients with elevated FOXA1 levels (28% in FOXA1 low vs. 8% in FOXA1 intermediate plus high, HR=4.0, p=0.011).

In the full population, RT-treated patients (n=312) fared better than the unirradiated (n=206) with invasive event rates of 6% and 10%, respectively (HR=0.41, p=0.021). By comparison, the patients with high marker-based invasive event risk (PR-/FOXA1 low and PR+/FOXA1 high, n=191) had a remarkable response to RT--event rate reduced from 19% to 4% (HR=0.13, p<0.001). In contrast, the remainder of patients (n=327) showed no evident RT response--event rate increased from 5% to 6%. In an interactional Cox analysis, the high-risk group (HR=5.0, p=0.0045) and RT response within that group (HR=0.10, p=0.020) were significant, but baseline RT response was not (p=0.72).

Discussion: These results indicate a complex interaction between PR and FOXA1, in which the prognosis for invasive and DCIS events flips both within and between the event types. Thus, the biology that drives these events differs and, in order to predict both event types, risk models must include biomarkers in context. In addition, PR/FOXA1 identify a risk group with remarkably strong RT response with the remaining patients exhibiting no measurable response.
**Title:** Integration of breast cancer index (BCI) with clinicopathological factors for prediction of distant recurrence in ER+ breast cancer

Ivana Sestak¹, Yi Zhang², Brock E Schroeder², Paul E Goss³, Mitch Dowsett⁴, Dennis C Sgroi⁴, Catherine A Schnabel² and Jack Cuzick¹.

¹Centre for Cancer Prevention, Queen Mary University, London, United Kingdom; ²bioTheranostics, Inc, San Diego, CA; ³Massachusetts General Hospital, Boston, MA and ⁴Royal Marsden Hospital, London, United Kingdom.

**Body:**

**Background:** BCI is a genomic signature that significantly predicts risk of both early (0-5 years) and late (5-10 years) distant recurrence (DR) in hormonal receptor-positive, lymph node negative (LN-) breast cancer. In LN- disease, tumor size (TS) and grade (TG) are important independent prognostic factors of breast cancer recurrence. In this analysis, the effect of integrating these traditional clinicopathological factors on the ability of BCI to predict distant recurrence was evaluated in the translational arm of the Arimidex, Tamoxifen, Alone or in Combination trial (TransATAC).

**Methods:** 709 primary tumor samples from hormonal receptor-positive, LN- patients treated with 5 years of tamoxifen or anastrozole were examined. Multivariate Cox proportional hazards regression was used to fit 2 models: 1) BCI+TS; 2) BCI+TS+TG. To facilitate comparison across models, cut points were chosen based on the pre-specified 10 year DR rates of <10% (low), 10-20% (intermediate) and >20% (high). Kaplan-Meier (KM) estimates of 10 year DR and hazard ratios (HR) were examined. Change in likelihood ratio $\chi^2$ ($\text{LR-}\chi^2$) values were used to quantitate relative prognostic information beyond standard clinicopathological variables (CTS, Clinical Treatment Score).

**Results:** In the univariate analysis, all models were significantly prognostic for 10 year DR risk; BCI was somewhat less predictive vs BCI+TS, whereas TG did not provide significant additional value beyond BCI+TS [HR (95% CI): 3.26 (2.29-4.63), 2.72 (2.11-3.50), 2.72 (2.11-3.50); LR-\(\chi^2\) (p value): 45.54 (p<0.0001), 54.71 (p<0.0001), 57.27 (p<0.0001) for BCI, BCI+TS, BCI+TS+TG, respectively]. Adjusted HRs beyond CTS demonstrated highly significant and comparable prognostic ability [HR (95% CI): 2.35 (1.61-3.42), 2.06 (1.48-2.86); LR-\(\chi^2\) (p value): 20.75 (p<0.0001), 18.96 (p<0.0001)] for BCI and BCI+TS, respectively. BCI and BCI+TS categorized similar proportions of patients into respective risk groups, and KM analysis comparing BCI vs BCI+TS risk categories showed similar rates of 10 year DR (Table 1).

**Comparison of Risk Categorization and 10 year DR**

<table>
<thead>
<tr>
<th></th>
<th>BCI</th>
<th>BCI+TS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Patients</td>
<td>10 year DR</td>
</tr>
<tr>
<td>Low Risk</td>
<td>53.7%</td>
<td>5.3%</td>
</tr>
<tr>
<td>Intermediate Risk</td>
<td>29.3%</td>
<td>15.5%</td>
</tr>
<tr>
<td>High Risk</td>
<td>17.0%</td>
<td>28.0%</td>
</tr>
</tbody>
</table>

In cross stratification analysis between BCI and BCI+TS, no significant re-classification was observed (Table 2), however 14.5% of BCI and 9.7% of BCI+TS intermediate risk patients were re-stratified as low risk.

**Re-classification of BCI and BCI+TS for all patients**

<table>
<thead>
<tr>
<th>BCI+TS</th>
<th>Low</th>
<th>Intermediate</th>
<th>High</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>358</td>
<td>30</td>
<td>0</td>
<td>388 (54.7%)</td>
</tr>
<tr>
<td>Intermediate</td>
<td>20</td>
<td>163</td>
<td>23</td>
<td>206 (29.1%)</td>
</tr>
<tr>
<td>High</td>
<td>3</td>
<td>14</td>
<td>98</td>
<td>115 (16.2%)</td>
</tr>
</tbody>
</table>
Discussion: Integration of tumor size, but not grade, statistically enhanced the prognostic ability of BCI to predict 10 year DR risk in patients with ER+, LN- early stage breast cancer. However, there was limited clinical impact on risk stratification, indicating that the prognostic information provided by these clinicopathological factors is effectively captured by BCI alone.
Body: Background: Since its introduction in 2004, the 21 gene assay (Oncotype Dx recurrence score [ODXRS], Genomic health Inc., Redwood City CA) has been endorsed by ASCO and routinely used in guiding clinical decisions on chemotherapy for ER positive, node negative breast cancer patients. Many investigators have tried to identify more effective and economical methods that have predictive powers similar to the 21 gene assay, using readily available, high quality clinical-pathological information from breast cancer patients. In 2013, Klein et al reported that the Estimated Recurrence Scores (ERS) from three pathology-generated equations derived by linear regression analysis (Magee equations) demonstrate relatively high concordance with the ODXRS. The current study aims to evaluate the clinical and prognostic utility of the three Magee equations. Methods: 560 ER positive patients with clinical follow-up information and diagnosed between 1997 and 2012 in our institution were included. Attempts were made to obtain the Modified ERS from all three Magee equations, which were calculated from Nottingham grade (3-9), modified H-score of ER and PR (average staining intensity x percent positive tumor cells, 0-300), HER2 (negative, Equivocal, positive), tumor size (cm), and % labeling for Ki-67; and a mean modified ERS from each case was used for statistical analysis. Results: For this cohort of patients, the mean age was 60 years, with a mean tumor size of 2.17cm, 71% of the tumors were histologic grade 1 and 2, 63% were node negative. All patients were ER positive, 85% were PR positive, and 10% were HER2 positive. The Modified ERS was not associated with patient age, tumor size, and histologic grade; however, nuclear grade and expression of ER, PR, HER2 and Ki-67 were significantly associated with the Modified ERS. Although nodal status was not included in these equations, high Modified ERS (>30) was strongly associated with nodal status, with only 19% for node negative tumors, and 33%, 40%, 58% for node positive tumors, with positive nodes ranging 1-3, 4-9, and 10 and greater, respectively. Among the three components of Nottingham grade, only nuclear grade showed significant association with Modified ERS. Only 4% of ER 71-100%, 2% of PR 71-100%, 8% of HER2 negative, and 5% of Ki-67 <15% had Modified ERS greater than 30. This Modified ERS was significantly associated with progression free survival for this entire cohort of patients (p-value 0.0031) and with node negative patients (p value=0.0069), but not with node positive patients (0.8697). Conclusion: Our data demonstrates that Modified ERS can effectively stratify ER positive patients into different prognostic subgroups, especially in node negative patients. Further larger studies are warranted to study its possible role in node positive patients.
Title: Prevalence of circulating tumor cells (CTCs) after five years of zoledronate treatment in the adjuvant SUCCESS-A study

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¹Ludwig-Maximilians-University, Munich; ²University of Erlangen; ³University of Ulm; ⁴Luisenkrankenhaus Düsseldorf; ⁵Diakoniekrankenhaus Henriettenstiftung Hannover; ⁶Practice Prof. Tesch Frankfurt; ⁷Practice for Obstetrics and Gynecology Fuerstenwalde; ⁸Practice Dr. Forstbauer Troisdorf; ⁹University of Duesseldorf; ¹⁰University Hamburg-Eppendorf; ¹¹University of Heidelberg and ¹²University Medical Center Charite, Berlin, Germany.

Body: Aim: The prognostic value of CTCs at primary diagnosis has recently been confirmed by the SUCCESS A Study (Rack et al. JNCI 2014). Key questions on the role of adjuvant bisphosphonate treatment, including patient populations deriving benefit and optimal timing/scheduling of therapy are still controversial. Aim of this study was therefore to evaluate CTCs in the context of two different zoledronate regimens.

Methods: The SUCCESS A trial is a large, randomized, open-label, 2x2 factorial design Phase III study in patients with high risk breast cancer (stage N1 or T2–T4 or grade 3 or age ≤ 35 or hormone-receptor negative). Patients were first randomized to adjuvant chemotherapy treatment with 3 cycles of Epirubicin-Fluorouracil-Cyclophosphamide (FEC) followed by either 3 cycles of Docetaxel or 3 cycles of Gemcitabine-Docetaxel. In addition, patients were randomized to 2 years vs. 5 years of zoledronate treatment. CTC status 5 years after primary diagnosis was assessed using the FDA-approved CellSearch System (Veridex, USA), and CTC positivity was defined as ≥ 1 CTC. We studied the influence of zoledronate treatment duration on CTC prevalence at 5 years in addition to well-known patient and tumour characteristics using a multiple logistic regression analysis on the ITT population.

Results: Data on CTC status at 5 years after primary diagnosis were available for 728 (19.4%) out of 3754 randomized patients. 310 patients had been randomized to 2 years of zoledronate treatment and 418 patients had been randomized to 5 years of zoledronate treatment. In these patients a difference in CTC positivity after 5 years could not be shown between patients randomized to 2 or 5 years of zoledronate (p = 0.13, Wald test). The adjusted odds ratio for 2 years vs 5 years was 0.65 (95%CI: 0.37 to 1.13).

Conclusions: The final survival analysis of the SUCCESS A trial will give further insight, whether CTCs can be used as early predictive marker for the efficacy of adjuvant zoledronate treatment.
High expression of LMTK3 is an independent factor indicating a poor prognosis in Japanese estrogen receptor α-positive breast cancer patients

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Body: Over 70% of breast cancers are estrogen receptor α (ERα)-positive, and endocrine therapy targeting estrogen action decreases mortality from breast cancer. However, their efficacy of endocrine therapy is limited by intrinsic and acquired resistance. Recently, a novel protein kinase that regulates ERα activity, lemur tyrosine kinase-3 (LMTK3) has been identified. In this study, we investigated whether LMTK3 mRNA expression and its polymorphisms are associated with prognosis in Japanese breast cancer patients during long-term follow-up. First, we investigated the relationship between mRNA expression of LMTK3 and patient outcome in 242 breast cancers (median follow-up, 6.8 years). The effects of several variables on survival were tested by Cox proportional hazards regression analysis. Next, we performed to analyze LMTK3 rs9989661 and rs8108419 genotyping in 641 breast cancer tissues (median follow-up, 9.2 years) to clarify the prognostic role of these polymorphisms. We showed that high expression levels of LMTK3 mRNA were significantly associated with a shorter overall survival (OS) in all patients. We then, analyzed the impact of LMTK3 mRNA expression on the prognosis of breast cancer according to ERα status. Both disease-free survival and OS were significantly shorter in ERα-positive patients with high LMTK3 mRNA expression receiving adjuvant endocrine therapy than in those patients with low LMTK3 mRNA expression. Notably, the level of LMTK3 mRNA expression was not associated with prognosis in ERα-negative breast cancer patients. Univariate and multivariate Cox regression analysis of factors associated with OS revealed that high LMTK3 mRNA expression was an independent poor prognostic factor in ERα-positive breast cancer patients.

Univariate and multivariate Cox regression analysis of factors associated with overall survival in ERα-positive breast cancer patients

<table>
<thead>
<tr>
<th>Variables</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables n (%)</td>
<td>P value</td>
<td>P value</td>
</tr>
<tr>
<td>Tumor size</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 2cm</td>
<td>51 (31)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>&gt; 2cm</td>
<td>112 (67)</td>
<td>0.264</td>
</tr>
<tr>
<td>Node status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>79 (47)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>Positive</td>
<td>72 (43)</td>
<td>0.003</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,2</td>
<td>128 (77)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>3</td>
<td>27 (16)</td>
<td>0.485</td>
</tr>
<tr>
<td>LMTK3 mRNA expression</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>129 (77)</td>
<td>1 (reference)</td>
</tr>
<tr>
<td>High</td>
<td>38 (22)</td>
<td>0.002</td>
</tr>
</tbody>
</table>

HR, hazard ratio; CI, confidence interval.

We did not find any correlation between LMTK3 genotypes and prognosis of breast cancer patients in our series. Our results suggest that higher LMTK3 expression might be one possible mechanism for endocrine resistance in ERα-breast cancer patients.
to be confirmed by further research.
Membranous ERα-36 expression is an independent predictor of poor prognosis in operable breast cancer

Loay Kassem¹, Soleilmane Omarjee², Sylvie Chabaud³, Emilie Lavergne³, Christelle Faure⁴, Frédéric Beurrier⁴, Olivier Tredan⁵, Laura Corbo², Isabelle Treilleux⁶ and Muriel Le Romancer². ¹Cairo University Teaching Hospital, Cairo, Egypt; ²Inserm U1052/CNRS UMR5286/University of Lyon, Centre de Recherche en Cancérologie de Lyon, Lyon, France; ³Biostatistics Unit, Centre Léon Bérard, Lyon, France; ⁴Centre Léon Bérard, Lyon, France; ⁵Centre Léon Bérard, Lyon, France and ⁶Centre Léon Bérard, Lyon, France.

Background
ERα-36 is a splice variant of ER-α with molecular weight of 36-kDa that lacks transactivation domains, and is expressed in the cytoplasm and cell membrane of ER (ERα66) negative as well as ERα66 positive breast cancer cells. It is also thought to predict resistance to tamoxifen therapy. Here we investigate its prognostic significance, its association with other clinico-pathologic factors and correlation with other biomarkers of the PI3K/AKT/mTOR pathway.

Methods
We studied ERα-36 expression on TMA blocks prepared from samples of 160 consecutive operable breast cancer patients who presented at CLB between 1998 and 2001. The intensity of the staining and the percentage of tumor cells stained for each biomarker (ERα-36, PI3K, pAKT, p4EBP1, pS6RP and LKB1) were integrated into a single score and a cutoff was defined for high versus low expression. Correlations were done between ERα-36 expression and the clinico-pathological parameters and other biomarkers using Pearson’s chi-square test. Kaplan-Meier method was used to estimate distant metastasis free survival (DMFS), disease free survival (DFS) and overall survival (OS) and the difference between the groups was evaluated with log-rank test. Cox regression model was used to adjust for other prognostic parameters in the multivariate analysis.

Results
Median age at diagnosis was 56.9 years (range: 30 to 87 years). The maximum tumor size was larger than 2 cm in 57.5% of cases and axillary lymph nodes (LN) were positive (N1a to N3) in 52.5% of cases. 16.3% of the patients had SBR grade I, 44.4% had grade II and 39.4% had grade III tumors. ERα66 was positive in 91.2%, PgR in 74.7% and HER2 was over-expressed in 15% of the cases. High ER-α36 expression in the cell membrane was observed in 65 patients (40.6%). ERα-36 expression was independent of the ERα66, PgR or HER2 expression and was not associated with age, tumor size, SBR grade or axillary LN invasion. There was no correlation between ERα-36 expression and PI3K, pAKT, p4EBP1, pS6RP or LKB1 expression. ERα-36 expression in tumor cells was a predictor of poor prognosis regarding DMFS (HR=2.02; 95% CI: 1.2 to 3.4; p=0.008), DFS (HR=1.7; 95% CI: 1.05 to 2.7; p=0.031) and OS (HR=1.8; 95% CI: 1.02 to 3.2; p=0.043). In the multivariate analysis and after adjustment for age, tumor size, SBR grade and LN invasion, ERα-36 remained an independent predictor of shorter DMFS (p=0.016) and DFS (p=0.052) in addition to SBR grade and axillary LN metastasis. The ERα-36 expression predicted shorter DMFS for patients who received tamoxifen as the only adjuvant systemic treatment (p=0.022) and also for those who received other hormonal therapy and adjuvant chemotherapy (p=0.039).

Conclusion
Immunohistochemically detected membranous ERα-36 expression can be a poor prognostic factor for patients with operable breast cancer that is independent from the traditional clinico-pathologic parameters and from PI3K/AKT/mTOR pathway activation status.
Title: TIF1γ interacts with the TGFβ1/SMAD4 signaling leading to poorer outcome in operable breast cancer

Loay Kassem¹, Laurent Fattet², Jonathan Lopez², Goulvent Thibault², Emilie Lavergne³, Sylvie Chabaud³, Nicolas Carrabin⁴, Nicolas Chopin⁴, Thomas Bachelot⁵, Germain Gillet⁴, Isabelle Treilleux⁶ and Ruth Rimohk³. ¹Cairo University Teaching Hospital, Cairo, Egypt; ²Inserm U1052/CNRS UMR5286/Université de Lyon, Centre de Recherche en Cancérologie de Lyon, Centre Léon Bérard, Lyon, France; ³Biostatistics Unit, Centre Léon Bérard, Lyon, France; ⁴Centre Léon Bérard, Lyon, France; ⁵Centre Léon Bérard, Lyon, France and ⁶Centre Léon Bérard, Lyon, France.

Body: Background
The Transforming growth factor β (TGFβ) signaling has a paradoxical role in cancer development and outcome. It protects against tumorigenesis by inhibiting cell growth and promoting apoptosis, but at advanced stages, it promotes tumor progression. Besides, the prognostic significance of the TGFβ1, SMAD4 in breast cancer patients is also an area of many contradictions. Transcriptional intermediary factor 1γ (TIF1γ) is thought to interact with the TGFβ/SMAD signaling through different mechanisms. Our study aimed at defining the prognostic significance of TGFβ1, SMAD4 and TIF1γ expression in breast cancer patients in addition to detection of possible interactions among those markers that might affect the outcome and explain the contradictory results.

Methods
Immunohistochemistry was performed on TMA blocks prepared from samples of 248 operable breast cancer patients who presented at CLB between 1998 and 2001 using. The intensity and the percentage of stained tumor cells were integrated into a single score (0-6) and a cutoff was defined for high or low expression for each marker. Correlation was done between the TGFβ1, SMAD4 and TIF1γ expression with the clinico-pathologic parameters using Pearson’s chi-square test. Kaplan-Meier method was used to estimate distant metastasis free survival (DMFS), disease free survival (DFS) and overall survival (OS) and the difference between the groups was evaluated with log-rank test.

Results
223 cases were assessable for TIF1γ, 204 for TGFβ1 and 173 for SMAD4. Median age at diagnosis was 55.8 years (range: 27 to 89 years). Tumors were larger than 20 mm in 49.2% and 45.2% had axillary lymph node (LN) metastasis (N1a to N3). 19.4% of the patients had SBR grade I tumors, 46.8% grade II tumors and 33.9% grade III tumors. ER was positive in 85.4%, PR in 75.5% and Her2-neu was over-expressed in 10% of the cases. Nuclear TIF1γ, cytoplasmic TGFβ1, nuclear and cytoplasmic SMAD4 staining was high in 35.9%, 30.4%, 27.7% and 52.6% respectively. TIF1γ expression was associated with younger age (p=0.006), higher SBR grade (p<0.0001), more ER negativity (p=0.035), and tumors larger than 2 cm (p=0.081), while TGFβ1 was not associated with any of the traditional prognostic factors.

TGFβ1 expression in tumor cells was a marker of poor prognosis regarding DMFS (HR=2.28; 95%CI: 1.4 to 3.8; p=0.002), DFS (HR=2.00; 95% CI: 1.25 to 3.5; p=0.005) and OS (HR=1.89; 95%CI: 1.04 to 3.43; p=0.037). TIF1γ expression carried a tendency towards poorer DMFS (p=0.091), DFS (p=0.143) and OS (p=0.091). In the multivariate analysis TGFβ1 remained an independent predictor of shorter DMFS, DFS and OS after adjustment for age, tumor size, SBR grade and LN invasion. Moreover, the prognostic significance of TGFβ1 was more obvious in the TIF1γ high patient subgroup than in the patients with TIF1γ low expression. The subgroup expressing both markers had the worst DMFS (HR=3.2; 95%CI: 1.7 to 5.9; p<0.0001), DFS (HR=3.02; 95%CI: 1.6 to 5.6; p<0.0001) and OS (HR=2.7; 95%CI: 1.4 to 5.4; p=0.005).

Conclusion
There is a crosstalk between the TIF1γ and the TGFβ1/SMAD4 signaling that deteriorate the outcome of operable breast cancer patients and when combined together they can serve as an effective prognostic tool for those patients.
Title: Reappraisal of conventional risk stratification for local recurrence based on clinical outcomes in 285 resected phyllodes tumors of the breast

Cha Kyong Yom¹, Wonshik Han², Sung-Won Kim³, So Yeon Park⁴, In-Ae Park⁵ and Dong-Young Noh². ¹Myongji Hospital, Goyang, Korea; ²Seoul National University College of Medicine, Seoul, Korea; ³Seoul National University Bundang Hospital, Seongnam, Korea; ⁴Seoul National University Bundang Hospital, Seongnam, Korea and ⁵Seoul National University College of Medicine, Seoul, Korea.

Body: Background A second resection has been recommended, to ensure a surgical margin of ≥ 1cm for the effective treatment of PTB, but the outcomes of an extensive series of cases casts this clinical approach in doubt. Objective To identify the local recurrence risk factors of phyllodes tumor of the breast (PTB) and determine future optimal surgical treatment according to verified risks. Methods All cases given a diagnosis of PTB and resected between 1989 and 2008 were retrospectively evaluated. Clinicopathologic data and clinical outcomes were analyzed and stratified according to the risks for local recurrence (LR). Results All 285 cases occurred and 200 (70.2%) categorized as benign, 51 (17.9%) as borderline, and 34 (11.9) as malignant. Median follow-up was 6.7 years and during follow-up, there were 20 LRs. All benign PTBs recurred as benign PTB lesions. Mitosis (p = 0.007) and tumor size (p = 0.029) were independent prognostic factors for LR in multivariate analysis. Neither margin status (p = 0.773) nor type of surgery (p = 0.922) had any significance for LR.

<table>
<thead>
<tr>
<th>Subgroup of patients</th>
<th>No recurrence(%)</th>
<th>Recurrence(%)</th>
<th>Univariate</th>
<th>Multivariate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>HR p</td>
<td>HR p</td>
<td></td>
</tr>
<tr>
<td>Mitosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1–4/10HPF</td>
<td>170(96.0)</td>
<td>7(4.0)</td>
<td>0.025</td>
<td>0.007</td>
</tr>
<tr>
<td>5–9/10HPF</td>
<td>33(84.6)</td>
<td>6(15.4)</td>
<td>4.259</td>
<td>0.009</td>
</tr>
<tr>
<td>≥10/10HPF</td>
<td>15(88.2)</td>
<td>2(11.8)</td>
<td>3.461</td>
<td>0.122</td>
</tr>
<tr>
<td>Size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤2cm</td>
<td>75(98.7)</td>
<td>1(1.3)</td>
<td>0.035</td>
<td>0.029</td>
</tr>
<tr>
<td>2cm&lt; and ≤5cm</td>
<td>136(89.5)</td>
<td>16(10.5)</td>
<td>8.599</td>
<td>0.037</td>
</tr>
<tr>
<td>&gt;5cm</td>
<td>51(98.1)</td>
<td>1(1.9)</td>
<td>1.564</td>
<td>0.752</td>
</tr>
<tr>
<td>Operation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WLE</td>
<td>229(93.1)</td>
<td>17(6.9)</td>
<td>0.957</td>
<td>0.922</td>
</tr>
<tr>
<td>VABS</td>
<td>23(92.0)</td>
<td>2(8.0)</td>
<td>1.247</td>
<td>0.769</td>
</tr>
<tr>
<td>Mastectomy</td>
<td>13(92.9)</td>
<td>1(7.1)</td>
<td>1.055</td>
<td>0.959</td>
</tr>
<tr>
<td>Margin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clear</td>
<td>200(93.5)</td>
<td>14(6.5)</td>
<td>0.846</td>
<td>0.773</td>
</tr>
<tr>
<td>Close/Involvement</td>
<td>39(92.9)</td>
<td>3(7.1)</td>
<td>1.132</td>
<td>1.253</td>
</tr>
</tbody>
</table>

In the risk stratification for LR, PTBs sized 2 - 5cm with ≥ 10 mitoses / 10 HPF had the highest LR rate (60%) compared with all other groups (p < 0.001). Conclusions Only PTB with 2 - 5 cm and frequent mitoses, is it recommended to follow to ascertain a wide excision and clear margin of 1 cm, it necessary by means of a 2nd surgery could be considered to avoid the risk of LR in this distinct group.
Title: Neutrophil-to-lymphocyte ratio is correlated with response to neoadjuvant chemotherapy as an independant prognostic indicator in breast cancer patients

Yi Chen¹, Yan Nie¹, Xiaoyun Xiao¹ and Erwei Song¹. ¹Sun Yat-sen Memorial Hospital, Sun Yat-sen University, Guangzhou, Guangdong, China.

Body:

Background
High neutrophil-to-lymphocyte ratio (NLR) may be related to increased mortality in patients with lung, colorectal, stomach, liver, and pancreatic cancer. To date, the utility of NLR to predict the response to neoadjuvant chemotherapy (NAC) has not been studied. Therefore, the aim of our study was to determine whether the NLR is a predictor of response to NAC and to investigate the prognostic impact of the NLR in patients of breast cancer who received NAC, in view of disease-free survival (DFS) and overall survival (OS).

Methods
We retrospectively studied patients who received NAC and subsequent surgical therapy for stage II–III invasive breast carcinoma treated at Sun Yat-sen Memorial Hospital between 2001 and 2010. The correlation of NLR with pathological complete response (pCR) rate of invasive breast cancer to NAC was analyzed. Survival analysis was used to evaluate the predictive value of NLR.

Results
A total of 215 patients were eligible for analysis. The pCR rate in patients with lower NLR(NLR<2.05) is higher than those with higher NLR(2.05≤NLR) (24.5% vs.14.3%, p=0.042). Those with higher NLR (NLR≥2.1) were statistically significantly had more advanced stages of cancer and higher disease-specific mortality. Through a multivariate analysis, including all known predictive clinicopathologic factors, NLR≥2.1 was a significant independent parameter for DFS (HR:1.57, 95%CI:1.05-3.07, p=0.028) and OS (HR:2.21,95%CI=1.01-4.39, p=0.047). Patients with higher NLR (NLR≥2.1) showed significantly lower disease-free survival rate and overall survival rate than those with lower NLR (NLR <2.1)(Log-rank p=0.0186 and 0.0242, respectively).

Conclusion
NLR<2.05 is statistically asso-ciated with pCR rate, suggesting that NLR may be an important factor predicting a response to NAC in breast cancer patients. NLR is an independent predictor of DFS and OS in breast cancer patients with NLR≥2.1 who received NAC. We suggest prospective studies to evaluate NLR as a simple prognostic test for breast cancer.
Title: Prospective comparison of conventional clinicopathological factors, uPA/PAI-1 and EndoPredict®-clin score (EPclin) for adjuvant clinical decision making in ER-positive, HER2-negative breast cancer: Progesterone receptor expression is strongly associated with risk stratification according to EP clin

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Body: Background: Recently, EPclin, a second generation multigene test has been introduced into clinical practice. Aim of this prospective study is to compare conventional clinicopathological factors, uPA/PAI-1 and EPclin and to determine, how these parameters influence adjuvant treatment decisions. Methods: 217 consecutive cases of ER-pos, HER2neg, breast cancer cases were enrolled in this study. Conventional clinicopathological factors, uPA/PAI-1 and EPclin were obtained by central pathology assessment of the patients' surgical specimen. Decision for type of adjuvant treatment was made twice – once with and once without EPclin result- after case discussion in an interdisciplinary tumor conference. Results: 217 Patients (pt) have been evaluated. Tumor grading within the presented cohort was as follows: G1: 44 pt (20%), G2: 146 pt (68%), G3: 27 pt (12%). 59 pt (27%) had positive axillary lymph node involvement. 57 pt (26%) were pT1a/b. EPclin could be assessed in all patients. 85 pt (39%) were classified as "high risk" whereas 132 pt (61%) were classified as "low risk". uPA/PAI was obtained from 123 pt (57%). 64 pt (52%) out of these had high uPA/PAI-1 levels whereas 59 pt (48%) showed low uPA/PAI-1 levels. Level of progesterone receptor (PR) expression was obtained from 216 pt. 39 pt (18%) had low or absent PR expression (PR ≤ 20%). 26 pt (67%) of this low PR expression group were classified as EPclin high risk. Furthermore 118 pt (67%) of the high PR expression group (177 pt) were classified as "low risk" via EPclin (p<0,0001). In 89 cases (41%) treatment decision was influenced by EPclin: In 73pt (34%) adjuvant chemotherapy (ctx) was omitted whereas in 16 pt (7%) ctx was added following the EPclin result. Conclusions: This prospective study shows for the first time, that PR expression is strongly associated with risk score evaluated by EPclin testing. In comparison with uPA/PAI-1 and conventional clinicopathological factors EPclin is the more powerful tool to help reduce unnecessary adjuvant chemotherapy.
Title: Clinical significance of adiponectin receptor 1 (AdipoR1) expression in invasive breast cancer

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Body: Background: Recent studies have demonstrated that obesity is associated with an increased risk of breast cancer, but the mechanisms underlying this relationship remain to be fully elucidated. Adiponectin is one of major adipokines secreted from adipose tissue. Adiponectin serum levels have been shown to be inversely correlated with breast cancer risk. This protein is believed to act through AdipoR1 and has been suggested to play an important role in cancer development. The purpose of this study was to quantitatively evaluate the expression of AdipoR1 in invasive breast cancer tissue compared to normal breast tissue. And then, we analyzed clinical significance of AdipoR1 in invasive breast cancer.

Materials and Methods: Tissues were obtained from 269 patients who underwent curative surgery with no prior treatment for invasive ductal carcinoma from Jan. 2003 to Dec. 2008 in Hallym Sacred Heart Hospital. A tissue microarray (TMA) containing 269 invasive ductal carcinomas as well as 269 adjacent normal breast tissues was established from paraffin-embedded archived tissue for further analysis. AdipoR1 expression was investigated in epithelium and stroma by immunohistochemistry, using 1:1600 dilution of rabbit anti-adiponectin receptor antibody, and correlated with clinical and pathologic tumor parameters.

Results: In 269 patients, median follow-up period was 57 months. AdipoR1 was detected in epithelial and stromal component of both normal breast and invasive ductal carcinoma tissues. In epithelium, immunoreactivity for AdioR1 was much lower in cancer tissue than normal one (24.5% versus 72%). This trend was quite similar in stroma, although the gap between cancer and normal was a bit narrow (48.7% versus 77.6%). AdipoR1 was more expressed in stroma than in epithelium among invasive breast cancer (48.7% versus 24.5%). In clinicopathologic features, mean age at diagnosis of AdipoR1 expression group in both epithelium and stroma was older than negative group. Furthermore, in epithelial component, HER-2/neu overexpression rate was slightly higher in AdipoR1 negative group than in positive one (27.8% versus 15%, p=0.059). And, Ki67 was more expressed in AdipoR1 negative group than in positive one (49.5% versus 34.5%, p=0.051). However, in stroma component, there was no other difference between AdipoR1 expression and clinicopathologic parameters. In survival analysis, AdipoR1 expression group in stroma showed significantly better disease free survival (DFS) than negative group (p=0.02). DFS curve according to AdipoR1 expression in epithelium showed a quite similar trend with ones in stroma (p=0.06).

Conclusions: This study showed that AdipoR1 expression was suppressed in both epithelium and stroma of invasive breast cancer tissue compared to normal breast tissue, and AdipoR1 expression group in stroma showed significantly better DFS than negative group. Although it is earliest days yet to make conclusion, loss of AdipoR1 expression in stroma, possibly epithelium either, appears to play a role of independent risk factor to predict disease progression, somehow.
Title: Quantitative measurement of HER2 levels by multiplexed mass spectrometry from FFPE tissue predicts survival in patients treated with anti-HER2 based therapy

Paolo Nuciforo¹, Sheeno Thyparambil², Claudia Aura¹, Ana Garrido-Castro¹, Vicente Peg¹, Jose Jimenez¹, William Hoos², Jon Burrows², Todd Hembrough², Jose Perez-Garcia¹, Javier Cortes¹ and Maurizio Scaltriti³. ¹Vall d'Hebron Institute of Oncology, Barcelona, Spain; ²Oncoplex Diagnostics, Rockville, MD and ³Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Introduction
Approximately 20% of breast cancer patients overexpress HER2 and are treated with anti-HER2 therapies. However, there is a great deal of disparity of HER2 levels in the patients that are classified as HER2 positive (IHC3+). Techniques like FISH or IHC do not allow for HER2 quantification and a significant proportion of patients are wrongly classified as HER2 positive. Liquid Tissue-Selected reaction monitoring (LT-SRM) is a multiplexed mass spectrometric technique that can objectively quantify levels of Her2 and other targets from formalin fixed paraffin embedded (FFPE) sections. Given the different available anti-HER2 therapies (trastuzumab, TDM1, lapatinib and pertuzumab) with different modes of action, it would be beneficial for a clinician to understand the levels EGFR and HER3 so as to personalize the therapy. In this work, we have used LT-SRM to quantitate HER2, EGFR and HER3 from FFPE samples (one slide) of patients treated with anti-HER2 agents and correlated the levels of these proteins to clinical outcome.

Methods
FFPE sections from 60 HER2 positive (IHC3+) primary breast cancer patients were microdissected and proteins were solubilized and digested by trypsin in Liquid Tissue® buffer. Of the 60 samples, 24 were from metastatic setting and 36 from adjuvant setting. After trypsin digestion, internal standards were added and absolute quantitation for multiple proteins was performed using selected reaction monitoring (SRM) mass spectrometry. In addition to LT-SRM, FISH for HER2 was also conducted.

Results
HER2 quantitation by LT-SRM revealed receptor level ranges from 283 to 14938 amol/µg. ROC analysis was conducted and a cut-off of 2758.75 amol/µg gave the optimal sensitivity and specificity. Survival analysis revealed statistically significant DFS (4.40 years vs 3.38 years; p=0.013) and OS (4.43 years vs 4.03 years; p=0.039) in patients expressing ≥ 2758.75 amol/µg in the adjuvant setting and also statistically significant OS (5.51 years vs 3.37 years; p=0.037) in the metastatic setting. Correlation of HER2 FISH and levels of HER2 is ongoing. Approximately 41% of samples expressed EGFR (range 45 to 2317 amol/µg) and similarly 51% of the samples expressed HER3 (range 84 to 360 amol/µg) with 18% of samples expressing all three targets. Correlation of EGFR/HER3 expression with clinical outcome is ongoing.

Conclusion
We used an objective multiplex non-antibody based method to quantify multiple targets from FFPE tissue. Clinical correlation analysis of HER2 revealed improved OS and DFS in samples with high HER2 protein levels. Currently, we are expanding these studies to a larger set of samples and taking into account also the expression of other markers such as EGFR and HER3. This approach can potentially identify those tumors that are more dependent on these receptors for survival and also those patients that are exquisitely sensitive to anti-HER2 therapy.
Title: Axillary lymph node metastasis after neoadjuvant chemotherapy: A central prognosis determinant of triple-negative breast cancers

Helene Bonsang-Kitzis¹, Lisa Belin², Jean-Yves Pierga³, Leonor Chaltiel², Bernard Asselain³, Roman Rouzier¹, Paul Cottu³, Marie-Paule Sablin⁵, François-Clement Bidard³, Marick Laé⁴, Xavier Sastre⁴, Gilles Houvenaeghel⁶ and Fabien Reyal¹,⁶. ¹Institut Curie, Paris, France; ²Institut Curie, Paris, France; ³Institut Curie, Paris, France; ⁴Institut Curie, Paris, France; ⁵Institut Paoli Calmettes, Marseille, France and ⁶RT2Lab Team, Institut Curie, Paris, France.

Body: Triple-negative breast cancer (TNBC), defined by the combination of estrogen receptor negative, progesterone receptor negative and HER2 negative status, corresponds to 15%-20% of breast cancers and is an aggressive disease. Neoadjuvant chemotherapy (NAC) is increasingly used for TNBC patients and also viewed as a research tool to assess the efficacy of new drugs and/or new treatment schemes. Our aim was to identify in a large set of TNBC patients treated by NAC, subgroups of patients with different prognosis outcome.

Patients and Methods
We analyzed a retrospective cohort of 326 patients diagnosed with infiltrating TNBC and treated at Institute Curie (Paris-France) between 2002 and 2012. Patients had at the time of diagnosis a unique tumor and no history of cancer. We excluded patients with T4 tumors as patients with synchronous metastasis. All patients received NAC followed by a surgery with or without radiotherapy. Metastasis-free interval was defined as the time from NAC until first occurrence of metastasis. Survival analysis was carried out using the Cox proportional hazard model. A decision tree (with the following rules: significative log-rank test p-value and at least ten patients in each subgroup defined by the discrimination) was established by recursive partitioning to identify homogeneous subgroups and have a better representation of the prognostic model. A similar cohort of 83 patients with infiltrating TNBC treated at Institute Paoli Calmettes (Marseille-France) between 2004 and 2012 was analyzed. Survival and interval rates of the subgroups defined as in the training decision tree were calculated by the Kaplan–Meier method and were compared using the log-rank test.

Results
The only prognostic factor that remained significant after multivariate analysis was the presence of axillary lymph node disease after NAC (adjusted HR=3.48 [2.08-5.84], p=2.25 10-6). In order to highlight factors dismissed in the multivariate analysis a tree partitioning was performed. The first significant feature was the lymph node disease after NAC. Then within the node negative branch, the next feature was the BMI. Obese patients had a poorer prognosis (pN-/BMI superior to 30.RR=2.64 [1.28-5.55]). Within the node positive branch the next feature was the Elston Ellis grade. Patients with grade 1-2 tumors had a better prognosis (pN+/Grade1-2.RR=1.29 [0.39-4.26]). Within grade 3 tumors, the menopausal status was significantly related to the prognostic (pN+/Grade3/post menopausal status.RR=3.57 [1.69-7.77] versus pN+/ Grade3/pre menopausal status.RR=9.68 [5.71-18.31]). This tree partitioning was validated within an independent population of 83 TNBC.

Conclusion
The identification of TNBC patients prognostic factors represents a major challenge for the development of new therapeutic programs. This study highlights the strong association between the lymph node involvement after NAC and a poor prognosis outcome. It reveals two hormonal environmental markers (BMI and menopausal status) which also play an important role in the prognosis of such patients. Prospective studies should be implemented for such high-risk patients after NAC (pN+, Elston Ellis Grade 3 and non menopausal status) to validate new therapeutic solutions.
Title: Inverse relationship between ER+/PR- breast cancer and OncotypeDx scores and concordance between IHC and RT-PCR

Lubna N Chaudhary¹, Zeeshan Jawa¹, Aniko Szabo¹, Alexis Visotcky¹ and Christopher R Chitambar¹. ¹Medical College of Wisconsin, Milwaukee, WI; ²Medical College of Wisconsin, Milwaukee, WI; ³Medical College of Wisconsin, Milwaukee, WI; ⁴Medical College of Wisconsin, Milwaukee, WI and ⁵Medical College of Wisconsin, Milwaukee, WI.

Body: Background
Estrogen receptor-positive/progesterone-receptor negative breast cancer (ER+/PR- BC) is recognized as a distinct clinical entity that is associated with potentially more aggressive tumor biology and early resistance to endocrine therapy when compared to ER+/PR+ BC. The OncotypeDx assay provides risk stratification and prognostic information for hormone receptor (HR)-positive invasive BC; however, the association between the PR status determined by immunohistochemistry (IHC) and by OncotypeDx genomic analysis and the impact on the recurrence score (RS) is less clear.

Methods
We designed an analysis to determine whether OncotypeDx scores differ between ER+/PR+ and ER+/PR- tumors. Three hundred and fifty patients (pts) with HR positive, node-negative invasive BC who underwent OncotypeDx testing at our institution between Dec 2006-Oct 2013 were included in our study. We examined whether there was discordance in the HR status on IHC staining reported by our pathologists and the RT-PCR analysis reported as part of the OncotypeDx assay. The data were analyzed by ANOVA-F test and t-test. Chi-square test was used to analyze the discrepancies between the two methods of tumor genomic profile assessment.

Results
The mean age at diagnosis was 58 yrs (SD ±10.1 yrs). Three hundred and one pts had ER+/PR+ and 47 pts had ER+/PR- tumors by IHC. Pt characteristics are shown in Table 1. PR- tumors had significantly higher OncotypeDx scores than PR+ tumors (24.7 ±8.53 vs 17.3 ±7.38; Mean ±SD; p <0.001), predicting a greater 10-yr risk of distant recurrence. Two hundred and eighty four pts had HR status reported by OncotypeDx assay. Comparison of IHC and OncotypeDx assay revealed that 20 pts (8.1%) who were ER+/PR+ by IHC had ER+/PR- tumors by RT-PCR. Of the ER+/PR- cases by IHC, 12 (31.6%) were ER+/PR+ and 2 (5.3%) pts were ER-/PR- by RT-PCR (p <0.001, Table 2). Independent evaluation for ER and PR expression showed a concordance of 99.3% for ER and 88.7% for PR between the two methods.

Conclusion
Our study shows that ER+/PR- BC tumors are associated with a significantly higher OncotypeDx scores; this interprets into a higher risk of recurrence. Our data also show that there was a high concordance of 99.3% for ER between IHC and RT-PCR analyses whereas the concordance for PR was much lower at 88.7%.

Patient characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>ER+/PR+ N=303 (%)</th>
<th>ER+/PR- N=47 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td></td>
</tr>
<tr>
<td>Mean</td>
<td>58</td>
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<tr>
<td>M status</td>
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<tr>
<td>M</td>
<td>207 (68.3)</td>
<td>39 (83)</td>
</tr>
<tr>
<td>PM</td>
<td>96 (31.7)</td>
<td>8 (17)</td>
</tr>
<tr>
<td>OncotypeDx score</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>301</td>
<td>47</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>17.3 ± 7.38</td>
<td>24.7 ± 8.53</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IDC</td>
<td>248 (82.4)</td>
<td>44 (93.6)</td>
</tr>
<tr>
<td></td>
<td>IHC N (%)</td>
<td>RT-PCR ER+/PR+ N(%)</td>
</tr>
<tr>
<td>----------------</td>
<td>-----------</td>
<td>----------------------</td>
</tr>
<tr>
<td>ER+/PR+</td>
<td></td>
<td>226 (91.9)</td>
</tr>
<tr>
<td>ER+/PR-</td>
<td></td>
<td>20 (8.1)</td>
</tr>
<tr>
<td>ER-/PR-</td>
<td></td>
<td>0</td>
</tr>
</tbody>
</table>

N= number, SD= standard deviation, M= menopausal, PM= premenopausal, IDC= invasive ductal carcinoma, ILC= invasive lobular carcinoma, AI= aromatase inhibitor, Tam= tamoxifen, ♦= statistically significant

Comparison between IHC and RT-PCR

<table>
<thead>
<tr>
<th>Stage</th>
<th>ILC N (%)</th>
<th>Stage</th>
<th>RT-PCR N (%)</th>
<th>Stage</th>
<th>RT-PCR N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1a</td>
<td>2 (0.7)</td>
<td>T1a</td>
<td>2 (4.2)</td>
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<tr>
<td>T1b</td>
<td>49 (16.2)</td>
<td>T1b</td>
<td>9 (19.1)</td>
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<tr>
<td>T1c</td>
<td>174 (57.5)</td>
<td>T1c</td>
<td>25 (53.3)</td>
<td></td>
<td></td>
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<tr>
<td>T2</td>
<td>74 (24.5)</td>
<td>T2</td>
<td>11 (23.4)</td>
<td></td>
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</tr>
<tr>
<td>T3</td>
<td>2 (0.7)</td>
<td>T3</td>
<td>0</td>
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</table>

Surgery

<table>
<thead>
<tr>
<th>Surgery</th>
<th>ILC N (%)</th>
<th>Surgery</th>
<th>RT-PCR N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LUMPECTOMY</td>
<td>198 (65.6)</td>
<td>LUMPECTOMY</td>
<td>30 (63.8)</td>
</tr>
<tr>
<td>MASTECTOMY</td>
<td>102 (33.8)</td>
<td>MASTECTOMY</td>
<td>17 (36.1)</td>
</tr>
</tbody>
</table>

Chemotherapy

<table>
<thead>
<tr>
<th>Chemotherapy</th>
<th>ILC N (%)</th>
<th>Chemotherapy</th>
<th>RT-PCR N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>YES</td>
<td>93 (30.4)</td>
<td>YES</td>
<td>32 (68.1)</td>
</tr>
<tr>
<td>NO</td>
<td>210 (69.6)</td>
<td>NO</td>
<td>15 (31.9)</td>
</tr>
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</table>

Endocrine tx

<table>
<thead>
<tr>
<th>Endocrine tx</th>
<th>ILC N (%)</th>
<th>Endocrine tx</th>
<th>RT-PCR N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AI</td>
<td>167 (55.4)</td>
<td>AI</td>
<td>30 (65.2)</td>
</tr>
<tr>
<td>TAM</td>
<td>89 (29.2)</td>
<td>TAM</td>
<td>9 (19.5)</td>
</tr>
<tr>
<td>Sequential AI &amp; TAM</td>
<td>36 (10.9)</td>
<td>Sequential AI &amp; TAM</td>
<td>4 (8.8)</td>
</tr>
</tbody>
</table>

N= number, SD= standard deviation, M= menopausal, PM= premenopausal, IDC= invasive ductal carcinoma, ILC= invasive lobular carcinoma, AI= aromatase inhibitor, Tam= tamoxifen, ♦= statistically significant
Title: Routine histopathological variables predict Oncotype DX risk categories

Hidetaka Kawabata¹, Keiichi Kinowaki², Nobuko Tamura¹, Yoko Kobayashi¹, Masami Kadowaki¹, Daishu Miura¹, Toshimi Takano³, Akihiko Shimomura⁴, Tsuguo Iwatai², Yuko Kitawaki² and Takeshi Fujii².¹ Toranomon Hospital, Tokyo, Japan; ²Toranomon Hospital; ³Toranomon Hospital; ⁴National Cancer Center Hospital, Tokyo, Japan and ⁵BioMedical Research Centre, University College London, London, United Kingdom.

Body: Background: Oncotype DX (ODX) is a clinically validated, commercially available multi-gene assay that provides prognostic and predictive information in estrogen receptor (ER)-positive, Her2-negative breast cancer. Because health care system does not cover this expensive assay in many countries, physicians and researchers try to use routine histopathological variables predict ODX Recurrence Score (RS). Hyunseok Kim et.al presented at ASCO that Estimated Recurrence Score (ERS) model could identify those patients most likely to benefit from including ODX RS results in therapeutic decision-making (J Clin Oncol 32:5s, 2014 suppl; abstr 559).

Methods: We retrospectively reviewed histopathological slides between July 2007 and April 2014 from Toranomon Hospital patients (n=149) with early stage ER-positive, Her2-negative breast cancer, for whom ODX was ordered. We developed an original linear regression model using routine histopathological markers (Allred Score for progesterone receptor (PR), nuclear grade (NG), and Ki67) to calculate an Estimated Recurrence Score (ERS), and correlated it with the observed ODX RS assay result. ERS=20.46+0.298xKi67–1.48xPR+1.41xNG predicts the ODX RS with an R² of 0.48. In order to internally validate this model in the Toranomon Hospital cohort in Japan, we adapted Hyunseok’s algorithm.

Results: 132 patients had an observed RS ≤ 25 (89%) and 17 patients had an observed RS above 25 (11%). When the ERS was < 21 (n=124), we accurately classified 97% of them (120) who were found to have a low risk (observed RS ≤ 25). Similarly, 83% of those (5/6) with an ERS > 30 fell into a high risk category with observed RS > 25.

Table 1 (TAILORx risk categories)

<table>
<thead>
<tr>
<th>Toranomon cohort</th>
<th>ODX ≤25</th>
<th>ODX RS&gt;25</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERS &lt; 21</td>
<td>120</td>
<td>4</td>
<td>124</td>
</tr>
<tr>
<td>ERS(21-30)</td>
<td>11</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td>ERS(&gt; 30)</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>132</td>
<td>17</td>
<td>149</td>
</tr>
</tbody>
</table>

We also presented the comparison of ERS and observed RS based on original risk categories.

Table 2 (Original risk categories)

<table>
<thead>
<tr>
<th>Toranomon cohort</th>
<th>ODX RS&lt;18</th>
<th>ODX RS 18-30</th>
<th>ODX RS&gt;30</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERS(&lt;18)</td>
<td>66</td>
<td>33</td>
<td>0</td>
<td>99</td>
</tr>
<tr>
<td>ERS(18-30)</td>
<td>17</td>
<td>25</td>
<td>2</td>
<td>44</td>
</tr>
<tr>
<td>ERS(&gt;30)</td>
<td>0</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>83</td>
<td>59</td>
<td>7</td>
<td>149</td>
</tr>
</tbody>
</table>

Conclusions: We developed an ERS model to find those patients most likely to benefit from ODX RS results in clinical practice setting. Although further external and prospective validation is mandatory, preliminary result supports the usefulness of this ERS.
model.
Title: Integrated stathmin 1 and its multiple serine phospho-sites status predict the clinical outcome for patients with breast cancer

Xia-Ying Kuang¹, Xin Hu¹, Zhi-Jie Zhang², Shan Li¹ and Zhi-Ming Shao¹. ¹Fudan University Shanghai Cancer Center, Shanghai, China and ²School of Public Health, Fudan University, Shanghai, China.

Body: Background
Stathmin 1 (STMN1), an important protein which destabilizes microtubules, is known to be overexpressed across a broad range of human malignancies. The activity of STMN1 can be modulated by its phosphorylation of its multiple Serine residues (Ser16, Ser25, Ser38, and Ser63). In this study, we detected the expression pattern of STMN1 and the phosphorylation status of its Serine phospho-sites respectively, to explore their clinical implications and establish a prognostic model for metastatic risk of breast cancer.

Methods
In the training set, STMN1 expression and phosphorylation status of its four Serine phospho-sites were assessed, respectively, by immunohistochemistry via a tissue microarray (TMA) consisting of 250 patients who had histologically confirmed as primary breast cancer and underwent mammectomy in the Department of Breast Surgery in Shanghai Cancer Center during 2001 to 2006. Then we collected another cohort with 60 patients operated in the same Department between 2009 and 2011 for a validation.

Results
The median follow-up in the training cohort was 96 months (range 2-144 months). In Kaplan–Meier analysis, STMN1 and Ser38 were strongly associated with poorer disease-free survival (DFS, \( P=0.034 \) for STMN1, \( P=0.005 \) for Ser38), whereas Ser16 and Ser63 were associated with better DFS (\( P=0.007 \) for Ser16, \( P=0.041 \) for Ser63), Ser25 had no significant association with DFS. Then the variables with \( P \) value less than 0.2 under univariate analysis were used to build the multivariate Cox proportional hazards models. It revealed that expression of STMN1 (HR=1.811, 95%CI 0.929-3.533, \( P=0.081 \)), phosphorylation of Ser25 (HR=1.949, 95%CI 1.077-3.527, \( P=0.027 \)) and Ser38 (HR=2.143, 95%CI 1.131-4.059, \( P=0.019 \)) were prognostic factors for poor DFS. Whereas phosphorylation of Ser16 (HR=0.338, 95%CI 0.175-0.653, \( P=0.001 \)) and Ser63 (HR=0.431, 95%CI 0.224-0.827, \( P=0.011 \)) were associated with improved DFS. Furthermore, we combined those five markers and some well-known clinical characters to establish a prognostic model:

\[
h(t,x)=h_0(t)\exp(0.549*STMN1-1.084*Ser16+0.667*Ser25+0.762*Ser38-0.842*Ser63+0.559*(menopausal status)\text{-0.431}*(\text{pathological stage})_{\text{I/II}}\text{-}0.758*(\text{pathological stage})_{\text{III}}\text{-}0.809*(\text{tumor size})_{\text{I/II}}\text{-}1.805*(\text{tumor size})_{\text{III}}\text{-}1.361*(\text{lymph node status})_{\text{I/0}}\text{-}1.693*(\text{lymph node status})_{\text{II/0}}\text{-}2.091*(\text{lymph node status})_{\text{III/0}}.\]

Applying this model, we could achieve the probability of the occurrence of disease events for individuals. The area under the receiver operating curve for DFS of patients in the validation set was calculated, showing an excellent predictive ability (area under curve=0.75).

Conclusions
Our results revealed expression of STMN1 and phosphorylation status of its multiple Serine residues in breast cancer had significant predictive value in DFS. Moreover, those five markers and some other clinicopathological characters could be integrated into the model to improve the identification and prediction of high-risk breast cancer patients at the time of primary diagnosis, which can enable clinicians to target those likely to metastasize for appropriate treatment.
Title: Validation of the CancerMath prognostic tool for breast cancer in Southeast Asia

Hui Miao¹, Helena M Verkooijen¹,², Mikael Hartman¹,³, Nur Aishah Taib⁴, Hoong-Seam Wong⁵, Cheng-Har Yip⁴, Ern-Yu Tan⁶, Soo-Chin Lee⁷ and Nirmala Bhoo-Pathy⁵. ¹Saw Swee Hock School of Public Health, National University of Singapore, Singapore; ²University Medical Center, Utrecht, Netherlands; ³National University Hospital System, Singapore, Singapore; ⁴University of Malaya, Kuala Lumpur; ⁵National Clinical Research Centre, Kuala Lumpur, Malaysia; ⁶Tan Tock Seng Hospital, Singapore, Singapore and ⁷National University Hospital System, Singapore, Singapore.

Body: Background: CancerMath is a set of web-based prognostic tools for non-metastatic breast cancer patients. It predicts probability of nodal and nipple involvement as well as overall and cancer-specific survival with and without adjuvant therapy at any of the 15 years after diagnosis. But it has not been externally validated outside United States. This study assessed its performance in a Southeast Asian setting.

Methods: Using Singapore Malaysia Hospital Based Breast Cancer Registry, clinical information were retrieved from 7064 Stage I to stage III breast cancer patients who were diagnosed between 1990 and 2011 and underwent surgery. Probability of positive nodes and overall survival at any of the 15 years after diagnosis were computed using the CancerMath algorithm. Predicted and observed outcomes were compared. Discriminative ability was evaluated by area under a receiver operating characteristic curve (AUC). And calibration was assessed by plotting observed survival against predicted survival for each decile of the predicted survival and a 45 degree line was added to illustrated perfect agreement.

Results: Nodal status calculator predicted 40.6% of patients to be node positive which was similar to the observed 43.6%. The AUC was 0.71 (95% CI, 0.70-0.72). For outcome calculator, the observed and predicted overall survivals was 83.4% vs 87.3% at 5 year after diagnosis and 70.4% vs 75.3% at 10 year after diagnosis. The difference appeared smaller for patients from Singapore (5-year and 10-year predicted-observed= 2.5% and 0% respectively) comparing to patients from Malaysia (5-year and 10-year predicted-observed= 5.8% and 8.0% respectively), as well as for patients diagnosed in more recent years. The AUC for 5-year and 10-year overall survival was 0.77 (95% CI: 0.75-0.79) and 0.74 (95% CI: 0.71-0.76). For cases with relatively good and moderate prognosis, CancerMath predictions were similar to observed outcome as the plotted dots in the calibration plot were close to the 45 degree reference line from the fourth decile onwards. For therapy calculator, overestimation of survival persisted for most demographic, pathologic and treatment subgroups, especially for women younger than 40 years, with larger and high-grade tumor and with more lymph nodes involved. The AUC for 5-year overall survival is 0.73 (95% CI: 0.70-0.77). And with the exception of two groups, all plotted dots were below the 45 degree reference line in the calibration plot .

Conclusion: The discriminative performance and calibration of CancerMath was fair in this validation study. The greater discordance observed in Malaysian patients might be explained by different life expectancy, adverse tumor characteristics, compliance to treatment and lifestyle after diagnosis between Singapore, Malaysia and United States. The results suggested that direct application of CancerMath should only be limited to certain subgroups in Southeast Asia.
Title: The association of serum albumin and glycated hemoglobin with all cause mortality in patients with breast cancer

Vinay N Minocha¹, Kavita Pal¹ and Robert Zaiden¹. ¹University of Florida College of Medicine, Jacksonville, FL.

Body: Background: There has been increasing data within the oncology literature to support an inverse relationship between serum albumin levels and survival with breast cancer. Various studies have also indicated that diabetes is associated with a higher mortality in patients with breast cancer. Given the need to develop objective ways to identify patients with a poorer than expected outcome, the aim of this study is to determine whether serum albumin and glycated hemoglobin at diagnosis bears any relationship with all cause mortality in patients with all stages of breast cancer.

Methods: We performed a retrospective study of patients diagnosed with breast cancer of all stages at University of Florida Health Center, Jacksonville between August 2007 and October 2013. The data for this study was obtained from a combination of the hospital tumor registry and the electronic medical record. We collected data including age, sex, stage of cancer, histological type, tumour size and grade, lymph node status, estrogen (ER), progesterone (PR) and HER2/Neu receptor status. We collected baseline serum albumin levels and glycated hemoglobin (HbA1C) values recorded within 3 months of diagnosis of breast cancer. Wilcoxon’s rank sum test was used to test whether cancer stage differed between survival outcomes. Spearman correlation was used to assess the relationship between cancer stage and serum albumin and HbA1C at time of diagnosis. Logistic regression was used to model the impact of HbA1c, serum albumin and age at time of diagnosis on the probability of survival.

Results: The number of patients with breast cancer included in our study was 294. Of these patients, 219 patients had serum albumin values available at diagnosis and 69 patients had glycated hemoglobin values at diagnosis. The correlation between serum albumin and stage is statistically significant (P < 0.0001).

<table>
<thead>
<tr>
<th>Spearman Correlation Coefficients</th>
<th>P-Value</th>
<th>Number of Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Albumin Stage</td>
<td>-0.378</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>217</td>
</tr>
<tr>
<td>HbA1C</td>
<td>0.004</td>
<td>0.9731</td>
</tr>
<tr>
<td></td>
<td></td>
<td>69</td>
</tr>
</tbody>
</table>

The negative correlation means that higher levels of serum albumin are associated with lower stages. Serum albumin at diagnosis had a significant effect on the probability of survival (p=0.0005). For every unit increase in serum albumin, the odds of survival increase (OR=4.938, 95% CI: 2.023, 12.050). Alternatively, for each unit decrease the odds of dying increase by about five fold. Hypoalbuminemia has a significant effect on survival (p=0.0020). When serum albumin is >3.5, the odds of death decrease (OR=0.176 95% CI: 0.058, 0.528). Neither HbA1C nor age had a significant effect on survival (P=0.7635, 0.0858 respectively).

Odds Ratios for probability of survival

<table>
<thead>
<tr>
<th>Serum Albumin</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR</td>
<td>Lower Limit</td>
</tr>
<tr>
<td>Serum Albumin</td>
<td>4.938</td>
<td>2.023</td>
</tr>
</tbody>
</table>
Conclusion: In the present study, a low serum albumin level at the time of diagnosis, either as a continuous or categoric variable, was significantly associated with poorer survival in patients with breast cancer. Furthermore, a lower serum albumin is associated with a higher stage of breast cancer. In contrast, glycated hemoglobin (HbA1C) at the time of diagnosis did not have a significant effect on survival.
Title: Prognostic models in male breast cancer

Carmen C vanderPol¹, Miangela M Lacle¹, Arjen J Witkamp¹, Robert Kornegoor², Miao Hui³, Christine Bouchardy⁴, Elsken vanderWall¹, Helena M Verkooijen¹ and Paul J vanDiest¹. ¹University Medical Center, Utrecht, Netherlands; ²Gelre Ziekenhuizen, Apeldoorn, Gelderland, Netherlands; ³Saw Swee Hock School of Public Health, National University of Singapore, Singapore and ⁴Institute for Social and Preventive Medicine, Geneva University, Geneva, Switzerland.

Body: Male breast cancer (MBC) is a rare disease and its treatment is largely extrapolated from its female counterpart. Accurate prognostication is essential for advising on adjuvant systemic treatment and informing patients. Several predictive models are available for female breast cancer (FBC) including, subsequently, the Morphometric Prognostic Index (MPI), Nottingham Prognostic Index (NPI), Adjuvant! and Predict. The aim of this study was therefore to compare the prognostic performance of these models in a group of 166 early MBC patients.

The MPI describes a "good"- (MPI<0,60) and a "poor" prognostic group (MPI>=0,60) by using a formula with mitotic activity index, tumour size and lymph node status. The NPI is calculated by a formula including size, number of lymph nodes with metastases and tumour grade and divides patients into three groups; "good"- (NPI <= 3,4), "intermediate"- (3,4<NPI>=5,4) and "poor" prognosis (NPI>5,4). For the programs Adjuvant!Online and Predict, similar groups with "good"-, "intermediate"- and "poor" prognosis were defined by using tertiles. The prognostic performance of each test was studied by using the logrank-test and comparison between the models was done by using C-statistics.

The mean age was 66,4 years old and the median survival was 4,6 years with a mean of 5,8 years overall survival. Survival of the highest predicted group was higher (MPI: 87%, NPI: 90%, Adjuvant!Online: 91% and Predict: 88%) than for the moderate groups (NPI: 76%, Adjuvant!Online: 77% and Predict: 75%) and lowest for the poor predicted groups (MPI: 51%, NPI: 43%, Adjuvant!Online: 45% and Predict: 42%), with p-values that were highly statistically significant.

In terms of discrimination, all models were moderately able to discriminate between good and poor survivors (C-statistics; MPI: 0,674, NPI: 0,678, Adjuvant!Online: 0,717 and Predict: 0,711).

In conclusion, the MPI, NPI, Adjuvant! and Predict, prognostic models that were originally developed and validated for FBC, perform fairly well for MBC. These models may therefore help in MBC prognostication and decisions on adjuvant systemic therapy.
Title: Prognostic value of lysyl-tRNA synthetase in patients with breast cancer

Sung Gwe Ahn¹, Hak Min Lee¹, Jong Tae Park¹, Hak Woo Leeⁱ, Seung Ah Lee², Sun-Hee Leem³, Joon Jeong¹ and In-Sun Chu⁴.
¹Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Korea; ²Eulji General Hospital, Eulji University College of Medicine, Seoul, Korea; ³College of Natural Science, Dong-A University, Busan, Korea and ⁴Korean Bioinformation Center, Korea Research Institute of Bioscience and Biotechnology, Daejon, Korea.

Body: Introduction

Lysyl-tRNA synthetase (KRS), a cytosolic protein synthesis enzyme, performs dynamic functions as a result of various stimuli, moving to the nucleus or the extracellular space. Recent studies suggest that KRS controls cell migration and is associated with the process of metastasis. The potential prognostic value of KRS has not previously been reported for human breast cancer. In this study, we evaluated the influence of KRS on breast cancer prognosis in patients.

Methods

To evaluate the prognostic value of KRS, we used a cohort at a single institute (Gangnam Severance Hospital) in Seoul, Korea. The cohort consisted of 297 patients treated between January 1997 and December 2007, with gene-expression profiling (GEP) performed using tumor samples. Total RNA (500 ng) was used for labeling and hybridization, in accordance with the manufacturer’s protocols (Illumina, San Diego, CA). Survival was compared according to KRS expression using the log-rank test.

Results

In 297 patients with GEP, at a median follow-up of 7.83 years, women with higher KRS-mRNA expression had worse breast cancer-specific survival (P = 0.049). Higher expression of KRS was associated with estrogen receptor (ER) negativity (P < 0.001), high histologic grade (P = 0.001), high Ki67 (P = 0.001), and higher proportion of the triple-negative subtype (P < 0.001). To validate the prognostic impact of KRS in external data, we conducted survival analysis using online gene-expression array data (www.kmplot.com). There was a significant correlation between KRS expression level and overall survival, as well as between disease-free survival and metastasis-free survival.

Conclusion

In patients with breast cancer, KRS expression adversely influences prognosis. Our findings support the hypothesis that KRS is a potential therapeutic target.
Title: Prognostic factors and survival outcome in malignant and borderline phyllodes tumor of the breast

Liang Huang\textsuperscript{1,2}, Yin Liu\textsuperscript{1,2}, Sheng Chen\textsuperscript{1,2} and Zhiming Shao\textsuperscript{1,2}. \textsuperscript{1}Fudan University Shanghai Cancer Center/Cancer Institute, Shanghai, China and \textsuperscript{2}Shanghai Medical College, Fudan University, Shanghai, China.

Body: Background: Although phyllodes tumors (PT) are rare fibroepithelial breast tumors representing less than 1\% of all breast malignancies, these tumors often develop rapidly and have high risk of local recurrence and distant metastasis. The objective of this study was to determine pathological parameters and clinical characteristic that possibly influence outcome of PT patients.

Patients and methods: From 2003 to 2013, 103 female patients diagnosed with PT were classified to borderline and malignant subtypes in Shanghai Cancer Center. All patients underwent wide local excision of the tumor or mastectomy. The medical records were reviewed in relation to ultrasound report, the surgical management, follow-up record and the histological features of the tumor. Clinical and pathological variables were investigated using univariate and multivariate survival analyses.

Results: 103 patients with PT (61 borderline, 42 malignant PT) were followed up for median 38 months (range 6-118 months). The median age was 43 years (range 17-88 years), and the average tumor size was 5.9 cm by ultrasound (range 0.8-23cm). Patients younger than 40y had higher rate of malignant PT (57.2\% vs. 29.5\%, p=0.005). 34 patients had local recurrence, 13 patients in borderline subtype and 21 patients in malignant subtype. 3 patients with malignant PT had distant metastasis. Borderline PT patients achieved more favorable prognosis compared with malignant PT patients (p = 0.002, OR: 3.027, 95\% CI: 1.509-6.072). However, there was no relationship between large pathological tumor size (≥5cm) and higher local recurrence (p = 0.924, OR: 1.034, 95\% CI: 0.521-2.052).

Conclusion: Our findings revealed younger PT patients had higher rate of pathological malignant subtype. We conclude that patients with malignant PT had higher local recurrence. Big tumor was not likely to increase the incidence of recurrence compared with small tumor.

Baseline characteristics in borderline and malignant subtypes

<table>
<thead>
<tr>
<th></th>
<th>Borderline PT (n=61)</th>
<th>Malignant PT (n=42)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥40y</td>
<td>43</td>
<td>18</td>
<td>0.005</td>
</tr>
<tr>
<td>Age &lt;40y</td>
<td>18</td>
<td>24</td>
<td></td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>premenopausal</td>
<td>39</td>
<td>30</td>
<td>0.427</td>
</tr>
<tr>
<td>postmenopausal</td>
<td>22</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Ultrasound size</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥5cm</td>
<td>34</td>
<td>22</td>
<td>0.737</td>
</tr>
<tr>
<td>≥5cm</td>
<td>27</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Previous history of breast surgery</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Yes</td>
<td>17</td>
<td>11</td>
<td>0.851</td>
</tr>
<tr>
<td>No</td>
<td>44</td>
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<tr>
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<td>25</td>
<td>21</td>
<td>0.405</td>
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<tr>
<td>&lt;5cm</td>
<td>35</td>
<td>21</td>
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Title: Epithelial-mesenchymal transition phenotype in triple negative breast cancer: ZEB1 as a potential biomarker for poor clinical outcome

Min Hye Jang1, Hyun Jeong Kim1, Eun Joo Kim1, Hee Jin Lee3 and So Yeon Park1,2. 1Seoul National University Bundang Hospital, Seongnam, Korea; 2Seoul National University College of Medicine, Seoul, Korea and 3Asan Medical Center, Seoul, Korea.

Body: Background: Triple negative breast cancer (TNBC) is a heterogeneous group of disease. TNBC is closely related to epithelial-mesenchymal transition (EMT) and breast cancer stem cell (BCSC) phenotype. Recent studies have shown that TNBC can be classified into six subtypes including basal-like, mesenchymal-like and mesenchymal stem-like. However, clinical significance of EMT phenotype in TNBC is not clear.

Methods: We performed immunohistochemical analyses of EMT markers (expression of vimentin, smooth muscle actin, osteonectin and N-cadherin; loss of E-cadherin), BCSC markers (CD44+/CD24- and ALDH1) and EMT inducers (CD146 and ZEB1) in 173 TNBCs using tissue microarrays, and correlated their expressions with clinicopathologic features of the tumor.

Results: Expression of vimentin, CD44+/CD24- and CD146 was significantly higher in basal-like TNBCs (TNBC with expression of CK5/6 and/or EGFR) than in non-basal-like TNBCs. Expression of EMT markers was commonly correlated with high histologic grade, CD44+/CD24- phenotype and metaplastic carcinoma. CD146 expression was related to the expression of EMT markers and CD44+/CD24- phenotype. ZEB1 expression showed an association with the expression of smooth muscle actin, but not with BCSC markers. And it was closely correlated with high histologic grade and metaplastic carcinoma. In survival analyses, although expression of EMT and BCSC markers was not associated with the survival of the patients, ZEB1 expression was found to be an independent prognostic factor for poor disease-free survival of the patients.

Conclusion: EMT phenotype can be a signature of certain subgroup of TNBC. Especially, ZEB1 expression can be used as a potential biomarker to define a subgroup of TNBC associated with poor clinical outcome.
**Title:** Prognostic impact of disseminated tumor cell in breast cancer patients: 10 years follow up results

Ku Sang Kim¹, Jihyun Sung¹, Heeseung Lee¹ and Yongsik Jung¹. ¹Ajou University School of Medicine, Suwon- Si, Gyeonggi-Do, Korea.

**Body: Background:** Breast cancer survival rates are greatly influenced by distant metastasis. In breast cancer, cytokeratin 19 (CK19) is the most commonly used marker for detection of tumor cells disseminated in the lymph nodes, peripheral blood, and bone marrow. We previously reported that the presence of bone marrow CK19 has a correlation with distant disease-free survival (DDFS), and applied this as a predictive clinical value in both DDFS and overall survival (OS) in a 10-year follow-up.

**Materials and Methods:** From 1999 to 2003, 254 breast cancer patients underwent bone marrow aspirations intra-operatively to detect CK19 by nested reverse transcriptase polymerase chain reaction (nRT-PCR). The correlation between the clinical features and the presence of bone marrow CK19 was reviewed and analyzed using 10-year follow-up data on distant recurrence and survival.

**Results:** Sixty-three (26.2%) patients were in the CK19 positive group and 177 (73.8%) patients were in the CK19 negative group. The DDFS rates in the CK19 positive group and CK19 negative group at the 10-year follow-up were 68.3% (43/63) and 86.4% (153/177), respectively (HR 2.69, 95%CI: 1.49-4.88, p=0.001). For OS rates, 68.3% (43/63) of the CK19 positive group and 83.6% (148/177) of the CK19 negative group survived (HR 2.16, 95%CI: 1.23-3.83, p=0.008). Analyzing survival rates by bone marrow CK19 status at each stage, only in stage II patients did we see significantly lower DDFS (61.3% vs. 92.3%, p=0.0002) and OS (67.7% vs. 91.0%, p=0.001) rates in the CK19 positive group compared with the CK 19 negative group.

**Conclusions:** The presence of bone marrow CK19 has been shown in the 10-year study to lead to unfavorable outcomes in DDFS and OS rates. Therefore, CK19 could be a useful predictive marker for the distant metastasis of breast cancer, especially in stage II.
2014 San Antonio Breast Cancer Symposium

Publication Number: P4-11-41
Average Grade: 6.17

Title: Pretreatment neutrophil/lymphocyte ratio and overall survival in African American and white breast cancer patients

Joseph C Rimando¹, Jeff Campbell¹, Jae Hee Kim¹ and Sangmi Kim¹. ¹Georgia Regents University Augusta, Augusta, GA.

Body: Previous studies have shown that the pretreatment neutrophil/lymphocyte ratio (NLR) is an independent predictor of mortality in breast cancer patients. Our aim was to further study the relationship between pretreatment NLR and overall survival in African American and white breast cancer patients treated at an academic cancer center. Electronic medical records were reviewed for 589 patients treated between 2002 and 2011, and pretreatment NLR data, determined at an average of 12 days prior to the initiation of cancer treatment, were available from 217 African American and 218 white patients. Other clinical and patient data were obtained from the hospital tumor registry, with annual follow-up for vital status. There were a total of 102 deaths over a mean follow-up of 59 months. For data analysis, patients were divided into quartiles based on their NLR (Q1: <1.54; Q2: 1.54≤2.0; Q3: 2.0≤2.79; and Q4: ≥ 2.79). Patients in the highest quartile of pretreatment NLR showed an increase in overall mortality compared to those in the lowest quartile, with a hazard ratio (HR) of 2.4 (p<0.005). After adjustment for age, tumor stage, and grade, the pretreatment NLR remained significantly associated with overall mortality (HR=2.1, p=0.03). Further adjustment for race, body mass index (BMI) and smoking increased the effect size (HR=3.2, p<0.01), but only half patients remained in the analysis due to incomplete data on BMI and smoking. In our study, race, but neither BMI nor smoking, was associated with pretreatment NLR values: African American patients had higher pretreatment NLR values than white patients regardless of stage. In a stratified analysis by race, HRs for overall mortality associated with the highest quartile of pretreatment NLR versus the remaining three quartiles combined were 2.5 (p<0.01) among African Americans and 1.6 (p=0.24) among white patients, although the interaction between race and pretreatment NLR was not statistically significant. Our data confirm previous findings that pretreatment NLR, a marker of systemic inflammatory response, is unfavorably associated with overall survival in breast cancer patients, and also suggest that the association may be more evident among African American patients whose levels were generally higher than white breast cancer patients. Future studies are warranted to investigate the prognostic utility of pretreatment NLR in different racial groups and whether a heightened systemic inflammatory response is an underlying contributor to racial difference in breast cancer outcomes.
2014 San Antonio Breast Cancer Symposium

**Publication Number:** P4-11-42  
**Average Grade:** 5.25

**Title:** Pathological response and its impact on survival with neoadjuvant chemotherapy according to intrinsic subtype in locally advanced breast cancers in North India: Is it different from the West?

Sushma Agrawal¹, PUNITA LAL¹ and SHALEEN KUMAR¹. ¹Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India.

**Body:** **Background:** Breast cancer patients commonly present in locally advanced stage (LABC) in our country. We propose to correlate the pathological response to neoadjuvant chemotherapy (NACT) and its impact on survival based on the intrinsic subtype of breast cancer in our population. **Materials and methods:** Consecutive patients of LABC who underwent NACT (taxane and or anthracyclines based) followed by definitive surgery and radiotherapy during the period January 2007 to December 2012 were grouped on the basis of intrinsic subtypes of tumor (Luminal A, Luminal B, Her-2 Type, Basal). The pathological response to NACT in tumour as well as axillary nodes [complete response (pCR), partial response (pPR)] was correlated with the disease free survival (DFS) and overall survival (OS) at 5 years in the four intrinsic subtypes using Kaplan Meier Analysis. **Results:** 208 patients were the subject of this analysis. The median age of patients was 46 years (range 24-81 years), 46% were premenopausal and 54% postmenopausal, 42% right sided and 58% left sided. The clinical prechemotherapy status of tumour and node at presentation was 15% T2, 40% T3, 45% T4 (9% T4a, 35% T4b, 1% T4c) 8% N0, 42% N1, 41% N2, 9% N3. The intrinsic subtype of our population at presentation was Luminal A (16%), Luminal B (23%), Her-2 Type (23%), Basal (37%). The overall pCR rate to NACT in tumour and in node was 31% and 45%. The pCR rate in tumour according to intrinsic subtype was 26%, 23%, 39% and 31.5% in Luminal A, Luminal B, Her-2 Type and Basal type respectively. The pCR rate in node was 26%, 38%, 41.6% and 59% in Luminal A, Luminal B, Her-2 Type and Basal type respectively. At a median followup of 34 months (range 6-84 mo) the 5 year DFS and OS was significantly higher in patients achieving pCR tumour or pCR node in Her-2 type and Basal subtype (Table 1). **Conclusions:** The pCR rate to NACT in tumour or node seems to be considerably higher in our population in Her-2 and basal subtypes than that reported in the western literature. pCR (tumour as well as node) as a surrogate for both DFS and OS at 5 years in Her-2 and basal subtypes of breast cancer has been validated.

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<tbody>
<tr>
<td>Luminal A</td>
<td>26</td>
<td>26</td>
<td>100 vs 67 (0.08)</td>
<td>88 vs 70 (0.49)</td>
<td>100 vs 72 (0.1)</td>
<td>85 vs 75 (0.7)</td>
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<tr>
<td>Luminal B</td>
<td>23</td>
<td>38</td>
<td>68 vs 56 (0.9)</td>
<td>87 vs 32 (0.11)</td>
<td>80 vs 57 (0.9)</td>
<td>85 vs 35 (0.35)</td>
</tr>
<tr>
<td>Her-2 Type</td>
<td>39</td>
<td>41.6</td>
<td>90 vs 44 (0.003)</td>
<td>83 vs 45 (0.009)</td>
<td>87 vs 59 (0.02)</td>
<td>90 vs 66 (0.03)</td>
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<tr>
<td>Basal</td>
<td>31.5</td>
<td>59</td>
<td>85 vs 50 (0.004)</td>
<td>46 vs 50 (0.26)</td>
<td>83 vs 50 (0.02)</td>
<td>975 vs 60 (0.19)</td>
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</tbody>
</table>

pCR(pathological CR), pPR(pathological PR)
Title: Characterizing the clinical presentation of individuals with pathogenic variants in a breast/ovarian cancer gene panel

Erica Vaccari¹, Lauren Yackowski¹, Melanie Hussong¹, Patricia Murphy¹, Maria L Cremona¹, Jessica Booker¹ and Kathleen Hruska¹. GeneDx, Gaithersburg, MD.

Body: Background: Hereditary breast and ovarian cancer (HBOC) is a common indication for referral to cancer genetic counselors. Next generation sequencing panels allow for the efficient evaluation of many genes associated with increased risk of these cancers. The purpose of this study is to compare clinical histories of those with pathogenic variants in high risk versus low/moderate risk genes in order to determine which patients might benefit from more extensive testing by a panel approach.

Methods: We queried the results of patients tested at GeneDx for a panel of 21 genes causing increased breast and/or ovarian cancer risk. Data regarding personal and family history of cancer provided on the test requisition forms were analyzed and classified according NCCN guidelines for testing criteria for HBOC syndrome.

Results: Of 1709 individuals referred for testing, 146 (8.5%) tested positive for a pathogenic variant. Of these, 33% percent were found to carry a pathogenic BRCA1/2 variant while 67% tested positive for a pathogenic variant in a gene other than BRCA1/2 (CHEK2: 17%; ATM: 12%; PALB2: 10%; BRIP1: 7%; BARD1: 3%; PTEN: 3%; each of FANCC, MSH2, MSH6, NBN, PMS2, RAD51C, RAD51D: 2%; MLH1: 1%). Eighty-six percent of these individuals were affected with cancer. In the probands with a pathogenic BRCA1/2 variant, 66% were diagnosed with breast cancer and 22% with ovarian cancer compared to the probands with a pathogenic variant in a gene other than BRCA1/2 of whom 84% had a history of breast cancer and 17% had ovarian cancer. The highest number of breast cancer diagnoses were found, in decreasing order, in association with pathogenic variants in BRCA1 (20), CHEK2 (20), ATM (17), PALB2 (14), BRCA2 (13), and BRIP1 (12). The greatest number of ovarian cancers were identified, in decreasing order, in association with pathogenic variants in BRCA2 (8), BRCA1 (3), CHEK2 (3), and ATM (3). Furthermore, all of the individuals with pathogenic variants met NCCN guidelines for HBOC.

Conclusion: A high yield of pathogenic variants were found in genes other than BRCA1/2. Data analysis also shows that individuals with a pathogenic variant in genes other than BRCA1/2 did not have a notably less severe clinical history than those pathogenic variants in BRCA1/2. As all individuals tested meet NCCN guidelines for HBOC testing, panel testing should be considered in this population.
Title: Spectrum of mutations identified in a 25-gene hereditary cancer panel for patients with breast cancer

Lavania Sharma¹, Kelsey Moyes¹, John Abernethy¹, Heidi McCoy¹, Jennifer Saam¹, Michelle Landon¹ and Richard Wenstrup¹.
¹Myriad Genetic Laboratories, Inc, Salt Lake City, UT.

Body: Introduction: With the advancements of next generation sequencing, patients with a personal and/or family history of cancer that may not be suggestive of one cancer syndrome may be offered testing for mutations in multiple cancer-predisposing genes simultaneously. The focus of this analysis was to determine the spectrum of gene mutations observed in patients with a personal history of breast cancer.

Methods: A commercial diagnostic laboratory database was queried for patients with a personal diagnosis of breast cancer who underwent a 25-gene hereditary cancer panel from September 4, 2013 through April 17, 2014. The panel includes highly penetrant cancer predisposing genes BRCA1, BRCA2, TP53, PTEN, MLH1, MSH2, MSH6, PMS2, EPCAM, APC, BMPR1A, CDH1, CDKN2A, MUTYH, SMAD4, STK11 and moderately penetrant genes CHEK2, PALB2, ATM, NBN, BARD1, BRIP1, CDK4, RAD51C and RAD51D. Sequencing and large rearrangement was performed for all the genes in the panel. All patient data regarding clinical history was obtained by health care provider report on the test requisition forms.

Results: A total of 3584 patients with a personal history of breast cancer were identified. Of these, 13.8% met the NCCN guidelines for genetic testing for both Hereditary Breast and Ovarian Cancer syndrome (HBOC) and Lynch syndrome (LS), while 80.3% met criteria for only HBOC and 1.2% met only for LS. 10.4% of females and 18.9% of males with breast cancer were positive for at least 1 deleterious or suspected deleterious mutation, of which mutations in BRCA1 and BRCA2 comprised 40.2%. In this cohort, 59.8% of the mutations were detected in other genes (see table below). Of the patients who did not meet either testing criteria, mutations were found in BRCA2 (2), APC (2), BARD1 (1), CHEK2 (1), MSH2 (1), NBN (1), and TP53 (1). Interestingly, we also identified 15 patients with two mutations in our cohort. BRCA1 or BRCA2 accounted for one of the mutations in 9 out of 15 patients and 6 patients had two mutations in other genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>% of Total Mutations</th>
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<tr>
<td>BRCA1</td>
<td>21.2%</td>
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<tr>
<td>BRCA2</td>
<td>19.0%</td>
</tr>
<tr>
<td>CHEK2</td>
<td>11.3%</td>
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<tr>
<td>ATM</td>
<td>9.7%</td>
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<tr>
<td>PALB2</td>
<td>7.2%</td>
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<tr>
<td>NBN</td>
<td>6.6%</td>
</tr>
<tr>
<td>APC</td>
<td>5.6%</td>
</tr>
<tr>
<td>BARD1</td>
<td>3.3%</td>
</tr>
<tr>
<td>PMS2</td>
<td>2.6%</td>
</tr>
<tr>
<td>BRIP1</td>
<td>2.3%</td>
</tr>
<tr>
<td>MSH6</td>
<td>2.0%</td>
</tr>
<tr>
<td>TP53</td>
<td>1.5%</td>
</tr>
<tr>
<td>MSH2</td>
<td>1.3%</td>
</tr>
<tr>
<td>RAD51C</td>
<td>1.3%</td>
</tr>
<tr>
<td>CDH1</td>
<td>1.0%</td>
</tr>
<tr>
<td>RAD51D</td>
<td>1.0%</td>
</tr>
<tr>
<td>CDKN2A</td>
<td>1.0%</td>
</tr>
<tr>
<td>PTEN</td>
<td>1.0%</td>
</tr>
<tr>
<td>Gene</td>
<td>Frequency</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>MLH1</td>
<td>0.5%</td>
</tr>
<tr>
<td>Biallelic MUTYH</td>
<td>0.3%</td>
</tr>
<tr>
<td>SMAD4</td>
<td>0.3%</td>
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**Conclusions:** Testing patients using a 25-gene panel identified 234 mutations outside of *BRCA 1* and *BRCA 2* (157). That is a 149% increase in mutations identified over *BRCA 1* and *BRCA 2* testing alone. Mutations in moderately penetrant breast cancer genes (including *CHEK2, ATM, PALB2* and *NBN*) comprised 34.8% of total mutations and 6.4% of mutations were in LS genes. Additionally, panel testing identified mutations in more than one gene in 4.0% of patients, which would not have been identified by single-syndrome testing. Panel testing provides a broader understanding of hereditary cancer in breast cancer patients both by identifying mutations in more genes and identifying patients with mutation in more than one gene. This approach can provide more guidance both for management of the patient and the patient’s family members.
Title: Triple-negative breast cancer: Frequency of inherited mutations in breast cancer susceptibility genes

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Body: Background: Guidelines recommend germline mutation testing of breast cancer predisposition genes in triple negative (TN) breast cancer cases with a family history of breast or ovarian cancer or when diagnosed under age 60. However, the prevalence of mutations in these genes among TN cases unselected for family history of breast or ovarian cancer is not known. Methods: To assess the frequency of mutations in 16 predisposition genes in TN cases we screened a large cohort of TN patients (n=1824) unselected for family history of breast or ovarian cancer from 12 centers and 824 study matched unaffected controls for mutations using a panel-based sequencing approach. Results: Deleterious mutations were identified in 15% of TN patients: 8.5% had BRCA1, 2.7% had BRCA2, and 3.6% had mutations in 12 other genes. Mutations in non-BRCA1/2 genes encoding proteins implicated in homologous recombination repair of DNA double strand breaks were detected at the same frequency as in breast cancer families. TN cases with mutations had high-grade tumors and were diagnosed at an earlier age than non-mutated cases. However, 10% of TN cases diagnosed at ≥60 years and 5% with no family history of cancer were also found to carry mutations. Inactivating mutations in non-BRCA1/2 predisposition genes were associated with moderate to high risks of TN breast cancer. Conclusions: National Comprehensive Cancer Network (NCCN) guidelines support clinical genetic testing of breast cancer predisposition genes in 95% of TN breast cancer patients carrying mutations in susceptibility genes. In contrast, National Institute of Health and Care Excellence (NICE) guidelines in the U.K. do not support genetic testing of a substantial proportion of TN patients with predisposing alleles. Frequency tables for inherited mutations in known predisposition genes based on age of diagnosis and family history of cancer will allow for selection of TN patients most likely to carry mutations in the predisposition genes.
Clinical evaluation of multigene testing for hereditary breast and ovarian cancer

Leif Ellisen¹, Allison Kurian², Stephen Lincoln³, Andrea Desmond¹, Meredith Mills², Kristen Shannon¹, Michelle Gabree¹, Michael Anderson³, Yuya Kobayashi³, Federico Monzon³ and James Ford². ¹Massachusetts General Hospital, Boston, MA; ²Stanford University, Stanford, CA and ³Invitae, San Francisco, CA.

Background: Advances in DNA sequencing technology have fueled the development of multigene panels for hereditary cancer testing. While such assays are potentially both practical and affordable for routine clinical genetic testing, there remains uncertainty regarding their proper application and interpretation. Among the unanswered questions are which patients are appropriate candidates for such expanded testing; what is the likelihood that finding deleterious variants will alter risk assessment and management, and what is the prevalence of variants of unknown significance (VUS) that may create uncertainty for providers and anxiety for patients.

Methods: 821 patients who met NCCN guidelines for BRCA1/2 testing for hereditary breast/ovarian cancer (HBOC) were prospectively recruited at two major academic medical centers. These patients received traditional BRCA1/2 tests as part of their clinical care and also were later tested for a panel of 29 known cancer risk genes. This panel also re-tested BRCA1/2. Both sequence changes and deletions/duplications were reported, and the panel-testing laboratory was blind to the earlier results. Panel testing results were validated by comparison with the earlier data or by independent testing using established technologies. Family history information was collected directly by genetic counselors.

Results: 13.8% of the patients carried BRCA1 or BRCA2 mutations, with >99% concordance between the traditional and panel results for these two genes. 53 (7.6%) of the BRCA1/2-negative patients carried mutations in other cancer risk genes. Some of these findings confer a high risk for breast/ovarian cancer (e.g. TP53), while others (i.e. CHEK2, BRIP1, PALB2, RAD51C, CDKN2A, ATM, and NBN) confer a moderate increase in risk. 10 of these 55 patients were positive for genes involved in Lynch syndrome (MLH1, MSH2, MSH6, and PMS2), although breast and ovarian cancer are not uniformly accepted as part of this syndrome. 40% of these patients were heterozygous carriers of pathogenic variants in MUTYH, which have a less clear impact on breast/ovarian cancer, although this rate (3%) is higher than the expected carrier frequency in this population. About 60% of patients had one or more VUS in the 29 genes. The rate of VUS was highly gene dependent, with ATM, APC, and PTCH1 providing the largest number. A small but significant subset of patients did not have personal or family histories consistent with the classical presentation of their identified mutation. Nevertheless, a majority of the non-BRCA1/2 findings would have prompted consideration of a management change for the tested patient even considering the personal and family cancer history.

Conclusions: Multigene panel testing for hereditary cancer risk assessment increased the yield of findings with potential clinical impact for almost 8% of patients. This is consistent with prior data from our laboratory and others. This study, carried out in a uniform clinical practice setting and with data collected directly by health care providers, provides a representative view of the benefit from such testing in an unselected patient population.
Title: Individuals with more than one pathogenic variant: Rationale for considering multi-gene panel testing for cancer susceptibility

Rachel Nusbaum1, Lisa Susswein1, Kathleen Hruska1, Melanie Hussong1, Windy Berkofsky-Fessler1, Mingjuan Liao1, Erica Rinella1, Nina Sanapareddy1, Joaquin Villar1, Haiyan Wan1, Zhixiong Xu1, Rebecca Y Bassett2, Elisabeth McKeen3, Constance Murphy4, Deborah Pencarinha4, Jessica Booker1, Maria L Cremona1, Patricia Murphy1 and Rachel T Klein1. 1GeneDx, Gaithersburg, MD; 2NYU Langone Medical Center, New York, NY; 3Jupiter Medical Center, Jupiter, FL and 4Wellmont Cancer Institute, Kingsport, TN.

Body: Introduction: Expansion of genetic testing technologies has brought multi-gene panels for cancer susceptibility into the clinic; however, the clinical utility of these next-generation sequencing (NGS) panels is largely unknown. Hypothesis: We hypothesized that the use of multi-gene panels would yield a significant number of cases with more than one pathogenic variant.

Methods: We retrospectively queried oncology tests reported at GeneDx from August 2013 to April 2014 for individuals with more than one pathogenic variant. Next, we calculated the proportion of individuals with more than one pathogenic variant among all positive reports, excluding familial and stand-alone BRCA1/2 tests. We then extracted personal and family histories, including available segregation data, to categorize the phenotypes.

Results: Of 406 unique, unrelated individuals with pathogenic or likely pathogenic findings, 11 (2.7%) had more than one pathogenic variant. This total includes nine individuals with a mutation in more than one gene, as well as two individuals with two mutations in trans in the same gene. Ten of these 11 individuals were identified by multi-gene panel tests, one individual by step-wise (tiered) testing. Seven of 11 individuals were positive for a pathogenic variant in a traditional, highly-penetrant cancer susceptibility gene and another pathogenic variant in a gene with moderate cancer susceptibility, such as CHEK2 and ATM. Three of 11 probands had more than one primary tumor. Several of the families were significant for bilineal cancer risk.

Conclusions: Our data suggest that a traditional single gene approach to cancer testing may fail to identify all pathogenic variants related to the clinical presentation. The identification of a second risk factor for inherited susceptibility to cancer allows for appropriate testing and management considerations for family members. In conclusion, we provide early evidence for the consideration of multi-gene panel testing in a clinical oncology setting.
Title: Results of next-generation sequencing panels in a large community-based hereditary cancer risk program

J L Blum¹, C A Garby¹, A E Simmons¹, S A Walker¹ and L E Panos². ¹Baylor Charles A. Sammons Cancer Center, Baylor University Medical Center, Dallas, TX and ²Ambrey Genetic Laboratories, Aliso Viejo, CA.

Body: Background: Next-generation sequencing (NGS) allows for broader germline genetic testing for hereditary cancers. Since the Supreme Court decision of AMP v. Myriad on June 13, 2013, hereditary cancer multi-gene panels can now include BRCA1 and BRCA2, making these panels an option for first-tier testing. However, questions have been raised about the clinical utility and implications of extended panels for medical management given the inclusion of unknown to moderate penetrant genes.

Methods: We reviewed all patients who underwent multi-gene panel testing from July 1, 2013 through May 23, 2014. The indications for testing included personal and/or family history of breast or ovarian cancer. The panels were ordered in a single genetic counseling clinic within a large community-based cancer center.

Results: A total of 136 patients underwent panel testing via a single commercial laboratory. We identified 12 (8.8%) patients who were positive for a pathogenic or likely pathogenic mutation in a cancer susceptibility gene; 4 had prior negative BRCA1 and BRCA2 sequencing and deletion/duplication testing. These positive results included 4 BRCA2 mutations, 2 TP53 mutations, 1 CDH1 mutation, 2 ATM mutations, and 1 patient each with a CHEK2, NBN, or PALB2 mutation. Of the patients found to have a positive test result, 100% met the National Comprehensive Cancer Network (NCCN) guidelines for Hereditary Breast and Ovarian Cancer (HBOC) genetic testing. The CDH1 mutation carrier did not meet NCCN guidelines for hereditary diffuse gastric cancer testing and only one of the TP53 mutation carriers met NCCN guidelines for Li-Fraumeni syndrome. Within our cohort (136), 21 (15.4%) patients had a total of 25 variants of uncertain significance (VUS) and 103 (75.7%) patients had negative test results.

Conclusion: Testing through NGS panels identified 7/12 (58%) patients with a mutation which led to changes in current medical management and 3/7 (43%) had a mutation in a gene other than BRCA1 or BRCA2. Our findings suggest that there is clinical utility of NGS panels for use in this patient population despite the inclusion of unknown to moderate penetrant genes and a higher rate of VUS than single gene testing.
Body: Introduction: Next Generation Sequencing, or NGS technology is gaining acceptance in diagnostic laboratories for multigene panel testing. However questions remain about the sensitivity, specificity and clinical implications of this new technology and the expanded testing it enables. Moreover, questions are raised as to whether new laboratories using these methods, typically without the benefit of large historical proprietary databases, can provide similar assessments of pathogenicity as do the previously established providers. Expanding on our recently published work (Kurian et al., JCO 2014) we considered whether NGS testing in an independent laboratory can replace traditional BRCA1/2 tests in patients indicated for hereditary breast/ovarian cancer testing.

Methods: We recruited over 800 patients who were indicated for BRCA1/BRCA2 testing under clinical management guidelines, and collected over 200 additional samples to increase the power of this ongoing study. All were tested using an NGS-based multigene panel which reported both DNA sequence and copy-number alterations for BRCA1/2 and 27 other known cancer risk genes. Traditional genetic testing results using established technologies were also available for comparison. In this report we focus on the results for BRCA1/2 and 27 other known cancer risk genes.

Results: Sensitivity was high: 261 alterations (196 pathogenic and 65 others) were reported in the traditional genetic data, and all were detected by NGS when the corresponding test was available. In this set are 141 alterations considered technically challenging for NGS: insertions, deletions, and complex sequence changes, as well as very large (chromosome) and small (single exon) copy number changes. Specificity was also high: all NGS variants for which we sought confirmation using independent methods (n>2000) were confirmed, including 51 alterations not previously reported. Determination of pathogenicity was also highly concordant: in all but 2 cases positive reports agreed, and in these 2 cases data were available in the literature to support pathogenicity, although not at a level which meets recent guidelines from the American College of Medical Genetics (ACMG). It is not clear from the diagnostic reports exactly what evidence supported pathogenicity in the traditional data for these 2 cases.

Rates of Variants of Unknown Significance (VUS) in BRCA1/2 were somewhat different: about 7% of cases in the NGS data vs. about 4% of fully tested cases in the traditional data had an uncertain report. The root of this difference is also unclear as details are not provided in the traditional reports.

Conclusions: NGS can be a viable replacement for traditional genetic testing for hereditary breast and ovarian cancer. Interpretation concordance is high but fully evaluating the details of this is hampered by the limited reporting of proprietary data by some established laboratories. Recent efforts to establish large public databases of genetic information (particularly ClinVar) will promote greater transparency and accountability and thus can help improve access to high quality care for hereditary conditions.

Note: All of the variants in this study and their interpretations will be released to public databases by the time of the meeting.
2014 San Antonio Breast Cancer Symposium

Publication Number: P4-12-08
Average Grade: 3.50

Title: Utilizing next generation sequencing technologies for hereditary breast cancer risk assessments in a private oncology practice

Barbara Hamlington¹, Scot Sedlacek¹, Lucy Langer², Brittney Goetsch¹, Katie Lemas¹ and Sami Diab¹. ¹Rocky Mountain Cancer Centers, Greenwood Village, CO and ²Compass Oncology, Portland, OR.

Body: Background: The recent introduction of clinical Next Generation Sequencing (NGS) technologies has transformed the ability of health care providers to provide personalized hereditary breast cancer risk assessments to patients and their family members. However, along with the plethora of data derived from NGS technologies come challenges regarding how to interpret this data in a clinically meaningful way to guide medical management decisions.

Methods: A retrospective analysis was performed on a population of 242 patients with a personal or family history of breast and/or ovarian cancer and completed NGS genetic testing between March 2012 and May 2014 at Ambry Genetics. Data points included age, gender, primary diagnosis, type of NGS genetic test and result. Patients with prior genetic testing were not excluded from the analysis.

Results: Of the 242 patients who completed NGS genetic testing 199 (82%) had a personal history of cancer and 43 (18%) had a family history of cancer. Ten (5%) clinically significant deleterious mutations were found in the affected population; 3 MUTYH mutations were consider not clinically significant and excluded from the analysis. Four (40%) were CHEK2 mutations, 2(20%) BRCA1 mutations, 1(10%) NBN mutation, 1(10%) ATM mutation, 1(10%) MSH6 mutation and 1(10%) PTEN mutation. One patient with a history of ovarian cancer had two deleterious mutations in the BRCA1 and NBN genes, and three affected patients had a deleterious mutation in addition to a variant of uncertain significance (VUS); a patient with synchronous breast and ovarian cancers had a MSH6 mutation and a BRCA2 VUS, a patient with breast cancer had a CHEK2 mutation and a CHEK2 VUS, and a patient with breast cancer had a CHEK2 mutation and a MSH6 VUS. A total of 37 (19%) VUS were found in the affected population, including one patient with a history of breast cancer and a VUS in both ATM and MRE11A. A single (2%) clinically significant BRCA1 deleterious mutation and 6(14%) VUS were detected in the unaffected population.

Conclusions: NGS technologies allow for health care providers to complete more comprehensive hereditary breast cancer risk assessments for patients and their families. However, the high VUS rate (14-19%) and the possibility for multiple mutations and/or variants in a single patient create unique challenges when interpreting NGS genetic test results. Furthermore, the paucity of prospective studies and consensus guidelines limits the ability of the health care provider to interpret NGS genetic test results in a clinically meaningful way to guide patient management. This is highlighted by the case example above regarding the patient with synchronous breast and ovarian cancers who was found to have a MSH6 mutation and a BRCA2 VUS. Given that the patient does not meet Amsterdam criteria, the MSH6 mutations would have been missed had it not been for the NGS technology utilized for this patient. However, challenges still exist about how to interpret this data for both the patient and her family in a clinically meaningful way given that the family does not look like a classic Lynch Syndrome family.
Low-level constitutional mosaicism of a de novo BRCA1 gene mutation

Eitan Friedman¹, Noa Efrat², Lior Soussan-Gutman³, Addie Dvir³, Yulia Kaplan³, Tali Ekstein⁴, Keith Nykamp⁴, Martin Powers⁴, Marina Rabideau⁴ and Scott Topper⁴. ¹Oncogenetics Unit, Chaim Sheba Medical Center, Tel-Hashomer, Israel; ²Kaplan Medical Center, Rehovot, Israel; ³Oncotest-Teva, Teva Pharmeceuticals Industries, Petach Tikva, Israel and ⁴Invitae, San Francisco, CA.

Background:
Germline mutations in the BRCA1 and BRCA2 genes detected in some high-risk breast/ovarian families are used to estimate cancer risk, plan cancer early detection schemes, and make decisions about risk reducing surgeries. The gold standard for detecting BRCA sequence changes has long been Sanger sequencing, but recently next-generation sequencing (NGS) technologies have emerged as an accurate and efficient alternative, with improved sensitivity for detection of mosaic events. Here we report the detection of low-level constitutional germline mosaicism (∼5%) for a de novo pathogenic BRCA1 mutation detected using deep sequencing of three different non-cancerous tissues; and a corresponding high-level detection (∼50%) in cancerous breast tissue. This is the first reported case of multiple tissue constitutional mosaicism in BRCA1 at this level of detection.

Patient and Methods:
The patient is a woman of mixed Jewish Ashkenazi - Bulgarian heritage, diagnosed with a large (8x10 cm), triple negative, high-grade invasive breast cancer in the right breast at age 43 years. Her cancer family history includes a daughter with acute lymphatic leukemia at age 18 months, a brother with a Central Nervous System (CNS) tumor at age 45 years, a father with a malignant CNS tumor at age 58 years, a maternal grandfather with a malignant tumor in his 70's, and two of this maternal grandfather's sisters who were diagnosed with breast cancer. The patient underwent neoadjuvant chemotherapy followed by bilateral mastectomy (therapeutic mastectomy for the right breast and a contralateral risk-reducing mastectomy). The patient underwent germline testing using NGS, sequencing of 29 hereditary cancer genes in DNA extracted from blood using Invitae's Hereditary Cancer Panel. This testing was followed up with additional NGS testing of buccal and contralateral healthy breast tissue. The patient also underwent somatic mutation testing on her breast tumor.

Results:
NGS of DNA extracted from blood identified a pathogenic BRCA1 mutation, c.1953dupG (p.Lys652GlufX21), in 5% of reads (X450 coverage). No other pathogenic mutations were detected in other genotyped cancer susceptibility genes. Analysis of buccal tissue and normal breast tissue removed at initial surgery also identified this mutation in ~5% of reads, and confirm that this individual is a constitutional mosaic for this mutation. Genetic analyses were subsequently performed on the breast cancer tissue using Genome Health platform (Foundation One) and the same mutation, c.1943dupG was detected in 47% of molecules. Sanger sequencing of BRCA1 and BRCA2 in DNA extracted from peripheral blood had not detected this mutation. Analysis of the maternal DNA did not reveal this mutation, and analysis of the father was not possible.

Conclusion:
This is the first reported case of a de novo constitutional mosaicism in BRCA1 at this level of detection and confirmed across multiple tissue types, and highlights the need to perform deep sequencing in individuals clinically suspected of having cancer predisposition, prior to considering risk-reducing surgery.
Title: Uptake and outcomes of multiplex testing for breast cancer susceptibility

Angela R Bradbury¹, Linda Patrick-Miller², Brian L Egleston³, Amanda Brandt¹, Jessica Long¹, Jacquelyn Powers¹, Jill Stopfer¹, Laura DiGiovanni¹, Jamie Brower¹ and Susan M Domchek¹. ¹University of Pennsylvania, Philadelphia, PA; ²University of Chicago, Chicago, IL and ³Fox Chase Cancer Center, Philadelphia, PA.

Body: Background: New counseling models for multiplex genetic testing for breast cancer susceptibility are needed. Further, the risks, benefits and utilities of multiplex genetic panels are unknown.

Purpose: To obtain stakeholder feedback on an innovative tiered-binned model for pretest counseling and informed consent for multiplex testing and to evaluate the uptake of, cognitive and affective responses to and perceived utility of panel testing.

Methods: Patients previously BRCA1/2- or BRCA1/2 untested completed in-person pre-test (V1) and post-test counseling (V2) and surveys regarding the novel counseling model and evaluating cognitive and affective responses to, and perceived utility of the 26 gene Myriad MyRisk panel for cancer susceptibility.

Results: 49 patients (62% of eligible) enrolled and completed V1. 38% of decliners were not interested in panel testing. BRCA1/2- were more likely to proceed with MyRisk (89%) than BRCA1/2 untested (48%, p<0.01). Although not statistically significant, those who declined panel testing after V1 had higher anxiety, depression and cancer worry, but no difference in knowledge. They also had lower perceived utility (p=0.005). Most patients would not change anything about their V1 (86%) or V2 (91%) counseling. Surveys suggest that patients value the engagement, personalization, organization and visual aids of the novel counseling model. Potential refinements include enhancing assessments of informational overload, confusion and psychosocial needs, particularly with uncertain results. As shown in Table 1, event anxiety, depression, uncertainty and cancer worry did not change, while general anxiety decreased. Knowledge increased and perceived utility and satisfaction decreased significantly. Exploratory analyses by results to date (positive = 5; VUS = 9; negative =22), suggest no difference in uncertainty by test result. Patients with a positive result might experience greater event anxiety and have less decline in perceived utility (p=0.02) than those with a negative or VUS result.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post V1</th>
<th>Post V2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>General Anxiety</td>
<td>6.8 (3.9)*</td>
<td>6.1 (4.0)*</td>
<td>5.8 (4.2)*</td>
</tr>
<tr>
<td>General Depression</td>
<td>2.6 (3.0)</td>
<td>2.3 (2.6)</td>
<td>2.9 (3.6)</td>
</tr>
<tr>
<td>Event Anxiety</td>
<td>37.1 (9.6)</td>
<td>37.7 (9.5)</td>
<td>37.3 (9.4)</td>
</tr>
<tr>
<td>Cancer Worry</td>
<td>18.3 (15.7)</td>
<td>16.9 (14.1)</td>
<td>6.6 (14.7)</td>
</tr>
<tr>
<td>Knowledge (K) Total</td>
<td>61.8 (6.1)**</td>
<td>63.9 (6.4)**</td>
<td>66.3 (6.9)**</td>
</tr>
<tr>
<td>K-Inheritance</td>
<td>29.5 (3.2)</td>
<td>30.0 (3.2)</td>
<td>30.3 (3.7)</td>
</tr>
<tr>
<td>K-Benefits</td>
<td>12.0 (1.4)</td>
<td>12.3 (1.8)</td>
<td>12.4 (1.9)</td>
</tr>
<tr>
<td>K-Limitations</td>
<td>20.3 (3.2)**</td>
<td>21.6 (2.8)**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Group A</td>
<td>Group B</td>
<td>Group C</td>
</tr>
<tr>
<td>----------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>Satisfaction</td>
<td>20.4 (3.4)**</td>
<td>21.9 (3.0)**</td>
<td>23.6 (2.6)**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42.8 (3.8)</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>42.9 (3.6)*</td>
<td>41.4 (2.6)*</td>
</tr>
<tr>
<td>Uncertainty</td>
<td>7.5 (4.3)</td>
<td>6.9 (4.6)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>7.7 (4.0)</td>
<td>6.5 (4.5)</td>
<td>6.7 (4.6)</td>
</tr>
<tr>
<td>Perceived Utility</td>
<td>37.2 (7.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37.7 (7.0)*</td>
<td></td>
<td>33.8 (8.6)*</td>
</tr>
</tbody>
</table>

*p<0.05, **p<0.001

**Conclusion:** With a tiered-binned counseling model, patients experience increased knowledge. Uptake of panel testing varies by prior testing and potentially by patient affective factors. Most patients do not experience negative psychological responses, although this may vary by test result. Declines in satisfaction and perceived utility may also vary by test result and may reflect the current unclear utility and uncertainty of multiplex testing.
Patient perceptions of the impact of genetic testing for breast cancer risk on health insurance

Erin Hofstatter and Anees Chagpar. "Yale School of Medicine, New Haven, CT.

Introduction: Genetic testing for breast cancer risk is increasingly available, and patients’ perceptions regarding how results may affect their health insurance may affect their likelihood to opt for testing. We sought to determine the changes in patient perceptions regarding the impact of genetic testing on health insurance over a five year period from 2005 to 2010. Notably, during this time period two significant U.S. federal health care laws were enacted. First, the Genetic Information Nondiscrimination Act (GINA) passed in 2008, protecting against the use of genetic information for health insurance coverage. Second, the Patient Protection and Affordable Care Act (PPACA) passed in 2010, representing a major regulatory overhaul of the U.S. health care system, promising universal access to health care coverage.

Methods: The National Health Interview Survey is a population-based face-to-face survey conducted annually by the CDC. In 2005 and in 2010, a cancer supplement was fielded which asked respondents who had undergone genetic testing whether they felt this had, or would, affect their health insurance. We evaluated the cohort of participants who had undergone genetic testing for breast cancer, and used SUDAAN statistical software to evaluate changes in the proportion who felt genetic testing would adversely affect their health insurance, and factors associated with this perception.

Results: In 2005, 48 respondents had undergone genetic testing for breast cancer, representing 333,544 people in the population. Of these, 16.8% felt that genetic testing had, or would, affect their health insurance. In 2010, 16 respondents had undergone genetic testing for breast cancer, representing 93,301 people in the population. Of these, 17.4% felt that genetic testing had, or would affect their health insurance. In 2005, this perception varied based on insurance status, education, and region; but in 2010, perceptions of the impact of genetic testing on health insurance were unaffected by these factors.

<table>
<thead>
<tr>
<th></th>
<th>2005</th>
<th>p-value</th>
<th>2010</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td></td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Insurance</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uninsured</td>
<td>11.8</td>
<td>0.006</td>
<td>22.8</td>
<td>0.276</td>
</tr>
<tr>
<td>Medicaid</td>
<td>0</td>
<td></td>
<td>59.5</td>
<td></td>
</tr>
<tr>
<td>Medicare</td>
<td>28.7</td>
<td>0.031</td>
<td>7.0</td>
<td>0.463</td>
</tr>
<tr>
<td>Private</td>
<td>15.7</td>
<td></td>
<td>18.8</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>35.4</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; Grade 12</td>
<td>8.7</td>
<td></td>
<td>7.9</td>
<td></td>
</tr>
<tr>
<td>High School/GED</td>
<td>0</td>
<td></td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td>Some college</td>
<td>0</td>
<td></td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td>Associates</td>
<td>34.5</td>
<td></td>
<td>19.5</td>
<td></td>
</tr>
<tr>
<td>Bachelors</td>
<td>34.2</td>
<td></td>
<td>5.6</td>
<td></td>
</tr>
<tr>
<td>Masters/Prof/Doctorate</td>
<td>38.3</td>
<td></td>
<td>42.5</td>
<td></td>
</tr>
<tr>
<td>Region</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>35.3</td>
<td>0.077</td>
<td>30.3</td>
<td>0.122</td>
</tr>
<tr>
<td>Midwest</td>
<td>17.0</td>
<td></td>
<td>6.1</td>
<td></td>
</tr>
<tr>
<td>South</td>
<td>3.2</td>
<td></td>
<td>29.1</td>
<td></td>
</tr>
</tbody>
</table>
### Conclusion:

More people undergoing genetic testing for breast cancer risk in 2010 were concerned that this would adversely affect their health insurance than in 2005, and there was less variation in this perception based on insurance, education and region than in the earlier period. We conclude that, despite the passage of significant U.S. legislation including GINA and PPACA, nearly 1 in 5 people undergoing genetic testing remained concerned about potential risk to their health insurance coverage. Accurate and early identification of those people carrying a cancer genetic mutation is crucial to cancer prevention and treatment efforts; our results suggest a dire need for improved education regarding federal protections for genetic testing so that any barriers to seeking genetic counseling are eliminated.

<table>
<thead>
<tr>
<th>Age</th>
<th>2010</th>
<th>2005</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;55</td>
<td>26.2</td>
<td>14.0</td>
</tr>
<tr>
<td>&gt;55</td>
<td>12.2</td>
<td>21.1</td>
</tr>
</tbody>
</table>
Title: Next generation sequencing-based analysis of BRCA1 and BRCA2 genes: Applicability for fast diagnostics of large samples

Sun-Young Kong¹, Eunhae Cho², Junnam Lee², Myong Cheol Lim¹, Jahyun Jang², Boyoung Park¹, Kyong-Ah Yoon¹, Young-Ho Kim¹ and Eun Sook Lee¹. ¹Research Institute, Hospital, & National Cancer Control Institute, National Cancer Center, Goyang, Korea and ²Green Cross Genome, Yongin, Korea.

Body: Purpose
BRCA1&2 gene mutation test for hereditary breast ovarian syndrome (HBOC) has been conducted mostly by Sanger sequencing. Currently, the next generation sequencing (NGS) is rapidly incorporated to the fields of cancer research and clinical diagnostics. Here we evaluated NGS-based results of BRCA gene analysis and compared with the results of Sanger sequencing for future diagnostic applications.

Methods
The patients (n=100) who have genetic counseling and decided to perform BRCA test were included. All coding regions of BRCA1 and BRCA2 analyzed by both of Sanger sequencing and NGS using Access Array BRCA1, BRCA2 and TP53 kit (Fluidigm, USA). Access array kit was designed to multiplex 48 samples simultaneously for 184 amplicons and the average sequencing depth per base was 6,500X using MiSeq sequencer (Illumina, USA). We developed analysis pipelines to avoid false negative results and detect all pathogenic mutations. The reads were aligned to a reference genome (NCBI human genome assembly build 37) using the BWA-MEM, then all candidate variants called with minimum filtering parameter.

Results
Total of 765 variants were detected by NGS and among them 616 variants (frameshift:22, nonsense:8, splicing:3, missense:290, and synonymous variants:293) were identical with the results from Sanger sequencing. When we evaluated the results of Sanger sequencing as standard methods, the mean allele frequency showed difference as 41.7% and 12.0% for true positive heterozygous variants (616) and false positive variants (149), respectively.

Conclusions
There was no false negative of pathogenic mutations from NGS. The BRCA mutation detection using NGS represented potential applicability in clinical diagnosis.
Title: Experience of pre-operative genetic testing on surgical decision making in newly diagnosed breast cancer patients

Siddhartha Yadav1, Otavio Pereira-Rodrigues1, Lindsay Dohany2, Heidi Dreyfuss2, Jennifer Fulbright2, Ashley Reeves2 and Dana Zakalik2. 1Beaumont Health System, Royal Oak, MI and 2James and Nancy Grosfeld Cancer Genetics Center, Beaumont Health System, Royal Oak, MI.

Body:
Introduction:
BRCA 1 and 2 testing has been widely incorporated into clinical care for women at risk for hereditary breast cancer. Knowledge of BRCA mutation status prior to surgery may influence decision regarding type of surgery. This study analyzes the experience with pre-operative BRCA mutation testing in patients with suspected hereditary predisposition to breast cancer.

Methodology:
Records of 150 patients referred to Cancer Genetics between November 01, 2013 and April 30, 2014 for pre-operative genetic testing were analyzed. This cohort consisted of patients with newly diagnosed breast cancer who met current genetic testing criteria. Patients were excluded if their surgical records were not available or they had not yet completed surgery. A total of 80 patients who completed genetic evaluation and definitive surgery were evaluated. Data on demographic characteristics, tumor pathology, BRCA mutation status and surgical management was collected on all evaluable patients.

Results:
Records of 80 patients who underwent pre-operative genetic testing for hereditary breast cancer risk were evaluated. The median age at diagnosis was 51.5 years. Median time from biopsy to initiation of genetics referral was 10 days. From that point, the median time to cancer genetics appointment was 3 days. Median time from initial genetics visit to definitive surgery was 24 days. 7 (9%) patients underwent surgery within 10 days of the genetics appointment, and prior to receiving the results of the genetic tests.

Of the 80 patients, 5 (6%) tested positive for a BRCA mutation, 3(4%) had a BRCA variant of unknown significance(VUS), and the rest tested negative. 4 of the 5 BRCA mutation carriers underwent bilateral mastectomy, as did 2 of the 3 patients with a BRCA VUS. Of the 72 BRCA -ve patients, 22 (30%) underwent bilateral mastectomies, 42 (58%) underwent partial mastectomy, 7 (10%) patients underwent unilateral mastectomy, and 1 underwent bilateral partial mastectomy.

There was no significant difference on univariate analysis in the age, histopathology (grade, receptor status, lymph node status and margins) in the patients who underwent bilateral mastectomy and those who underwent breast conservation. Mean tumor size was 24.5 mm in the bilateral mastectomy group, compared to 15.8 mm in the unilateral mastectomy group(p=NS). The bilateral mastectomy group had a greater number of close relatives with breast cancer reflecting a more significant family history.

Conclusions:
This study demonstrates the feasibility and successful implementation of preoperative genetic testing in newly diagnosed breast cancer patients. The majority of mutation carriers underwent bilateral mastectomies as did a significant proportion of BRCA -ve patients. Larger tumor size and a more significant family history of breast cancer appeared to be associated with the decision to pursue bilateral mastectomy. Further studies are needed to better characterize the impact of preoperative genetic testing in newly diagnosed breast cancer patients.
Title: Distress as a measure of the psychological impact after disclosure of a BRCA1/2 positive test result

Joana Parreira¹, Susana Esteves¹, Fatima Vaz¹, Carla Simões¹, Paula Rodrigues¹, Ana Luis¹, Ana Clara¹, Sandra Bento¹ and Maria Jesus Moura¹. ¹Instituto Portugues Oncologia Lisboa Francisco Gentil, EPE, Lisboa, Portugal.

Body: Introduction and objectives- A positive result after BRCA1/2 screening can represent a difficult psychological experience. Previous studies have shown that the psychological outcomes following BRCA1/2 testing vary according to the previous individual and family experiences of each person. Objectives of this study were to measure the distress caused by the disclosure of a positive BRCA1/2 test result and to analyse the degree of BRCA1/2 carriers retention of information, transmitted at the post-test counselling interview.

Material and Methods- This is a prospective study. All consecutive individuals with a BRCA1/2 positive test were invited to participate, after the post-test counselling visit. Participation involved signing an informed consent form and agreeing to a structured post-test phone interview, one week and one month after disclosure of the test result. Phone interviews were done by nurses trained by the Psychological Unit of our centre. Measure instruments: 1) Emotional thermometer (ET) - analogical scale ranging from 0 (no distress) to 10 (maximum distress), measures distress during the previous week 2) Distress questionnaire (DQ)- 13 items to evaluate depression, anxiety and loss of emotional control, with a global score ranging from 3 (no distress) and 45 (maximum distress). 3) Knowledge of disease status and understanding of the individualized risk management plan- additional 4 items included at the end of DQ. Subgroup analysis was performed according to age, sex, previous cancer diagnosis and offspring existence using Wilcoxon rank sum test with continuity correction.

Results- From 177 eligible carriers, 28 were not included (14 for logistical reasons; 2 deaths; 1 refused; 3 progressive symptomatic disease ). A total of 149 carriers were included: 120 women (81%) and 29 men (19%); median age 43 yrs (21-74); 67 (45%) with a previous cancer diagnosis and 82 (55%) healthy at risk; 42 (28%) had no offspring and 102 (68%) were professionally active. The mean distress scores were 3.07 (SD 2.72) and 20.13 (SD 7.88) for ET and DQ instruments, respectively. Using the NCCN (2013) guidelines for ET classification, we found that 95 (64%) of our carriers did not have clinically significant distress. For the DQ (using a cut-off <20) this proportion decreased to 54% (81 pts).

Subgroup analysis: A statistically significant difference was found with both ET and DQ for higher distress levels in women than men (p=0.006 and p<0.001, respectively). There was no difference in either measure for age (≤ 50yrs vs > than 50yrs), previous cancer diagnosis or with vs no offspring. Levels of knowledge and understanding of individual risk management were high (average 18.7; maximum 20) and no correlation was found with distress levels. Twenty-eight (19%) carriers were found in need of specialized psychological/psychiatric support and were appropriately referred.

Conclusions- In our BRCA1/2 carrier population clinically significant distress was not frequent and only 19% needed specialized psychological/psychiatric support. Distress was higher in women than in men. Retention of information given during counselling was high, and there was no correlation between information retention and distress levels.
2014 San Antonio Breast Cancer Symposium

Publication Number: P4-12-15
Average Grade: 0

Title: Are we appropriately referring and testing breast cancer women for genetic mutations?

Pankhooori Saraf¹, Dipen Patel¹ and Alice J Cohen¹. ¹Newark Beth Israel Medical Center, Newark, NJ.

Body: Background – Annually it is estimated that Hereditary Breast and Ovarian Syndrome (HBOC) accounts for 5 - 6% of breast cancer in the US. The majority of HBOC are associated with gene mutations in BRCA 1 & 2; other mutations include p53, PTEN, CH1, STK11. These genes are tumor suppressor genes and play a role in the maintainence of genomic integrity. Women with BRCA mutations have a life time risk of developing breast cancer (50 -85%) and ovarian cancer (15-40%). Other cancers have been associated with these mutations as well. The estimated frequency of a mutation in the BRCA gene is 1/800-1/1000. As a primary oncologist it is our responsibility to screen breast cancer patients who may harbour such deleterious mutation and offer appropriate screening, counselling, testing and management. As tests are now available without trained genetic counsellors, the question remains are all appropriate patients being offered genetic testing to assist in treatment and does onsite genetic counselling improve testing rates.

Purpose of study – To evaluate the difference in identification of high risk breast cancer patients and the number of women who undergo genetic testing with or without an onsite genetic counsellor.

Methods – A retrospective chart review was performed of all newly diagnosed breast cancer patients from March 2012 – February 2014. Year 1 was without a genetic counsellor, March 2012 - February 2013 (Gr.1) and year 2 with an onsite genetic counsellor, March 2013 – February 2014 (Gr.2). Information collected included age, stage of breast cancer, receptor type, reason for referral or genetic testing based on NCCN criteria for HBOC testing and results of tests.

Results – 135 new breast cancer patients were identified and 125 were evaluable. In Gr 1, 27/72 (37.5%) met criteria for genetic testing. 15 were offered testing (55.5%). 5/15 (33.3%) completed testing with no patient positive for BRCA mutation; 10 patients were non compliant with recommended testing; 3 patients were tested outside of guidelines with negative results.

In Gr 2, 22/53 (41.5%) met criteria for genetic testing. 16 were offered testing (72.3%); 8/16 (50%) completed testing with 4 positive for a BRCA mutation; 8 patients were not tested (6 patients non compliant with recommended testing, 1 due to insurance issues and 1 refused).

See Table 1.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total number of patients</th>
<th>Genetic test criteria met</th>
<th>Offered testing</th>
<th>Actual testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gr 1</td>
<td>72</td>
<td>27 (37.5%)</td>
<td>15 (55.5%)</td>
<td>5 (33.3%)</td>
</tr>
<tr>
<td>Gr 2</td>
<td>53</td>
<td>22 (41.5%)</td>
<td>16 (72.3%)</td>
<td>8 (50%)</td>
</tr>
</tbody>
</table>

The most common reason for genetic testing was patients with primary breast cancer age < 45 years. Patients who met criteria but were not offered testing were those with age < 45 years and triple negative breast cancer. 5 patients in Gr 1 were seen by the genetic counsellor post treatment and all 5 were tested.

Conclusion – A greater number of women with newly diagnosed breast cancer were identified and offered genetic testing with an onsite genetic counsellor. The percentage of individuals tested increased with an onsite counsellor.
Trancriptome sequencing of the histologically normal breast epithelium of BRCA mutation carriers

Dadrie Baptiste¹, MiRan Choi², Zhiping Wang¹, Yunlong Liu¹, Milan Radovich¹ and Susan E Clare². ¹Indiana University School of Medicine, Indianapolis, IN and ²Feinberg School of Medicine, Northwestern University, Chicago, IL.

**Body:** Background: Meaningful progress in the prevention of breast cancer is unlikely until the risk of the development of breast cancer is translated into specific, quantifiable molecular alterations. Among those women at the greatest risk are BRCA mutations carriers: 55 to 65% of women who inherit a deleterious BRCA1 mutation and approximately 45 % who inherit a deleterious BRCA2 mutation will develop breast cancer by age 70 years. The purpose of this study was to identify the earliest transcriptional alterations present by examining the histologically normal breast epithelia of BRCA mutation carriers.

**Methods:** Epithelia were microdissected and the RNA isolated from the histologically normal breast of 16 frozen tissue cores from known BRCA mutation carriers, who were donors to the Susan G. Komen for the Cure Tissue Bank at the IU Simon Cancer Center. RNA-sequencing was carried out using the Life Technologies SOLiD XL 5500 Platform. RPKM gene expression values from the BRCA specimens and from Komen normal breast epithelium (controls; Pardo et al, Breast Cancer Research, 2014) were merged, quantile normalized, and batch effect corrected. Normalization and differential gene expression was performed using EdgeR v2.11

**Results:** 6583 RNAs were differentially expressed with the false discovery rate set at 0.1.

1. DNA damage is signaled by a tripartite system that includes kinases, phosphatases and proteins with modular domains, e.g., the BRCA 1 C-terminal (BRCT), that recognize phosphorylated linear motifs in other proteins. Several BRCT domains recognize motifs phosphorylated by kinases that are activated by DNA damage. To identify the proteins active in DNA damage signaling, Monteiro and colleagues generated a protein-protein interaction map for seven proteins with tandem BRCT (Cell Signaling, 2012). A significant proportion of the genes with decreased expression in our data set encode proteins that have been identified in this network.

2. Two of the top biological processes affected by the BRCA mutations in the histologically normal breast tissue are translation, and cellular protein metabolism.

3. There was significantly less expression of genes associated with other hereditary cancer syndromes inducing the genes responsible for Lynch Syndrome: MHL1, MHS2, MSH6 and PMS2; and the multiple endocrine neoplasia genes RET and MEN1.

4. High grade serous carcinoma of the ovary, the histologic subtype associated with BRCA mutation, may originate in the distal Fallopian tube. The list of differentially expressed genes from microdissected breast epithelia was compared with that derived from gene array profiling of microdissected Fallopian tube epithelium from histologically normal BRCA1 mutation carriers and controls. 795 genes were common to both gene sets, of which 354 were regulated similarly, i.e., increased or decreased.

**Conclusions:** There are significant transcription alterations in the histologically normal breast tissue of BRCA mutation carriers. These data will have to be corroborated at the protein level and functional level. Once substantiated, they have potential to stratify risk and to serve as targets for prevention.
BRCA1/2 founder mutations among high-risk HBOC patients from Northeast of Brazil

Ivana L Nascimento¹,², Gabriela ES Felix¹, Camila A Sandes¹, Taisa B Machado-Lopes¹, Thais F Bomfim¹, Roberto Meyer¹, Maura Romeo¹, Betania Toralles¹ and Kiyoko A Sandes¹. ¹Instituto de Ciencias da Saude-UFBA, Salvador, Bahia, Brazil and ²Nucleo de Oncologia da Bahia, Salvador, Bahia, Brazil.

Body: Background: Genetic susceptibility to Hereditary Breast and Ovarian Cancer (HBOC) syndrome could be test by the screening of BRCA1 and BRCA2 genes. Thus, knowing that each population has its degree of heterogeneity and the Brazilian population is one of the most heterogeneous of the world, we aimed to verify the frequency of BRCA1/2 founder mutations in high-risk HBOC patients from Bahia, the biggest State of the Northeast of Brazil.

Methods: It was analyzed the DNA of 99 unrelated high-risk HBOC patients, considering the criteria of NCCN v.1.2010. These patients were tested for the next founder mutations: BRCA1 c.211A>G (Galician), BRCA1 943ins10 (African), BRCA1 3450del4 (Hispanic) and BRCA2 c.156_157insAlu (Portuguese) by sequencing or PCR/RFLP. All positive results were confirmed by two sequencing reactions. The genetic ancestry was analyzed with a panel of 9 ancestry informative markers (AIMs). The clinical and epidemiological data were collected during the genetic counseling and were analyzed in Epi Info™ 7, while the frequency of the AIMs was analyzed in GENEPOP, and in ADMIX95 and STRUCTURE the genetic admixture was estimated.

Results: All patients had personal and/or familial history of breast and/or ovarian cancer. Most of the patients self-reported as mulatto (62.63%), followed by black (21.21%), white (10.10%) and others (6.06%). The African, Amerindian and European ancestry contribution estimated were 35.33%, 11.31% and 53.35% respectively ($r^2 = 0.998$). Three mutations were detected: BRCA1 3450del4 in five patients (5.05%), BRCA1 c.211A>G in two patients (2.02%), and BRCA1 943ins10 in one patient (1.01%). All patients with these mutations were from State of Bahia and self-reported as mulatto or white.

Conclusion: Although the Portuguese settled Brazil, here it was observed that this population seems to have high Spanish ancestry contribution, due to the high frequency of the BRCA1 3450del4 and c.211A>G (7.07%). It is also interestingly the frequency of BRCA1 943ins10 (1.01%), because the State of Bahia has the higher rate of African-descendants in Brazil and most of the patients exhibited these physical traits (mulatto and black, 83.84%). Thus, high-risk HBOC patients from that region of Brazil could be screening first for the BRCA1 3450del4, BRCA1 943ins10, and BRCA1 c.211A>G, where they have founder effect.
Title: Whole exome sequencing reveals greater intratumor genetic heterogeneity in primary breast cancer arising in African American compared to Caucasian women

Tanya E Keenan¹, Edmund A Mroz¹, James W Rocco¹, Leif W Ellisen¹, Beverly Moy¹ and Aditya Bardia¹. ¹Massachusetts General Hospital, Boston, MA.

Body: Introduction: Multiple studies have reported that African American women have higher mortality from breast cancer than Caucasian women. While the higher mortality could be due to several factors including access to care, relatively little is known about tumor genetic differences that may contribute to this disparity. A particularly uncharted area of investigation is intratumor genetic heterogeneity arising from various subclones within a tumor. The primary objective of this study was to characterize the differences in mutation patterns and evaluate intratumor heterogeneity in primary breast cancer between African American and Caucasian women.

Methods: Somatic mutations based on whole exome sequencing of primary breast cancers were analyzed using data from The Cancer Genome Atlas (TCGA). Individuals with races that were Native American, Asian, or not reported were omitted. Logistic regression analyses were conducted to compare the odds ratio (OR) with 95% confidence interval (CI) of non-silent gene mutations by race. Total non-silent gene mutations and genetic heterogeneity, measured by the mutant-allele tumor heterogeneity (MATH) algorithm (Mroz et al. Cancer 2013), were compared by race with Mann-Whitney two-sample tests and linear regression analyses, respectively. All regression analyses were adjusted for age and stage. A p value of 0.05 was considered statistically significant.

Results: The analytical dataset comprised non-silent somatic mutations in unique genes of primary breast cancers from 799 women (689 Caucasian; 110 African American). TP53 mutations were significantly more prevalent in African Americans than Caucasians (OR 1.7; CI 1.1-2.7, p = 0.01). However, there was no difference in TP53 mutation prevalence after further adjustment for triple negative status (OR 1.2; CI 0.7-2.0, p = 0.53). Total non-silent gene mutations per patient were significantly greater in African Americans compared to Caucasians (Table, p = 0.01). Similarly, MATH was significantly greater in African Americans than Caucasians (Table), specifically 5.3 units (CI 2.6-7.9, p <0.001) greater after adjustment for age and stage. Further adjustment for triple negative status showed that MATH was still 4.2 units (CI 1.6-6.9, p = 0.002) greater in African Americans than Caucasians.

Somatic mutations and genetic heterogeneity by race

<table>
<thead>
<tr>
<th></th>
<th>Caucasians</th>
<th>African Americans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total non-silent mutations per patient (median, interquartile range)</td>
<td>33 (21-60)</td>
<td>39.5 (26-74)</td>
</tr>
<tr>
<td>Intratumor genetic heterogeneity (mean, 95% CI)</td>
<td>39.0 (38.0-40.0)</td>
<td>43.9 (41.3-46.5)</td>
</tr>
</tbody>
</table>

CI, confidence interval.

Conclusions: Primary breast cancers in African Americans exhibit significantly greater total non-silent somatic mutations and intratumor genetic heterogeneity but not higher frequency of TP53 mutations, suggesting African Americans have genetically more complex tumors as compared to Caucasian women. Additional research is needed to determine if the observed tumor genetic differences could contribute to the known racial disparity in breast cancer mortality.
Household net worth is associated with racial disparities in hormonal therapy adherence among women with early stage breast cancer

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Background: Non-adherence to adjuvant hormonal therapy is common and has been associated with both increased copayment amount and black race. Studies suggest controlling for wealth may eliminate racial disparities for a variety of medical condition. We investigated the impact of personal finance on disparities in adherence rates.

Patients and Methods: Using de-identified, integrated pharmacy and medical claims data from the Optum insurance claims database, we identified women >50 years old on hormonal therapy for early breast cancer with at least 2 mail order prescription refills between 1/1/07 and 12/31/11. Variables evaluated included demographic and clinical information, annual household income, estimated household net worth (<$250,000, $250,000-750,000, >$750,000), insurance type, and monthly copayment amounts (<$10, $10-20, >$20). Non-adherence was defined as a medication possession ratio <80% of eligible days during a 2-year period. Logistic regression analyses were conducted by sequentially including sociodemographic variables, copayment amount, and each of the economic variables. Due to the strong correlation between net worth and income, and variance of income with age, net worth was included in multivariate models; an additional analysis was done stratifying patients by net worth.

Results: We identified 15,522 subjects who initiated hormonal therapy; 25% were non-adherent during the study period. Adherence was 67% with net worth <$25,000 and 81% with net worth >$750,000 (p<0.001). In a univariate analysis, adherence was negatively associated with black race (OR 0.76, p<0.001), advanced age, comorbidity, extent of surgery, Medicare insurance, and higher copayment. Adherence was positively associated with higher household income (OR 1.3, p<0.001) compared to lowest income and with medium net worth (OR=1.26, p<0.001) and higher net worth (OR 1.5, p<0.001) compared to the lowest net worth group. The negative association of black race and adherence (OR 0.76) was reduced by the sequential addition of sociodemographic variables (OR 0.78, p<0.001), copayment (OR 0.80, p=0.004), and net worth (0.87, p=0.08). In the analysis stratified by net worth, black race was no longer associated with decreased adherence. In patients within the high net worth category, high copayment amount was also no longer associated with decreased adherence (OR 1.0, p=0.77).

Conclusions: We have shown that financial factors, and in particular net worth, partially explain the lower hormone therapy adherence rate in black women compared to white women. These results suggest economic factors may contribute significantly to disparities in the quality of breast cancer care.
Title: United States breast cancer mortality trends in young women according to race

Foluso O Ademuyiwa¹, Feng Gao¹, Lin Hao¹, Daniel Morgensztern¹, Rebecca L Aft¹ and Cynthia X Ma¹.¹Washington University School of Medicine, St Louis, MO.

Body: Purpose
Studies have shown that there is a negative prognostic impact of young age at diagnosis on clinical outcome in women with breast cancer (BC). We sought to determine if there is a differential effect of race in this high-risk population of women and examined mortality trends according to race and diagnosis age.

Methods
Using the Surveillance, Epidemiology, and End Results (SEER) program 1990-2009, women diagnosed with invasive BC under the age 50 were identified. Clinicopathologic characteristics, overall survival, and BC specific survival rates were compared between racial groups. After adjustments for stage, ER, PR, histology, age, diagnosis year, tumor size, nodal status, and grade, multivariate logistic regression analyses determined the risk-adjusted likelihood of survival for whites and blacks. Annual hazard rates (HR) of BC deaths after diagnosis according to race and calendar period, and adjusted relative hazards of death for white and black women stratified by age at diagnosis were computed.

Results
Overall, 162,976 women were identified, including 126,573 whites (77.7%), 20,405 blacks (12.5%), and 15,998 of other races (9.8%). At a median follow-up of 85 months, five year disease specific survival rates were 90.1% for whites, and 79.3% for blacks. During the study period, the annual HR for death in whites decreased by 26% at 5 years after diagnosis, in contrast to the rates in blacks women decreasing by only 19%. With 1990 as referent, adjusted relative hazards for death in 2005 for white and black women <40 years were 0.55 (95% CI 0.46-0.66) and 0.68 (95% CI 0.49-0.93) respectively. In whites and blacks ≥40, 2005 hazards were 0.53 (95% CI 0.47-0.60) and 0.78 (95% CI 0.61-0.99).

Conclusion
Among young women diagnosed with BC, blacks have a worse outcome than whites. Mortality declines in both racial groups have been observed over time, although more rapid gains have occurred in whites. Emphasis should be placed on improving outcomes for young BC patients.
Clues to the abundance of triple negative breast cancer in women of African descent

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**Body: Background:** Triple negative breast cancer (TNBC) is over-represented in indigenous African women and in women of African descent. The majority of women diagnosed with breast cancer in Nigeria, Uganda and Kenya have TNBC. Dr. Lisa Newman and colleagues reported TNBC rates of 83.3% in Ghana, 41.4% among African-Americans (AA) and 15.4% for Caucasian-Americans (C)(Stark et al, Cancer, 2010). The abundance of TNBCs in both African and African-American women has yet to be explained and the incidence rates suggest that there may be a genetic predisposition to this particularly aggressive form of breast cancer.

Lipocalin 2 (LCN2), also known as NGAL, is a small-secreted glycoprotein. It chelates iron making this element unavailable to infectious agents that require it for growth including salmonella and tuberculosis bacilli, and Plasmodium parasites. Inactivation of the tumor suppressor gene “hypermethylated in cancer 1” (HIC1) results in upregulation of LCN2 at the mRNA and protein levels. Recently, it was shown that HIC1 is silenced in TNBC when but not in the luminal and HER2 subtypes (Cheng et al, Cancer Research, 2013). The purpose of this study was to determine if the expression of either of these two genes, LCN2 and HIC1, differs in breast epithelia as a function of race.

**Methods:** Multiple epithelial cell lines from normal, healthy breast tissue donated to the Susan G. Komen for the Cure Tissue Bank at the IU Simon Cancer Center were established (Sauder et al, BMC Cell Biology, 2014). Six epithelial cell lines from AA and six from C donors were matched for age, menstrual status and Gail risk score. RNA and DNA were extracted from the cultured cells. Genome-wide gene expression was assayed using the IlluminaHumanWG-6 v3 Expression Bead Chip. Differential gene expression was determined using PADE (PAirwise Differential Expression; Expression Analysis, Inc). SNPs were identified using the Illumina Human 1M-Duo BeadChip.

**Results:** The expression of LCN2 in the normal breast epithelia of AA donors was 20-fold that in C donors (p=0.01). The expression of HIC1 was 1.94-fold less in AA but this did not reach statistical significance (p=0.29). Analysis of HIC1 DNA data was complicated by the small number of samples and the paucity of probes within HIC1 on the Human 1M-Duo BeadChip. The search, therefore, was extended 10,000 bases upstream. There appears to be a heterozygous deletion in two of the AA and one of the C donors in this region of the genome. We then searched the ENSEMBLE database to determine if there are any SNPs in HIC1 or its promoter region which differ by Race; only rs8065350 did so. This SNP is in the promoter region of this gene and occurs within the ENCODE Chip-Seq. tracks of REST/NRSF and Elf1.

**Conclusions:** LCN2 expression may provide a survival advantage to individuals residing in regions of the world where iron-requiring infectious agents are endemic. Iron and folic acid supplementation in a clinical trial in East Africa resulted in significantly more deaths and hospitalizations from infectious diseases. LCN2 expression may provide innate immunity while at the same time predisposing women to TNBC in an as of yet undetermined manner. It is interesting to note that LCN2 expression is increased in prostate carcinoma in AA men when compared to C.
Title: Characteristics of benign breast disease and subsequent risk of breast cancer differ by age among African Americans

Michele L Cote¹, Julie J Ruterbusch¹, Sudesha Bandyopadhyay², Quratulain Ahmed², Barra Alosh², Eman Abdulfatah², Haitham Arabi² and Rouba Lynn Ali-Fehmi². ¹Wayne State University, Karmanos Cancer Institute, Detroit, MI and ²Wayne State University, Detroit, MI.

Body: Introduction: A history of benign breast disease (BBD) is common and certain established pathologic features are associated with increased breast cancer risk. These associations have been reported primarily from studies of white women, where incidence of BBD peaks in the 4th or 5th decade of life. Previous work in an African American (AA) cohort of women with BBD showed AA women were younger at their first BBD diagnosis. Thus investigating whether different features of benign lesions may be associated with age and/or subsequent breast cancer risk in this population is warranted.

Methods: Benign breast biopsies from 1,867 AA women with BBD diagnosed from 1997-2003 were microscopically reviewed for 15 benign features (apocrine metaplasia (AM), ductal hyperplasia (DH), atypical ductal hyperplasia (ADH), lobular hyperplasia (LH), calcifications (Calc), cysts, duct ectasia (DE), fibroadenoma (FA), fibrosis, intraductal papilloma (IDP), radial scar (RS), sclerosing adenosis (SA), columnar cell alterations (CC), mucocele-like tumors (MLT), and atrophy), and followed for subsequent breast cancer in metropolitan Detroit, Michigan. Data from 439 women under 40 and 1,428 women 40 and older at BBD diagnosis were available for analysis, with a mean follow-up time of 14 years. Differences between age categories for BBD features were compared using chi-square tests, and risk of breast cancer was estimated with odds ratios (OR) and 95% confidence intervals (95% CI) calculated from logistic regression analysis.

Results: Women 40 and over were more likely to be diagnosed with nearly all of the benign characteristics compared to younger women, including: AM, DH, Calc, cysts, CC, and ADH (all p-values <0.001). Younger women were more likely to present with FA (56% to 43%, p-value<0.001) and no atrophy (44% versus 24%, p-value<0.001) compared to older women. Risk of subsequent breast cancer was associated with cysts (OR=3.95, 95% CI: 1.09, 14.29) in the younger age group, but not the older (OR=1.25, 95% CI: 0.76, 2.03). CC were also associated with breast cancer risk in the young (OR=5.35, 95% CI: 1.45, 19.73) but not the older women (OR=1.44, 95% CI: 0.82, 2.50). RS were associated with increased risk in both groups, but only statistically significant for the older women (OR=3.60, 95% CI: 1.54, 8.39), and not the younger women (OR=6.64, 95% CI: 0.73, 60.67). Similarly, risk of cancer was associated with a diagnosis of ADH in both groups, but only statistically significant among older women (OR=3.02, 95% CI: 1.34, 6.79), and not younger women (OR=6.75, 95% CI: 0.71, 64.43).

Conclusions: Characteristics of BBD differ by age, with more women over the age of 40 being diagnosed with various conditions. Risk of subsequent cancer also varies, although RS, CC and ADH appear to increase risk in both age groups.
Title: Understanding disparities in breast cancer care in Memphis, Tennessee

Elena M Paulus¹, Frances E Pritchard¹, Simonne S Nouer², Elizabeth A Tolley², Brandon S Boyd¹, Jesse T Davidson¹, Gitonga Munene¹ and Martin D Fleming¹. ¹University of Tennessee Health Science Center, Memphis, TN and ²University of Tennessee Health Science Center, Memphis, TN.

Body: Background: Recent literature highlights the troubling racial divide in breast cancer mortality that continues to widen in most major cities across the country. Although significant progress has been made in improving overall breast cancer survival, disparities among racial, ethnic, and underserved groups still exist. Previous studies examine the breast cancer mortality disparity in the 50 largest U.S. cities, and Memphis demonstrates the largest breast cancer mortality disparity for African Americans (AA). The goal of this investigation is to quantify racial disparities in the context of breast cancer treatment in order to reduce disparities in recurrence and mortality for breast cancer in the city of Memphis, Tennessee.

Methods: Patients with a biopsy-proven diagnosis of breast cancer over a 10 year period ending December 31, 2012 were obtained from the tumor registry of a university hospital system. Females of Caucasian and African-American race were included, while males, patients less than eighteen years of age, and patients with unknown histology, clinical stage, or type of surgery were excluded. Primary outcomes measured included overall survival and recurrence. Secondary outcomes examined were stage at diagnosis by race and time from diagnosis to surgery.

Results: 3072 breast cancer patients were reviewed (41% AA). AA patients were more likely to have advanced (Stages II, III, or IV) clinical stage of breast cancer at diagnosis versus Caucasian patients. Of the 113 recurrences, 62% occurred in AA. Of the 676 deaths, 54% occurred in AA. After adjusting for race and clinical stage of breast cancer, AA breast cancer patients had a 2.0 higher odds of recurrence when compared to Caucasian breast cancer patients (95% CI 1.4, 3.0). AA breast cancer patients were 1.5 more likely to die compared to Caucasian breast cancer patients (95% CI: 1.2, 1.8), after adjusting for race, age at diagnosis, clinical stage of breast cancer, ER, PR, and HER2 status, and recurrence. AA women with stages 0, I, II, and III breast cancer all had a statistically significant longer median time from diagnosis to surgery (TDS) than Caucasian women.

Conclusions: African-American patients were more likely to have advanced clinical stages of breast cancer at diagnosis versus Caucasian patients on a citywide level in Memphis. African-American breast cancer patients have a higher odds of recurrence and mortality when compared to Caucasian breast cancer patients, after adjusting for appropriate demographic and clinical attributes. Several factors have been suggested for the disparities including racial differences in access to and utilization of screening and treatment, risk factors distributed by race and socioeconomic status (SES), biological differences such as tumor aggressiveness, and cultural factors. More work is needed to develop, evaluate, and disseminate interventions to decrease inequities in timeliness of care for breast cancer patients.
Title: Race as an independent factor affecting post-mastectomy reconstruction in Asian women

Carmen F Fong1, Alyssa Gillego1, Theresa Shao1, Erika Reategui1, Catherine Campo1, Sarah Cate1, Christopher Mills1, Mark L Smith1, Gina Aharonoff1 and Susan K Boolbol1. 1Mount Sinai Beth Israel Medical Center, New York, NY.

Body: BACKGROUND:
Racial disparities exist in many areas of breast cancer treatment. Multiple factors influence whether Asian women with breast cancer undergo immediate reconstruction after mastectomy. This study aims to evaluate breast reconstruction trends at a comprehensive cancer center and to determine whether race is an independent predictor of breast reconstruction.

METHODS:
Using an IRB-approved, prospectively maintained database, post-mastectomy reconstruction rates were determined for 2003 to 2013. This database was compiled from three university-affiliated hospitals serving a diverse urban population. A total of 5379 patients were identified who were treated for breast cancer during the 10-year period. The odds-ratio for immediate breast reconstruction was compared among different races. The data was examined to identify factors influencing the decision for post-mastectomy reconstruction, including age, stage of presentation, marital status, and family history of breast cancer.

RESULTS:
Thirty percent (n=1614) of women treated for breast cancer underwent mastectomy, while seventy percent (n=3765) received breast-conserving therapy. In the mastectomy group, a unilateral procedure was performed in 93.1% (n=1503) of women and a bilateral procedure in 6.9% (n=111). The immediate reconstruction rate after mastectomy was 70% (n=1130), with only 30% (n=484) of women not undergoing reconstruction. Of the women undergoing mastectomy, 58.5% were white, 14.9% were black, 13.1% were Hispanic, 7.5% were Asian and 6% of women did not report race. Of the women who underwent breast reconstruction after mastectomy, 60.5% were white women, 13.4% were black women, 13% were Hispanic women, 6.9% were Asian women and 6.2% of women did not report race. The immediate reconstruction rate by race was 72.2% for white women, 63.2% for black women, 69.8% of Hispanic for women, and 64.8% for Asian women. The remainder did not receive breast reconstruction. The unadjusted odds ratio (OR) for immediate breast reconstruction, including both unilateral and bilateral cases, for black versus white women was 0.62 (95% confidence interval 0.46-0.84; P=0.001). The OR for breast reconstruction for Hispanic versus white women was 0.88 (95% confidence interval; 0.63-1.22; P=0.45). The OR for unilateral breast reconstruction for Asian versus white women was 0.66 (95% confidence interval 0.44-0.99; P=0.04.)

CONCLUSION:
Reconstruction rates vary by race, with Asian women being less likely to undergo immediate breast reconstruction after mastectomy. Compared to white women, Asian and black women were both significantly less likely to have immediate breast reconstruction following mastectomy. Many variables may contribute to this disparity in breast cancer care. Language and cultural beliefs may be unique factors that influence Asian women’s decision for post-mastectomy reconstruction and warrant further study.
Title: The effect of socioeconomic status and race on breast cancer tumour biology and stage at diagnosis


Body: Introduction: Mortality from breast cancer has declined over the last 2 decades. However, not all racial groups have benefited equally. African American women continue to die from breast cancer at higher rates than do white women. While racial disparities exist in breast cancer outcomes, they may largely be explained by socioeconomic factors. The effect of poverty on more aggressive breast cancer has not been well studied. We evaluated the association of race, insurance, and age on breast cancer stage and tumour biology within an urban inner city safety net hospital.

Methods: A retrospective review of a prospective breast cancer database was used to identify 535 women with stage 0 to IV breast cancer seen at UF Health Jacksonville from the period January 2009-March 2013. Age, race and insurance status at the time of diagnosis were used as covariates for defining disparities in stage at diagnosis and tumour biology. Tumour profile was defined as four groups: luminal A (ER+, PR+, HER-2 neg), luminal B (ER+, PR neg, HER-2 neg), HER-2/neu positive, and triple negative. Insurance categories were defined as Commercial, Medicare, Medicaid/Charity.

Results: There was equal racial distribution between African American (47.3%) and white women (47.5%) with 5.2% other races. A relatively large number of patients had Medicaid/Charity coverage (37.1%), followed by Medicare at 32.7%, and 30.3% had commercial insurance. The mean age was 58.8 (SD=12.9). There was no significant association between race and stage at diagnosis within our patient population (p=0.869). However, women with Medicaid/Charity coverage were diagnosed at more advanced stage compared to women with other insurnace (adjusted p-value <=0.011). Stage III disease for Medicaid/Charity was 23.1% vs 13.4% for commercial & 12.7% for Medicare coverage. Luminal A was the most prevalent tumour biology overall (58%), but triple negative tumours were more frequent in the black population (26.6%) vs white (13.1%) vs other races (17.9%). More aggressive tumour biology was found in black women compared to white women (adjusted p-value=.0007), and those with Medicaid/Charity coverage compared to other insurance (adjusted p-value <.0001). For example, 28.1% of Medicaid/Charity patients had triple negative tumours vs 14.8% of commercial and 14.9% of Medicare population. Younger women were diagnosed at later stages (r=-0.196, p<.0001) and had more severe tumour subtypes (r=0.235, p<.0001).

Conclusion: UF Health Jacksonville encounters a uniquely equal racial distribution of women with breast cancer. Within our population, women with lower socioeconomic status based on insurance type present with more advanced stage at diagnosis independent of race. Black race and Medicaid/Charity coverage was significantly associated with more aggressive tumour biology. These results suggest that socioeconomic factors may have a significant influence on the breast cancer disease process and may contribute to racial disparities in breast cancer outcomes.
**Title:** Breast cancer histological subtypes by race/ethnicity

Carol Parise¹ and Vincent Caggiano¹. ¹Sutter Institute for Medical Research, Sacramento, CA.

**Body:**

**BACKGROUND**

It is generally accepted that Asian/Pacific Islanders with breast cancer have the same or better survival than whites. Infiltrating duct carcinoma (IDC) is the most common histologic subtype of breast cancer but little is known about whether this subtype varies by race/ethnicity. The purpose of this study was to determine if there was a difference in incidence of IDC among different race/ethnicities.

**METHODS**

We identified 197,570 cases of AJCC stages 1-4 first primary female invasive breast cancer from the California Cancer registry 2000-2011. The race/ethnicity distribution was as follows: Whites n=130,634; Blacks n=11,927; Hispanics n=32,291; Asian/Pacific Islanders (API) n=22,027; and American Indians n= 691. Histology was classified using ICDO-3. The distribution of infiltrating ductal, infiltrating lobular, mixed ductal/lobular, mucinous, tubular, and medullary cancer was examined using contingency tables and Pearson Residuals. Odds ratios were computed for infiltrating ductal versus all other histologies for each race/ethnicity using whites as the comparator with logistic regression analysis adjusting for age, stage, socioeconomic status, grade, and ER/PR/HER2. The stage X race interaction was tested.

**RESULTS**

Whites had fewer than expected cases of IDC (z = -8.5) and APIs had more than expected (z = 9.2). Blacks (z = 5.6) and Hispanics (z = 6.1) had a higher number of IDC cases than expected and also had a higher than expected number of cases of medullary (black: z=12.8; Hispanic: z =10.7). The proportion of American Indians with each of the subtypes was similar to what would be expected.

The stage X race interaction was statistically significant so analyses were conducted separately for each stage. APIs had an increased risk of IDC in all stages except for stage 4. Hispanics had an increased risk in stage 3 but a decreased risk in stage 4. American Indians had the same risk as whites for all stages. Blacks had decreased odds of IDC in stage 1 but increased odds in stage 3. (Table 1)

**Table 1. Odds ratios and 95% confidence intervals of infiltrating duct carcinoma from the California Cancer Registry 2000-2011.**

<table>
<thead>
<tr>
<th></th>
<th>White OR (95% CI)</th>
<th>Black OR (95% CI)</th>
<th>Hispanic OR (95% CI)</th>
<th>Asian/Pacific Islander OR (95% CI)</th>
<th>American Indian OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage 1</td>
<td>1.00</td>
<td>0.90 (0.81, 0.99)</td>
<td>0.98 (0.92, 1.05)</td>
<td>1.20 (1.12,1.29)</td>
<td>1.31 (0.89, 1.92)</td>
</tr>
<tr>
<td>Stage 2</td>
<td>1.00</td>
<td>0.99 (0.90, 1.09)</td>
<td>1.06 (1.00, 1.14)</td>
<td>1.39 (1.29, 1.50)</td>
<td>0.73 (.051, 1.02)</td>
</tr>
<tr>
<td>Stage 3</td>
<td>1.00</td>
<td>1.27 (1.10, 1.48)</td>
<td>1.24 (1.12, 1.37)</td>
<td>1.74 (1.52, 1.99)</td>
<td>1.02 (0.59, 1.76)</td>
</tr>
<tr>
<td>Stage 4</td>
<td>1.00</td>
<td>1.02 (0.79, 1.31)</td>
<td>0.78 (0.64, 0.94)</td>
<td>1.21 (0.94, 1.56)</td>
<td>0.48 (0.19, 1.19)</td>
</tr>
</tbody>
</table>

Confidence intervals that include 1.00 indicate that the odds of IDC were no different than the reference category.

**CONCLUSION**

The incidence of IDC is not the same for all race/ethnicities and depends on the stage of disease. APIs are at an increased risk for IDC in all except for the highest stage of disease.
INTRODUCTION: Despite extensive progress in breast cancer prevention, diagnosis and treatment over the past 20 years, African American women suffer disproportionate breast cancer morbidity and mortality. In 1998, breast cancer deaths among African American women were 28% higher than in white women and the five-year survival rate for African American women was 71% compared with 86% for white women. The purpose of this bibliometric study is to assess our national research efforts to combat this racial disparity by analyzing breast cancer research productivity in the United States focusing on African American women from 1992 to 2012.

METHODS: This retrospective bibliometric analysis of public data was exempt from Institutional Review Board approval. Articles with "Breast Neoplasm" as a major medical subject heading (MeSH) term published between 1992 and 2012 were identified in the National Library of Medicine MEDLINE database. In addition, articles with "African Continental Ancestry Group" which included African American as a major MeSH term were identified. Country of origin, methodology, journal name, first author specialty and funding sources were recorded. Growth in number of publications was analyzed using linear and nonlinear regression statistical analysis.

RESULTS: A total of 113,721 journal articles were identified with "Breast Neoplasm" as a major MeSH term worldwide, of which 34,155 (30.0%) were published from the United States. Among United States publications, 668 (2.0%) were specific to African ancestral populations. From 1992 to 2012, both African ancestral and non-African ancestral specific articles displayed linear growth patterns (p < 0.0001). National Institute of Health (NIH) funded studies displayed an exponential growth pattern (p < 0.0001) for African ancestral specific articles and displayed a linear growth pattern (p < 0.0001) for non-African ancestral articles. The largest specialty contributor of African ancestral specific articles was Epidemiology and Public Health (33.2%), followed by Medicine (internal medicine, family medicine, obstetrics & gynecology, nursing) (24.7%), Basic Sciences (17.4%), Surgery (15%), Medical and Radiation Oncology (4.7%), Pathology (2.9%) and Radiology (2.1%). African ancestral specific articles were most frequently published in Cancer (10.8%).

CONCLUSION: Among breast cancer publications from the United States, only 2% of the articles were specific to African ancestral population, which is concerning given recent data indicating the persistent ethnic disparity in survival. However, the trend in research productivity in this population is encouraging, largely due to the exponential growth of NIH-funded studies specific for African American women in the past 20 years. NIH-funded research accounts for 52% of all published African American specific breast cancer studies as compared to 40.5% of studies that are non-specific to this population. Ultimately, improvements in breast cancer incidence, mortality, and survival rates in African American women will undoubtedly result from quality research and should continue to be a priority in our nation’s breast cancer research agenda.
Title: Influence of socio-economic deprivation score on outcomes for patients diagnosed with early breast cancer over a 9 year follow up at Kings College Hospital, UK

Olga Oikonomidou1, Mercy Ofuya1 and Anne S Rigg1. 'Kings College Hospital, London, United Kingdom.

Body: Background: Socio-economic deprivation may have an influence on the outcome for patients diagnosed with breast cancer. The aim of this study was to investigate differences in survival outcome from breast cancer in women from different socio-economic backgrounds and to identify underlying factors that may contribute to any variation.

Material and methods: This is a retrospective analysis of data from female patients diagnosed with early breast cancer at Kings College Hospital, a teaching hospital in London, UK between 2004 and 2012. For this study, mean, standard deviation, range and 50th percentile were calculated for the variables investigated. Incidence of death was estimated for deprivation quintile, ethnicity and year of diagnosis. The Cox proportional hazards model was used to estimate the relationship between survival and the several variables (deprivation, income score and ethnicity). The survival rates between levels of deprivation were compared using Kaplan Meier survival curve and log-rank test.

Results: A total of 330 women with a diagnosis of early breast cancer were identified. Analysis of data on all patients (n=330) showed that the median survival time at which 50% of the patients survive is 4018 days (95% CI: 2556, 5479). Deprivation quintile ranged from levels 1 to 5 with level 1 defined as ‘affluent’ and level 5 being ‘most deprived’. These scores were reported for a total of 80 patients. Forty of these were alive and 40 had died from metastatic breast cancer. The two groups were matched for age, year of diagnosis and stage of disease. The highest incidence of death (19/33; 58%) was in level 5 of the deprivation quintile. There was no difference in patients' survival rates between the 5 levels of the deprivation quintile. There was no difference between survival rates in white ethnic group compared with the black ($\chi^2=0.03, p=0.86$). There was no evidence of significance in relationship between hazard of death and deprivation in black and white ethnic groups. Similarly, there was no evidence of significance in relationship between hazard of death and income score in black and white ethnic groups. Adjusting for ethnicity also showed that deprivation and income score had no effect on hazard of death.

Conclusion: According to our study higher degree of socio-economic deprivation is associated with higher mortality in breast cancer. Previous studies from different hospitals in UK have shown that black women have a significantly poorer outcome that white patients despite equal access to healthcare. Our study has shown that there is no correlation between ethnicity (white ethnic and black groups) and survival rates, hazard of death or deprivation in patients treated in Kings College Hospital.
2014 San Antonio Breast Cancer Symposium

Publication Number: P4-15-01
Average Grade: 5.00

Title: Distinct early proliferation response to neoadjuvant anti-HER2 antibody drug conjugate +/- endocrine therapy in early breast cancer in the WSG ADAPT HER2+/HR+ trial

Oleg Gluz1,2, Ulrike Nitz1,2, Kuemmel Sherko3, Kraemer Stefan4, Michael Braun5, Claudia Schumacher6, Bahriye Aktas7, Helmut Forstbauer8, Toralf Reimer8, Peter Fasching10, Jochem Potenberg11, Daniel Hofmann11, Ronald E Kates1, Rachel Wuerstlein1,12, Matthias Christgen13, Hans H Kreipe13 and Nadia Harbeck1,12. 1West German Study Group, Moenchengladbach, Germany; 2Ev. Hospital Bethesda, Breast Center Niederrhein, Moenchengladbach, Germany; 3Clinics Essen-Mitte, Clinics for Senology/Breast Center, Essen, Germany; 4University Clinics Cologne, Breast Center, Cologne, Germany; 5Rotkreuz Clinics Munich, Women Clinics, Munich, Germany; 6St Elisabeth Hospital, Cologne, Germany; 7University Clinics Essen, Women Clinics, Essen, Germany; 8Practice Network Troisdorf, Troisdorf, Germany; 9Clinics Suedstadt, Rostock, Germany; 10University Clinics Erlangen, Women Clinics, Erlangen, Germany; 11Ev. Waldkrankenhaus, Berlin, Germany; 12University Hospital Munich, Breast Center, CCC of LMU, Munich, Germany and 13Medical School Hannover, Institute of Pathology, Hannover, Germany.

Body: Background: HER2+, hormone receptor-positive (HR+) breast cancer is a distinct subtype associated with good prognosis but poor response to standard chemotherapy + anti-HER2 (single or dual blockade). Substantial overtreatment by poly-chemotherapy combined with anti-HER2 therapy is suspected in this subtype. Yet, the efficacy of combining endocrine therapy (ET) with anti-HER2 therapy or novel antibody drug conjugates like T DM1 without systemic chemotherapy remains unclear.

Methods: ADAPT HER2+/HR+ is a phase II, randomized, neoadjuvant, 3-arm trial (12 weeks) in patients with cT1c-cT3, cN0/+ HER2+, ER+ and/or PR+ early BC. Arm A: T-DM1 (3.6 mg/kg) alone; Arm B: T DM1 + ET (premenopausal: tamoxifen, postmenopausal: AI); Arm C: trastuzumab + ET. Postoperative chemotherapy is recommended together with completion of one year of trastuzumab. Initial and serial core biopsies were obtained prior to therapy and after 3 weeks. First translational analysis of the trial run-in phase (n=130) focuses on dynamics of HER2, Ki67, ER and PR.

Results: 162 tumors, HR+ and HER2+ by local pathology, were screened; n=130 were HR+ and HER2+ by central pathology and randomized at 40 trial sites in Germany between 11/2012 and 03/2014 (Arm A/B/C: 37/49/44). Median age was 49 years; 60% were cT2-3, 32% cN+, 75% central G3; median baseline Ki67 was 30%; 49 patients were treated by TAM and 44 postmenopausal patients by AI in ET containing arms. Three-week core biopsies were available in 117 patients (arm A/B/C: n=33/43/41), n=99 with invasive tumor tissue (61/76 (80%) in T-DM1 containing arms and 38/41(93%) in trastuzumab + ET arm). Three-week Ki67 could only be analyzed in n=73 (53% of patients in T-DM1-containing arms, 81% in the T+ET arm) due to a lacking amount of cells for counting (<500).

Median fractional decrease in proliferation (Ki67) after 3 weeks of therapy was 40% in the T-DM1 + ET arm (B) as compared to 14% and 25% in the T-DM1 (A) and T+ET (C) arms, respectively. Among postmenopausal patients, the contrast (52% (B) vs. 0% (A) and 28% (B)) was significant. Mean PR expression change (absolute, measured in %) was -15 in the B Arm vs. 7 and -7 in the A and C arms, respectively (p=0.04) – particularly in postmenopausal women ( 25 vs. +13 and -10, respectively, p=0.04). Baseline ER expression was positively associated with early proliferation response (fractional Ki67 decrease), e.g., by logistic regression using 30% decrease as response criterion. Data on the impact of gene mutations and further molecular markers on proliferation response will be available for presentation at the meeting.

Conclusions: In the unique neoadjuvant ADAPT HER2+/HR+ trial, the combination of T-DM1 with ET (particularly AI) seems to be associated with a strong early proliferation response and PR expression drop in anti-HER2 antibody (drug conjugate) +/- ET, without systemic pre-operative chemotherapy. Interim analysis is scheduled after 130 completely treated patients. The high percentage of non-invasive tissue biopsied after 3 weeks in the T-DM1 arms is intriguing, as it would be consistent with higher pathological complete response rates.
2014 San Antonio Breast Cancer Symposium

Publication Number: P4-15-02
Average Grade: 2.20

Title: Clinical, biochemical, and genomic predictors of trastuzumab-related cardiotoxicity: Results of CATS

Shom Goel1, Hao Guo1, William Barry2, Jodi Lynch3,4, Patricia Bastick3, Lorraine Chantrill5, Belinda Kiely5, Richard Bell6, Ehtesham Abdi7, Josie Rutovitz6, Ray Asghari9, Anne Sullivan10, Michelle Harrison7, Maija Kohonen-Corish11 and Jane Beith2. 1Dana-Farber Cancer Institute, Boston, MA; 2Chris O’Brien Lifehouse, Sydney, NSW, Australia; 3St George Hospital, Sydney, NSW, Australia; 4Sutherland Hospital, Sydney, NSW, Australia; 5Macarthur Cancer Therapy Centre, Sydney, NSW, Australia; 6Andrew Love Cancer Centre, Geelong, VIC, Australia; 7Tweed Hospital, Tweed Heads, NSW, Australia; 8Northern Haematology and Oncology Group, Sydney, NSW, Australia; 9Bankstown Cancer Centre, Sydney, NSW, Australia; 10Concord Repatriation General Hospital, Sydney, NSW, Australia and 11Garvan Institute, Sydney, NSW, Australia.

Body: Background: Adjuvant therapy for HER2-positive breast cancer (HBC) often comprises anthracyclines (A) followed by trastuzumab (T). Trastuzumab-related cardiotoxicity (TRC) is T’s primary toxicity, and led 15% of patients in NSABP-B31 to stop T early. Earlier detection of TRC might enable timely therapeutic intervention, lowering the number of patients ceasing T prematurely. Small, retrospective, unvalidated studies have proposed various markers as predictors for TRC. The Cardiotoxicity of Adjuvant Trastuzumab Study (CATS) aims to assess a panel of clinical, biochemical, and genomic/immunologic markers as predictors for TRC.

Methods: HBC patients with a left ventricular ejection fraction (LVEF) ≥50% scheduled to receive adjuvant A followed by 12 months’ T were eligible. Serum N-terminal pro-B type natriuretic peptide (NT pro-BNP) and troponin I were measured centrally at baseline (T1), at completion of A (T2), and after 3 and 6 months of T. LVEF was measured at T1, T2, and then 3-monthly until completion of T. Germline SNPs in ERBB2 (V655I), FCGR2A (H131R), and FCGR3A (V158F) were assessed. TRC was defined as: cardiac death, NYHA class 3/4 heart failure, grade 3/4 arrhythmia/ischemia, drop in LVEF >15% from baseline, or drop in LVEF of >10% to <55%. 1 patient with LVEF <50% at T2 was excluded. Uni- and multivariate logistic regression were used to determine features associated with TRC. Serum markers were added to the model using forward selection.

Results: 17 centers enrolled 222 patients (217 evaluable). Patient characteristics are shown.

Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [median (range), yrs]</td>
<td>52 (28-77)</td>
</tr>
<tr>
<td>Baseline LVEF [median (range)]</td>
<td>64% (50-82%)</td>
</tr>
<tr>
<td>Left-sided tumors [n(%)]</td>
<td>105 (48%)</td>
</tr>
<tr>
<td>History of hypertension [n(%)]</td>
<td>48 (22%)</td>
</tr>
<tr>
<td>Doxorubicin [n(%), median total dose]</td>
<td>80 (37%), 242mg/m²</td>
</tr>
<tr>
<td>Epirubicin [n(%), median total dose]</td>
<td>137 (63%), 300mg/m²</td>
</tr>
<tr>
<td>Concurrent taxane with T [n(%)]</td>
<td>217 (100%)</td>
</tr>
</tbody>
</table>

TRC occurred in 33 patients (15.2%). In multivariate models, lower baseline LVEF (OR 3.23 for a 5% lower LVEF, [95% CI 1.96-5.33], p<0.0001) and greater absolute decline in LVEF from T1 to T2 (OR 3.25 for each 5% drop in LVEF, [95%CI 1.83-5.79], p<0.0001) were each independent predictors of TRC. NT pro-BNP increased from T1 to T2 (median Δ=1.31pmol/L, p<0.0001) and then fell to baseline levels. Troponin I increased from T1 to T2 in 64.5% of patients (p<0.0001) and remained above baseline levels post-A (p<0.0001). NT pro-BNP and troponin I at T1 were not predictive for TRC. There were trends for the absolute increase in NT-pro BNP and troponin I from T1 (pre-A) to T2 (post-A) to be associated with risk of TRC (p=0.08 and 0.09) in univariate analyses. Germline SNPs were not predictive of TRC.

Conclusion: This is the largest prospective study analyzing predictors for TRC, and the first studying immune-related SNPs in this context. Consistent with the literature, lower LVEF at baseline increases risk of TRC. Previously unreported, the absolute
decrease in LVEF after A is also predictive for TRC. The persistent elevation of troponin I 6 months after completing A demonstrates chronic cardiac stress during therapy. Ongoing modeling analyses (to be presented at the meeting) will determine whether longitudinal changes in serum biomarkers can predict subsequent development of TRC.
Title: Activating mutations in ERBB2/HER2 as found by FoundationOneTM represent potential therapeutic targets in breast cancer

Gary A Palmer¹, Jeffrey S Ross¹,², Kai Wang¹, Garrett M Frampton¹, Siraj M Ali³, Norma Palma¹, Deborah Morosini¹, Vincent A Miller¹, Roman Yelensky¹, Doron Lipson¹, Philip J Stephens¹ and Juliann Chmielecki¹. ¹Foundation Medicine, Cambridge, MA and ²Albany Medical College, Albany, NY.

Body: Background: Targeted ERBB2/HER2 inhibitors are FDA-approved for the treatment of breast and gastric cancers. Immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) to test for overexpression and amplification of ERBB2/HER2, respectively, are performed as part of routine clinical care. Recently, activating mutations in ERBB2 have been reported and may confer sensitivity to targeted agents. Testing for these mutations is not routine, and testing for amplifications is not done outside of approved indications. We explored the complete spectrum of activating ERBB2 mutations and amplifications across a collection of ~7,300 solid tumor specimens, including a large number of breast cancer specimens, to determine a) how many breast cancer patients could benefit from HER2-targeted therapy due to activating mutations in ERBB2 and b) how widespread ERBB2 alterations are across the solid tumor spectrum.

Methods: Extracted DNA from clinical tumor samples underwent comprehensive genomic profiling using FoundationOne®. 3769 exons of 236 cancer-related genes and 47 introns from 19 genes that are frequently rearranged in cancer were fully sequenced to high, uniform coverage and results were analyzed for base substitutions, insertions and deletions, amplifications/deletions, and rearrangements.

Results: Known oncogenic ERBB2 alterations were identified in approximately 6% of all solid tumors across 27 different histologies. Of all the ERBB2 alterations, activating mutations in ERBB2 were identified in 131 samples and amplifications were observed in 246 samples. Two samples harbored an ERBB2 rearrangement. Ten samples harbored multiple ERBB2 mutations, yet mutations and amplifications were mutually exclusive in 91% of mutated cases. ERBB2 amplification in breast and gastric cancers accounted for only 30% of these alterations. Breast cancer accounted for 37% of all the ERBB2 alterations detected, and included primarily amplifications. 25% of the alterations found in breast cancers were activating base substitutions. Standard tests for overexpression or amplification of ERBB2 would fail to detect these potentially treatable mutations. Non-amplification ERBB2 alterations were enriched in cases of relapsed lobular breast cancer. Multiple breast cancer patients with non-amplification ERBB2 alterations have responded to combinations of anti-HER2 targeted therapies.

Conclusions: Comprehensive genomic profiling through FoundationOne identifies 25% more breast cancers that may be susceptible to HER2 targeted therapy due to the presence of activating mutations in ERBB2. Also, many more solid tumor cases could potentially benefit as well from ERBB2 testing. The current policy of testing only breast and gastric tumors for only HER2 amplifications is greatly limiting the potential value of the ERBB2/HER2 inhibitors.
Title: Effect of pre-treatment with cyclophosphamide on MM-302 (HER2-targeted liposomal doxorubicin) deposition in HER2-positive metastatic breast cancer patients assessed by $^{64}$Cu-MM-302 PET/CT

Kathy Miller, Patricia M LoRusso, Pamela Munster, Ian Krop, Cynthia Ma, Helen Lee, Joe Reynolds, Karen Campbell, Victor Moyo, Bart Hendriks, Thomas Wickham, Barry A Siegel and Anthony F Shields. 1Indiana University Melvin and Bren Simon Cancer Center, Indianapolis, IN; 2Karmanos Cancer Institute, Detroit, MI; 3Helen Diller Family Comprehensive Cancer Center, San Francisco, CA; 4Dana-Farber Cancer Institute, Boston, MA; 5Washington University School of Medicine, St Louis, MO and 6Merrimack Pharmaceuticals, Inc, Cambridge, MA.

Body: Background: Liposomal encapsulation of doxorubicin has addressed the cardiotoxicity of free doxorubicin, but has not achieved an improvement in anti-tumor activity in metastatic breast cancer. MM-302 is a HER2-targeted liposomal doxorubicin, specifically designed to target tumor cells overexpressing HER2 and minimize uptake into normal cells such as cardiomyocytes, which express low levels of HER2. MM-302 and MM-302 plus trastuzumab are being studied in patients as part of an ongoing Phase 1 clinical trial. Published reports indicate that deposition into solid tumors is a rate-limiting step in liposome-mediated delivery of drug to tumor cells. In order to understand drug deposition into solid tumors, we have radiolabeled MM-302 with $^{64}$Cu for imaging by PET/CT. Further, preclinical work has demonstrated that pretreatment with cyclophosphamide has the ability to improve liposomal drug delivery by making the tumor microenvironment permissive for deposition and retention of liposomes. This Phase 1 study evaluates the delivery of $^{64}$Cu-MM-302 to solid tumors and the ability of cyclophosphamide pretreatment to increase delivery of $^{64}$Cu-MM-302 to tumors.

Methods: Patients aged $\geq$ 18 years with histologically confirmed HER2-positive advanced breast cancer that has progressed or recurred on standard therapy or for which no standard therapy exists, who have adequate performance status, bone marrow reserve and organ function, were eligible for the study. Patients received 30 mg/m$^2$ of MM-302 plus 6 mg/kg trastuzumab, q3w and 400 MBq (10.8 mCi; 3-5 mg/m$^2$, doxorubicin basis) of $^{64}$Cu-MM-302 (cycle 1 only) with or without pretreatment of 450 mg/m$^2$ cyclophosphamide. Patients underwent PET/CT on the day of administration and on day 2, day 3 or both. The primary goal of this analysis was to study delivery of $^{64}$Cu-MM-302 and the effect of cyclophosphamide pretreatment. Other endpoints being studied include safety, dosimetry, and treatment response.

Results: Combination of MM-302 with cyclophosphamide was well tolerated and no issues were reported related to $^{64}$Cu-MM-302 administration or imaging. Uptake of $^{64}$Cu-MM-302 in tumor lesions increased over time with significant deposition at 24 and 48 h while activity in the blood decreased over time. Median lesion deposition of $^{64}$Cu-MM-302 (as % i.d./kg) in the patients with cyclophosphamide pretreatment was higher than in those without pretreatment: 6.0 (n=5 patients/20 lesions) vs. 4.4 (n=4 patients/17 lesions) at 24 h and 7.6 (n=4 patients/16 lesions) vs. 4.0 (n=4 patients/17 lesions) at 48 h.

Conclusions: PET/CT imaged $^{64}$Cu-MM-302 deposition into diverse tumor lesions, including liver, brain and bone metastases. Our preliminary data thus far suggest that cyclophosphamide pretreatment increases delivery of $^{64}$Cu-MM-302 to patient tumors, as predicted by preclinical models. Correlation between $^{64}$Cu-MM-302 tumor deposition and lesion/patient response is currently being investigated.
Title: Effects on outcome of concomitant neoadjuvant chemotherapy-trastuzumab compared with sequential neoadjuvant chemotherapy followed by post-operative trastuzumab

Carlo Palmieri¹, Iain RJ Macpherson², Kelvin Yan³, Felipe Ades Moraes⁴, Pippa Riddle⁵, Riz Ahmed⁶, Waheeda Owadally⁶, Barabara Stanley⁷, Deep Shah³, Ondrej Gojis³, Adam Januszewski², Conrad Lewanski⁷, Rebecca Asher⁶, Daniel Lythgoe⁸, Evandro De Azambuja⁴, Mark Beresford⁵ and Sacha J Howell⁹.

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Body: Background:

Neoadjuvant chemotherapy delivered with trastuzumab (NCT) has been shown to increase the rates of pathological complete response (pCR) compared to neoadjuvant chemotherapy (NC) alone in women with HER2 positive breast cancer (BC). pCR in this setting has been associated with improved event free survival (EFS). However, no study has yet investigated the effect on outcomes of NCT compared to NC followed by adjuvant trastuzumab initiated postoperatively (NCAT). This study sought to investigate the impact on breast cancer outcomes of concomitant (NCT) versus sequential (NCAT) treatment in HER2 positive early breast cancer.

Methods:

Women with HER2 positive invasive breast cancers treated with neoadjuvant chemotherapy between 2006-2010 were identified at each of 7 European institutions and the case notes reviewed. Preoperative clinical, radiological and pathological details, treatment details and pathology results following breast surgery were reviewed. To be defined as NCT at least one dose of trastuzumab needed to be given preoperatively. pCR was defined as absence of invasive disease in breast and lymph nodes. Multivariable Cox regression and logistic regression were used to Survival outcomes for event-free survival (EFS) were calculated by log rank analysis model the influence of a number of factors on event-free survival (EFS) and pCR respectively.

Results:

236 patients were identified; 138 (58%) received NCAT & 98 (42%) received NCT. The median follow up for the whole group was 53.7 months (IQR 41.7-68.8), 61.5 months (IQR 50.3-78.5) for NCAT group and 44.8 months (range 37-53.9) for NCT group. The 5-year EFS for NCAT vs NCT was 59.3% (95% CI: 49.8-67.6) and 69.6% (95% CI: 51.5-82.0) respectively. The unadjusted hazard ratio (HR) for EFS with NCT compared with the NCAT was 0.63 (95% CI 0.37–1.08; p=0.091). NCT significantly increased the odds of having pCR relative to NC (OR: 4.39 (2.18-8.86); p<0.001), and pCR was associated with a significantly improved EFS, with an unadjusted HR of 0.23 (95% CI 0.08–0.64; p=0.002). Multivariable analysis revealed that treatment group, tumour size and ER status were significantly associated with EFS. NCT was associated with a reduced risk of relapse relative to NCAT (HR 0.48, 95% CI: 0.26-0.89). In ER negative tumours NCT was significantly associated with a reduced risk of an event relative to NCAT (HR:0.25; 95% CI, 0.10-0.62), this was not observed for ER positive tumours (HR: 1.07; 95% CI, 0.46-2.52).

Conclusion

Concomitant as compared to sequential trastuzumab is associated with improved outcomes in the neoadjuvant setting for women with ER negative tumours. These data further support the need for the early introduction of targeted combination therapy in women with ER negative/HER2 positive BC.
Title: Correlation between ERBB2 mRNA levels, HER2-dependence and susceptibility to trastuzumab in human breast cancer

Body: While results thus far demonstrate the clinical benefit of trastuzumab in breast cancer, some patients do not respond to this reagent. Identifying a robust clinical or molecular predictor of adjuvant trastuzumab benefit has proven challenging and even the most obvious candidate biomarker for a predictor of trastuzumab benefit, HER2 expression as assessed by immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH), has proven to be surprisingly ambiguous in realizing benefit from the antibody. Results of a recent study that profiled expression of select genes in archived formalin-fixed, paraffin-embedded (FFPE) tumor blocks from a randomized study of breast cancer patients treated with adjuvant trastuzumab support a relationship between ERBB2/ESR1 mRNA levels and trastuzumab sensitivity. Based on that observation, we conducted whole-genome profiling by DASL technology of archival FFPE tumor blocks from 53 HercepTest 3+/2+ FISH-positive patients treated with adjuvant trastuzumab in our Institute; 308 genes were significantly associated with relapse-free survival by Cox proportional hazard model ($\alpha<0.005$; permutation test $p<0.01$). We then developed a relapse risk 41-gene classifier (TRAR) able to discriminate cases mainly according to ERBB2 and ESR1 mRNA expression levels and to predict tumor relapse in both trastuzumab adjuvant and neoadjuvant settings. No correlation was found between ERBB2 mRNA and HER2 protein expression (the latter assessed by IHC on FFPE tumor sections using serial dilutions of anti-HER2 antibody), nor did HER2 protein expression correlate with relapse risk in these tumors. Analysis of HER2-amplified breast carcinoma cell lines revealed higher levels of ERBB2 mRNA in oncogene-addicted than in non-addicted cells in correlation with HER2 activation assessed by Western blot but not with total HER2 protein amount. Moreover, ERBB2 mRNA expression levels in HER2-amplified breast carcinoma cells correlated with trastuzumab-mediated cellular cytotoxicity (ADCC) in vitro ($r=0.96$, $p=0.038$), whereas no correlation was found between ADCC and membrane-associated HER2. Together, our findings strongly suggest that ERBB2 mRNA, but not protein levels, mirror HER2 activity and thus oncogene addiction of tumor cells and susceptibility to trastuzumab in amplified HER2-positive tumors. Supported by AIRC.
**Title:** HER2 positive (HER2/CEP17 ratio ≥ 2.0) invasive mammary carcinomas with average <4.0 HER2 and <2.0 CEP17 signals/cell: Clinicopathologic features, correlation with HER2 immunohistochemistry and response to neoadjuvant chemotherapy

Chad A Livasy¹, Kimberly Limentani², Benjamin C Calhoun³ and Steven Limentani⁴. ¹Levine Cancer Institute, Charlotte, NC; ²Cleveland Clinic, Cleveland, OH; ³Thomas Jefferson University Hospital, Philadelphia, PA and ⁴Levine Cancer Institute, Charlotte, NC.

**Body:**

**Background:** ASCO/CAP guidelines for the determination of HER2 amplification have recently been revised in an attempt to clarify which patients will benefit from HER2 directed therapy. Patients with HER2/CEP17 ratios ≥2.0 were eligible for the first generation trials of adjuvant trastuzumab regardless of the average number of HER2 signals/cell. Array CGH and FISH studies have since demonstrated the presence of complex segmental aneusomy of chromosome 17 in a subset of breast tumors. These focal copy number gains and losses can skew the HER2/CEP17 ratio and result in discordant results with HER2 immunohistochemistry (IHC). There is limited data on the clinicopathologic features, HER2 protein expression and response to therapy for tumors showing a HER2/CEP17 ratio ≥ 2.0 and average <4.0 HER2 signals/cell.

**Methods:** All cases with HER2/CEP17 ratio ≥ 2.0 and average <4 HER2 signals/cell were identified from our database from 2009-2013. HER2 IHC was performed and scored on all identified cases. Tumor grade, histologic subtype, hormone receptor status and menopausal status were recorded. For patients receiving neoadjuvant chemotherapy, response was measured using residual cancer burden (RCB) class and yAJCC pathologic stage.

**Results:** 70 (1.5%) of 4659 FISH cases met criteria for inclusion in the study. 22 (31%) of the 70 patients were premenopausal. All tumors were ductal (no special type) with the following features features: low-grade 14%, intermediate-grade 40%, high-grade 45%, estrogen receptor positive 79% and progesterone receptor positive 65%. HER2 protein evaluation demonstrated 52 (74%) negative (0-1+), 16 (23%) equivocal (2+) and 2 (3%) positive (3+) results. Most tumors (83%) showed 1+ or 2+ staining. 12 patients received neoadjuvant chemotherapy (see table). Six of these patients did not receive trastuzumab as part of their therapy due to congestive heart failure (n=1) or uncertainty about the patient's HER2 status (n=5).

**Neoadjuvant chemotherapy patient summary**

<table>
<thead>
<tr>
<th>Patient # (regimen)</th>
<th>Ratio</th>
<th>HER2 copy#</th>
<th>CEP17 copy#</th>
<th>HER2 IHC</th>
<th>ER/PR</th>
<th>yAJCC</th>
<th>RCB class</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1 (AC+TH)</td>
<td>2.2</td>
<td>3.9</td>
<td>1.8</td>
<td>1+</td>
<td>+/-</td>
<td>ypT0</td>
<td>N0(i+)</td>
</tr>
<tr>
<td>Patient 2 (TCH)</td>
<td>2.2</td>
<td>3.8</td>
<td>1.7</td>
<td>1+</td>
<td>+/-</td>
<td>ypT0</td>
<td>N0</td>
</tr>
<tr>
<td>Patient 3 (TCH)</td>
<td>2.2</td>
<td>3.8</td>
<td>1.7</td>
<td>2+</td>
<td>+/-</td>
<td>ypTis</td>
<td>N0</td>
</tr>
<tr>
<td>Patient 4 (AC+TH)</td>
<td>2.3</td>
<td>3.0</td>
<td>1.3</td>
<td>0</td>
<td>+/-</td>
<td>ypT2</td>
<td>N2a</td>
</tr>
<tr>
<td>Patient 5 (TCH)</td>
<td>2.2</td>
<td>3.7</td>
<td>1.7</td>
<td>1+</td>
<td>+/-</td>
<td>ypT1c</td>
<td>N0</td>
</tr>
<tr>
<td>Patient 6 (TCH)</td>
<td>2.2</td>
<td>3.9</td>
<td>1.8</td>
<td>2+</td>
<td>+/-</td>
<td>ypTis</td>
<td>N0</td>
</tr>
<tr>
<td>Patient 7 (TC)</td>
<td>2.0</td>
<td>3.4</td>
<td>1.7</td>
<td>0</td>
<td>+/-</td>
<td>ypT0</td>
<td>N0</td>
</tr>
<tr>
<td>Patient 8 (AC+T)</td>
<td>2.2</td>
<td>3.6</td>
<td>1.7</td>
<td>1+</td>
<td>+/-</td>
<td>ypT1c</td>
<td>N0</td>
</tr>
<tr>
<td>Patient 9 (TC)</td>
<td>2.1</td>
<td>3.9</td>
<td>1.9</td>
<td>1+</td>
<td>+/-</td>
<td>ypT0</td>
<td>N0</td>
</tr>
<tr>
<td>Patient 10 (AC)</td>
<td>2.5</td>
<td>3.4</td>
<td>1.4</td>
<td>1+</td>
<td>+/-</td>
<td>ypT1b</td>
<td>N1a</td>
</tr>
<tr>
<td>Patient 11 (TAC)</td>
<td>2.4</td>
<td>3.6</td>
<td>1.5</td>
<td>2+</td>
<td>+/-</td>
<td>ypT3</td>
<td>N1mi</td>
</tr>
<tr>
<td>Patient 12 (AC)</td>
<td>2.0</td>
<td>3.3</td>
<td>1.6</td>
<td>1+</td>
<td>+/-</td>
<td>ypT1c</td>
<td>N1a</td>
</tr>
</tbody>
</table>

*Congestive heart failure

**Conclusions:** HER2 FISH positive invasive mammary carcinomas with a HER2/CEP17 ratio ≥2.0 and average <4.0 HER2 signals/cell are rare (1.5% in this study) and frequently show 1+ or 2+ staining by IHC. Treatment decisions for these patients
were impacted by the uncertainty of HER2 status under the previous guidelines. In this small data set, these tumors demonstrated a good response to neoadjuvant trastuzumab-based chemotherapy and further support the recent modifications of ASCO/CAP guidelines to determine HER2 amplification.
**Title:** A phase 1b study of ONT-380, an oral HER2-specific inhibitor, combined with ado-trastuzumab emtansine (T-DM1), in HER2+ metastatic breast cancer (MBC)


**Body:**

Background: ONT-380 (also known as ARRY-380) is a potent, selective small molecule inhibitor of HER2 with 500-fold selectivity compared to EGFR. Preclinical studies have demonstrated synergistic activity with ONT-380 and chemotherapy or trastuzumab, as well as superior activity compared to lapatinib and neratinib in models of HER2+ CNS metastases. In a Phase 1 single agent study in HER2+ MBC, ONT-380 was well tolerated and provided clinical benefit with minimal EGFR-type toxicities, with an MTD of 600 mg BID using an API-in-capsule formulation. Based on the potential for dual blockade of HER2 to lead to clinical benefit, ONT-380 is being evaluated in combination with T-DM1 in patients previously treated with a taxane and trastuzumab for metastatic disease.

Methods: This 3+3 dose escalation study evaluates escalating doses of ONT-380 in a new tablet formulation combined with T-DM1 at 3.6 mg/kg IV once every 21 days. Prior treatment with trastuzumab and a taxane are required. Prior lapatinib or neratinib therapy and asymptomatic brain metastases (treated or untreated) are allowed. Previous T-DM1 is not permitted. Normal left ventricular ejection fraction and anthracycline exposure ≤ 360 mg/m² is required. Study assessments include safety, ONT-380 and T-DM1 PK, tumor response by RECIST 1.1, and CNS response by both modified RECIST and volumetric criteria. Dose escalation will be followed by enrollment of expansion cohorts in patients with and without CNS disease.

Results: As of 21 May 2014, 7 patients have been treated with ONT-380 at 300 mg BID for 1–5 cycles. One dose limiting toxicity (DLT) of Grade 3 ALT/AST elevation was seen in the ONT-380 300 mg BID cohort, requiring a dose reduction for both agents with subsequent cohort expansion to 6 patients. No further DLTs have been seen in this cohort, and no other dose reductions have been required. Most toxicities have been Grade 1 or 2, with the most common regardless of attribution being nausea, fatigue, diarrhea, and thrombocytopenia. Two Grade 3 AEs have been reported, including the DLT of ALT/AST elevation, and one event of thrombocytopenia, considered related to T-DM1 but not ONT-380. There has been no Grade 3 diarrhea and no SAEs. In the four patients evaluable for response to date, best response has been 1PR, 2 SD, and 1 PD. Three patients with prior CNS radiation have had continued reduction in CNS lesions on study. Initial PK data indicate greater ONT-380 exposure is achieved with the new tablet formulation compared to the earlier capsule formulation with no evidence of drug interaction with T-DM1.

Conclusions: Treatment with ONT-380 and T-DM1 has been associated with an acceptable safety profile, with only one DLT and minimal Grade 3 toxicity, including no Grade 3 diarrhea or rash. Early evidence of disease control has been seen, including in patients with CNS metastases. Dose escalation continues, and updated results will be presented for additional cohorts.
Body: Introduction

Trastuzumab emtansine (T-DM1) is an antibody–drug conjugate comprising trastuzumab, a stable linker, and the microtubule inhibitor DM1. In phase 3 studies of HER2-positive metastatic breast cancer (MBC), T-DM1 significantly increased progression-free survival (EMILIA and TH3RESA) and overall survival (EMILIA) vs. control regimens. Few patients (2–4%) treated with T-DM1 experience grade ≥3 increases in transaminases. Currently, there are no data on the pharmacokinetics (PK) of T-DM1 in patients with hepatic impairment. This international, multicenter, open-label, parallel group, phase 1 PK study (BO25499/NCT01513083) is designed to assess the PK of T-DM1 and relevant analytes in MBC patients with normal hepatic function and mild or moderate hepatic impairment; safety and efficacy will also be evaluated.

Methods

To obtain 8 evaluable patients, up to 10 patients each with HER2-positive MBC and ECOG performance status of 0–2 were enrolled in 1 of 3 independent cohorts based on hepatic function per Child-Pugh criteria: normal hepatic function, mild hepatic impairment (Child-Pugh A), and moderate hepatic impairment (Child-Pugh B). Patients with severe hepatic impairment (Child Pugh C) were ineligible. Patients received 3 cycles of T-DM1 3.6 mg/kg every 3 weeks. After 3 cycles, patients could continue to receive T-DM1 until disease progression, unmanageable toxicity, or study termination in the present study, or enroll in an extension study (BO25499/TDM4529g). PK samples were collected during cycle 1 (days 1 [predose, 30 m and 4 h postinfusion], 2, 3, 4, 8, 11, 15, and 18); cycle 2 (day 1 [predose, 30 m postinfusion]); and cycle 3 (days 1 [predose, 30 m postinfusion], 8, 15, and 22). T-DM1, total trastuzumab, DM1, MCC-DM1, and Lys-MCC-DM1 were measured using validated assays. Adverse events were graded per NCI CTCAE, v4.03. All analyses are descriptive. The clinical cutoff date for this interim analysis was January 30, 2014.

Results

PK data were fully evaluable for 10 out of 10 patients each in the normal and mild cohorts and for 6 out of 7 patients in the moderate cohort. Compared with the normal cohort, T-DM1 clearance at cycle 1 was ∼1.9- and 3.3-fold faster in the mild and moderate cohorts, respectively. The trend of faster clearance was less apparent for cycle 3 after repeated dosing, with similar T-DM1 exposures across the 3 cohorts. Plasma concentrations of DM1 and DM1-containing catabolites were largely comparable across the 3 cohorts. No new safety signals were seen relative to the known safety profile of T-DM1. Updated safety data will be presented.

Conclusions

There is a trend for faster clearance of T-DM1 at cycle 1 in patients with mild and moderate hepatic impairment vs. those with normal hepatic function, which can be partly explained by demographic and pathophysiological covariates such as tumor burden, albumin, and body weight. The study’s small sample size could also partly explain the variability. Work to better understand the mechanisms for the observed differences in clearance is ongoing. No increase in the systemic concentration of DM1 was observed in patients with mild or moderate hepatic impairment vs. those with normal hepatic function. No additional safety concerns were observed.
Title: Initial therapy among patients newly diagnosed with operable early stage human epidermal growth factor receptor 2-overexpressed (HER2+) breast cancer in the US: A real-world retrospective study

Stacey DaCosta Byfield², Philip O Buck¹, Cori Blauer-Peterson² and Sara A Poston¹. ¹GSK, Philadelphia, PA and ²Optum, Eden Prairie, MN.

Body: BACKGROUND: Prognosis and appropriate treatment of breast cancer patients (pts) is influenced by tumor molecular characteristics. However, few existing retrospective studies have investigated the treatment patterns and outcomes of breast cancer pts by tumor biomarkers. The objective of this study was to assess the current real-world treatment patterns associated with resected non-metastatic HER2+ breast cancer in the US.

METHODS: This was a retrospective study of physician-reported clinical information (including date and stage at diagnosis, HER2 status and hormone receptor (HR) status) for commercially insured breast cancer pts from the Oncology Management Registry linked with medical and pharmacy claims from a large, national health plan in the US from 01/2008 to 8/2013. The date of initial diagnosis was the index date. The inclusion criteria were: adult pts (≥18 years old), enrolled in the health plan for ≥6 months after the index date, diagnosed with HER2+ Stage I-III disease, known HR status, received breast cancer specific surgery (mastectomy or lumpectomy) and anti-cancer systemic therapy (ACST) and/or radiation within 6 months of index date. Pts with other primary cancers during the study period were excluded. The initial phase of care included initial therapy (surgery and ACST and/or radiation) until 30 days after the last therapy received (surgery, ACST or radiation) prior to a 90-day gap in treatment. Treatment patterns during the initial phase of care by HR status were examined.

RESULTS: Among 915 pts who met all study criteria, 662 (72%) and 253 (28%) were HR+ and HR-, respectively. Mean age was 52 years (standard deviation=9) and was not significantly different by HR cohort. Approximately 82% (n=749) were diagnosed with Stage I/II disease. Most pts (80%, n=732) received adjuvant therapy only, 19% (n=177) received both neo-adjuvant and adjuvant therapy, and <1% (n=6) were observed to have neo-adjuvant therapy only. Among pts who received neo-adjuvant therapy, mean time from diagnosis to ACST was 21 days (median=21). Among pts who received only adjuvant therapy, mean time from diagnosis to initial breast cancer specific surgery was 24 days (median=20). Overall, 72% of pts received HER2 targeted therapy (69% HR+, 80% HR-; p<0.01) during their initial phase of care. During neo-adjuvant therapy, 72% of pts received trastuzumab (67% HR+, 81% HR-; p<0.05). During adjuvant therapy, 72% of pts received trastuzumab (69% HR+, 81% HR-; p<0.05). The most common neo-adjuvant regimen regardless of HR status was carboplatin+docetaxel+trastuzumab (>40% pts). The most common regimens during the adjuvant therapy period were carboplatin+docetaxel+trastuzumab with or without hormone therapy (~30% of pts).

CONCLUSION: In this real-world population of commercially insured breast cancer pts treated for operable, early stage HER2+ disease in the US, 28% of pts did not receive targeted therapy. More pts with HR- status received targeted therapy than those with HR+ status. Further studies are warranted to examine whether pts that have not received targeted therapy are eligible and would benefit from an HER2 targeted approach.
Title: Advanced HER2 positive breast cancer treated with trastuzumab: Is combination with chemotherapy always needed? Randomized phase III trial SAKK 22/99

Olivia Pagani, Dirk Klingbiel, Thomas Ruhstaller, Franco Nolè, Serenella Eppenberger, Christian Oehlschlegel, Jürg Bernhard, Peter Brauchli, Dagmar Hess, Christoph Mamot, Elisabetta Munzone, Bernhard Pestalozzi, Manuela Rabaglio, Karin Ribi, Christoph Rochlitz, Karin Rothgiesser, Beat Thürlimann, Roger von Moos, Khalil Zaman, and Aron Goldhirsch.

1 Institute of Oncology of Southern Switzerland, Bellinzona, Ticino, Switzerland; 2 Swiss Group for Clinical Cancer Research Coordinating Center, Bern, Switzerland; 3 Kantonsspital, San Gallen, Switzerland; 4 European Institute of Oncology, Milan, Italy; 5 University Hospital, Basel, Switzerland; 6 International Breast Cancer Study Group, Bern, Switzerland; 7 Kantonsspital, Aarau, Switzerland; 8 University Hospital, Zurich, Switzerland; 9 Inselspital, Bern, Switzerland; 10 Kantonsspital, Chur, Switzerland and 11 University Hospital, Lausanne, Switzerland.

Body: <Background>
In advanced HER2+ breast cancer the impact of combining Trastuzumab (T) and chemotherapy (chemo) versus T alone followed by the addition of chemo at disease progression has not been properly studied.

Study design
The trial compared efficacy, toxicity and quality of life of sequential administration of T followed, at progression, by combination with chemo (T>TChemo) versus the upfront combination of T and chemo (TChemo) in patients with HER2+ advanced breast cancer.

Materials and methods
Eligibility: measurable/evaluable HER2+ advanced disease; ≤ 2 previous chemo; ECOG performance status <=1. Stratification: degree of HER2 overexpression, estrogen (ER) receptor status, 1st-line vs 2nd/3rd-line therapy, previous anthracyclines, institution. Primary endpoint: time to progression on combined TChemo (TTP-TChemo). Secondary endpoints: response rate, time to 1st progression and to treatment failure, overall survival, toxicity. Substudies: quality of life, predictive value of serum HER2 levels, association of HER2 immunoprofiles with outcome.

The estimated median TTP-TChemo in the control arm (TChemo) was 5-6 months. A 3 months' increase in the experimental T>TChemo arm was considered meaningful.

The chemo backbone was at investigator’s choice (taxanes, vinorelbine, cisplatin) and could be stopped after 6 cycles in responding patients. T was continued until progression. Treatment after progression under TChemo was by investigators’ decision.

Patients’ characteristics
From Sept 1999 – Jan 2013, 175 patients were enrolled. The trial was stopped prematurely due to insufficient accrual. Baseline characteristics were well balanced between arms: median age 55 years (32–79), ER and/or progesterone receptor positive 63%, ≥ 2 disease sites 91%, dominant bone 36% or dominant visceral disease 66%, 1stline therapy 72%.

Results
At the cutoff date (May 2014) 173 patients were evaluable: median follow up 77.7 months, 29 patients (17%) censored when receiving, at chemo stop, off-protocol treatment before progression (maintenance metronomic chemotherapy or endocrine therapy), 11 patients (6%) had no event at the end of follow-up.

TTP-TChemo was longer than expected in both arms (12.7 months T>TChemo, 10.3 months TChemo) and not significantly different (HR=0.7; 95% CI, 0.5–1.0; p=0.08). In the T>TChemo arm, median TTP before introduction of chemo was 3.7 months (95% CI 2.3–5.1). Overall survival was not significantly different, 35.6 months versus 36.3 months (HR=0.9; 95% CI, 0.6–1.3; p=0.50). Toxicity was mainly chemo related, consistent with the chosen regimen. Cardiac toxicity was mild (no grade 4, 1 cardiac failure NYHA III in the T>TChemo arm). No treatment-related death was reported.

Conclusions
The sequential administration of T and chemo showed a non-significant trend to longer TTP-TChemo compared to upfront combination therapy: it allows to delay chemo use and its toxicity and seems a reasonable approach. TTP-TChemo was better than projected in both arms.
The sequential strategy with double anti-HER2 targeting (T/Pertuzumab) is now under evaluation in 1st-line patients in the SAKK 22/10 trial.
Body: Background
Until recently, trastuzumab (H) combined with a taxane was standard first line systemic therapy for patients with Her2 positive metastatic breast cancer (MBC) who are not candidates for endocrine therapy. Clinical studies have shown that bevacizumab (A) enhances the activity of weekly paclitaxel (T), while preclinical data suggest that A can also augment anti-tumor activity of H. Here we report outcome data of a randomized phase II study in Her2 positive MBC on the combination of HAT versus sequential treatment starting with a chemotherapy-free approach with H and A (HA), followed by adding T at progression (HA-HAT).

Methods
Patients with Her2 positive MBC, eligible for first-line systemic therapy, were randomized (1:1) between HAT (3-weekly H 6 mg/kg (first dose 8/mg/kg) plus A 15 mg/kg both until progression and T 90mg/m2, day 1,8,15 every 4 weeks for a maximum of 6 cycles) and HA-HAT (doses HA were the same as in HAT with T for a maximum of 6 cycles added to HA at progression). Primary endpoint was progression-free survival rate at 1 year after randomization (PFR-1yr). A regimen yielding a PFR-1yr of around 40% was decided to warrant further exploration (p1). A Fleming one-stage design was, therefore, applied to both arms with p2=20%, alpha=0.05 and beta=0.10. Secondary endpoints included PFS, defined as the time from randomization to documented disease progression (PD) or death from any cause after patients had received HAT. In addition for the HA-HAT group, a PFS1 and PFS2 was established. PFS1 was defined as the time from randomization to PD or death from any cause, PFS2 as the time from starting treatment with HAT to PD or death from any cause.

Results
Between April 2009 and September 2013, 84 patients were randomized, 39 to HAT and 45 to HA-HAT. Baseline characteristics were similar for both arms: mean age 55 years (range 29-80 years), prior adjuvant chemotherapy in 35% and hormone receptor positive disease in 63%. The primary endpoint was met in both arms. At a median follow-up of 22 months, 26 patients in the HAT arm had progressed of whom 14 had died. The median PFS for the HAT arm was 19.4 months (95% confidence interval (CI) 14.6-25.2). In the HA-HAT arm 23 patients progressed under HA of whom 3 still have no 2nd progression after adding T. Eleven patients of the HA-HAT arm died of whom 2 due to toxicity (1 sigmoid perforation, 1 pneumonia). The median PFS1 was 14.9 months (95% CI 10.9-NA) The median PFS2 was 8.2 months (95% CI 5.9-NA). Overall PFS in the HA-HAT arm was 22.6 months (95% CI 15.4-NA). Grade 3 or higher toxicity was 3 times more often seen during treatment including T. More detailed information regarding toxicity will be shown at the meeting.

Conclusion
Both HAT and HA-HAT are active regimens in Her2 positive MBC. In particular the sequential approach with starting monoclonal antibodies only, yielding a median PFS of more than 1 year and a relatively mild toxicity profile, and adding chemotherapy at progression is worthwhile to be further explored.

Financial support Roche Netherlands.
Title: CDK8 inhibition potentiates anti-ER and anti-HER2 therapies in breast cancer

Martina S McDermott¹, Chang-uk Lim¹, Mengqian Chen¹, Alexander Chumanevich¹, James F Catroppò², Balazs Gyorffy³, David Oliver¹, Igor B Roninson¹ and Eugenia V Broude¹. ¹South Carolina College of Pharmacy, University of South Carolina, Columbia, SC; ²Microbiology and Immunology, University of South Carolina School of Medicine, Columbia, SC and ³Research Laboratory for Pediatrics and Nephrology, Hungarian Academy of Sciences, Budapest, Hungary.

Body: CDK8, along with its paralog CDK19, is a cyclin dependent kinase which, in contrast to other members of the CDK family does not regulate cell cycle progression. CDK8 acts as a pleiotropic transcription regulator potentiating the induction of transcription by several transcription factors. Immunohistochemical staining of breast tissue arrays and bioinformatics analysis of gene expression microarray data of breast cancer patients revealed that CDK8 is overexpressed in breast cancer and that higher CDK8 expression correlates with the failure of systemic therapy. Small-molecule selective inhibitors of CDK8 and CDK19 (Senexin A and Senexin B) inhibited the mitogenic effects of estrogen and estrogen-dependent transcription in estrogen receptor (ER)+ breast cancer cell lines. CDK8/19 inhibitors had a cytostatic effect on different ER+ cell lines, and this growth inhibition was synergistic with the effect of the anti-estrogen fulvestrant, particularly in ER+ cell lines resistant to estrogen deprivation. Some of the ER+ cell lines sensitive to CDK8/19 inhibition also express HER2, and therefore we tested CDK8/19 inhibitors in combination with the HER2 and EGFR tyrosine kinase inhibitor lapatinib and an anti-HER2 monoclonal antibody, a biosimilar of trastuzumab. CDK8/19 inhibition produced a synergistic decrease in cell growth with both HER2 inhibitors; this effect was especially pronounced with a trastuzumab biosimilar. Surprisingly, the synergistic effect with HER2 inhibitors was observed in both ER+ HER2+ and ER-HER2+ cell lines, suggesting an effect on a HER2-complementing molecular target other than ER. Interestingly, CDK8/19 inhibition also synergized with trastuzumab biosimilar in a breast cancer cell line that exhibits innate resistance to trastuzumab, suggesting that CDK8/19 inhibition can overcome trastuzumab resistance in breast cancer. These results suggest that combining anti-estrogen and anti-CDK8 therapy may be more effective than conventional hormone therapy for ER positive breast cancer and that combining anti-HER2 and anti-CDK8 therapy is a rational potential treatment for HER2+ breast cancer, regardless of ER status or sensitivity to trastuzumab.
Title: Preclinical data of SYD985 support the clinical investigation of this novel anti-HER2 antibody-drug conjugate in breast cancer patients with low levels of HER2 expression

Gijs Verheijden¹, Patrick Beusker¹, Ruud Ubink¹, Miranda van der Lee¹, Patrick Groothuis¹, Peter Goedings¹, David Egging¹, Marco Timmers¹ and Wim Dokter¹. ¹Synthon Biopharmaceuticals BV, Nijmegen, Netherlands.

Body: SYD985 is a novel anti-HER2 antibody-drug conjugate (ADC) in development for breast cancer. The ADC consists of three parts: i) the monoclonal antibody trastuzumab, ii) a linker that can be cleaved by tumor-resident proteases at the dipeptide valine-citrulline (vc) motif, and iii) the prodrug seco-DUocarmycin-hydroxyBenzamide-Azaindole. The average ‘drug to antibody ratio’ (DAR) of this ADC, also named trastuzumab vc-seco-DUBA, is ~2.8. After antibody-mediated binding of SYD985 to its molecular target HER2 on the surface of tumor cells, the ADC is internalized. Subsequently, the active duocarmycin is released and binds to DNA in the minor groove, followed by alkylation of the DNA and killing of the tumor cells.

In vitro and in vivo experiments were performed to (directly) compare various pharmacodynamic and pharmacokinetic properties of SYD985 with those of the most progressed (marketing-approved) HER2-targeting ADC, i.e. ado-trastuzumab emtansine (T-DM1). T-DM1 is currently indicated for second line treatment of breast cancer patients overexpressing HER2 tumor tissue. In in vitro experiments, SYD985 shows potencies similar to T-DM1 in cell lines expressing HER2 at high levels (IHC HER2 3+), whereas SYD985 is significantly more potent compared to T-DM1 in cell lines expressing low HER2 levels (IHC HER2 2+ or 1+). In line with these results, SYD985 has superior efficacy compared to T-DM1 in xenograft models in which patient-derived breast cancer tumor tissue with low HER2 levels (IHC HER2 2+/FISH-) have been used.

Pharmacokinetic experiments (in vitro and in vivo) have revealed that whereas in mice SYD985 has limited stability, in cynomolgus monkey SYD985 is very stable. Excellent stability in vitro is also observed in human plasma. Moreover, after i.v. administration of SYD985 in the cynomolgus monkey the amount of free toxin (DUBA) detected in the monkey plasma is many-fold lower than levels reported for the toxin (DM1) released from T-DM1.

Resuming, the preclinical data of SYD985 support clinical studies to investigate whether SYD985 has benefit in cancer patients with moderate or even low HER2 levels of the tumor tissue.
Eradication of HER2-overexpressing breast cancer through the Induction of tumor-specific T cell immunity by Tras-FL bispecific fusion protein

Fan Zhang¹, Lei Zhao¹, Junlan Yang¹ and Ruixia Linghu¹. ¹PLA Geneal Hospital, Beijing, China.

Overexpression of HER2 (or ErbB2), a member of the ErbB family of receptor tyrosine kinases, is found in 25% to 30% of human breast cancers. Trastuzumab, a humanized monoclonal antibody (mAb) directed against HER2, is the first anti-HER2 treatment approved for clinical use for patients with HER2-overexpressing metastatic breast cancer. Although trastuzumab has successed in the treatment of HER2-positive breast cancer, the acquired resistance is one of the prime obstacles for breast cancer treatment. There is an urgent need to enhance the efficacy of the current generation of anticancer antibodies. Flt3 ligand (FL), a soluble protein, has the ability to induce substantial expansion of dendritic cells (DCs) and regulate its development. In this study, we constructed a bispecific IgG-like fusion protein targeting both HER2 and Flt3 (Tras-FL BiFP) by using CrossMab technology. We found that the BiFP exhibited stabilities that were comparable to the parental antibody Trastuzumab, and were able to bind to both targets with unaltered binding affinity. Intriguingly, our data indicated that Tras-FL BiFP could not only eliminate breast cancer temporarily, but also potentiate tumor-specific T cell immunity, which affords a long-lasting protection from tumor recurrence. This protective effect was achieved by inducing a cellular immune response that required the presence of both CD4+ and CD8+ T cells. To investigate the correlation between the binding affinity to the target protein HER2 and the tumor-specific T cell immunity triggered by Tras-FL BiFP, we manipulated the binding affinity of Tras-FL BiFP by introducing several point mutations in the CDR of Tras-FL BiFP. Our data clearly showed that, although the binding affinity of BiFP from 10e-8 M to 10e-10 M could effectively induce tumor-specific T cell immunity, binding affinity improvement of BiFP could trigger more significantly T cell immunity against tumor recurrence. Our results showed that the expansion and infiltration of DCs into tumor tissues by Tras-FL BiFP could be an effective way to generate protective immune responses against cancer, suggesting that the Tras-FL BiFP could be a promising therapeutic agent against HER2-overexpressing breast cancer.
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Title: Combating trastuzumab resistance in HER2-overexpressing breast cancer by Tras-Per CrossMab

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Body: Background: Approximately 20% to 25% of invasive breast cancers have overexpression of the human epidermal growth factor receptor (HER)2 tyrosine kinase receptor. Vast number of studies have demonstrated that elevation of HER2 expression levels are associated with reduced disease-free and overall survival in metastatic breast cancer (MBC), and therapeutic strategies are being developed to target this oncoprotein. Although trastuzumab, a recombinant humanized monoclonal antibody (mAb) directed against an extracellular region of HER2, has revolutionized the treatment of HER2-positive breast cancer, the acquired resistance is one of the prime obstacles for breast cancer treatment. There is an urgent need to develop novel HER2 antibodies against trastuzumab resistance.

Methods: By using CrossMab technology, we converted trastuzumab and pertuzumab to a bispecific immunoglobulin G-like CrossMab (Tras-Per CrossMab) and evaluated the in vitro and in vivo anti-tumor activities against trastuzumab-sensitive and -resistant breast cancer.

Results: Our in vitro experimental data showed that Tras-Per CrossMab with the benefits of IgG architecture had similar high level of binding affinity as the parental antibodies. Notably, compared with the combination therapy of trastuzumab and pertuzumab, Tras-Per CrossMab could induce more potent cell death, anti-proliferation and antibody-dependent cell-mediated cytotoxicity (ADCC) against breast cancer. Further studies indicated that Tras-Per CrossMab with the ability to initiate higher level of cell death and ADCC than that of trastuzumab plus pertuzumab in trastuzumab-resistant breast cancer cells, could significant delay the progression of trastuzumab-resistant breast cancer.

Conclusion: Tras-Per CrossMab efficiently eliminated both trastuzumab-sensitive and -resistant breast cancer in vitro and in vivo, suggesting that HER2-HER3 heterodimers might still play an important role in the progression of trastuzumab-resistant breast cancer. Although combination of trastuzumab plus pertuzumab could markedly delay breast cancer progression in several clinical trials, Tras-Per CrossMab had higher levels of anti-tumor activities against breast cancer compared with that of trastuzumab plus pertuzumab, suggesting that comprehensive blockade of HER2 heterodimerization through Tras-Per CrossMab may be a promising therapeutic strategy against trastuzumab-resistant breast cancer.
Title: A novel targeted engineered toxin body for treatment of HER2 positive breast cancer

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Body: The HER2 receptor is overexpressed in 20-30% of breast cancers. HER2 overexpression in breast cancer has been successfully targeted by both antibody and antibody-drug conjugate (ADC) approaches. The recently approved trastuzumab-emtansine (T-DM1, Kadcyla) ADC has shown promising clinical results in patients refractory to trastuzumab (Herceptin). Because DM1 is a chemotherapeutic and refractory breast cancer patients have typically progressed through several rounds of chemotherapy, we are developing a new targeted therapy approach utilizing a novel mechanism of action. MT-2H74 is an engineered toxin body comprised of the trastuzumab single chain variable fragment (scFv) and a modified ribosome-inactivating protein derived from Shiga-like toxin 1 A (SLT-1A). We have proprietarily modified SLT-1A for reduced immunogenic potential and increased stability. MT-2H74 selectively binds HER2-expressing breast cancer cells, and exerts a potent HER2-specific cytotoxic effect on several tested cell lines. MT-2H74 demonstrates effective cell kill at picomolar concentrations on cell lines expressing high levels of HER2 and is not cytotoxic to HER2 negative control cell lines. Multi-drug resistance transporter 1 (MDR1) has been reported to confer resistance to chemotherapeutic and ADC treatments, such as direct T-DM1 cell kill; however, MT-2H74 displays cytotoxic activity against HER2 transfected NCI/ADR-RES cells known to express MDR1. Murine models are under investigation to determine therapeutic efficacy in vivo. MT-2H74 is a promising HER2-targeted therapeutic agent against breast carcinomas with a unique mechanism of action and is currently under further development.
Title: A phase 1b study of ONT-380, an oral HER2-specific inhibitor, combined with capecitabine and/or trastuzumab, in HER2+ metastatic breast cancer (MBC)

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Body: Background: ONT-380 (also known as ARRY-380) is a potent, selective small molecule inhibitor of HER2 with 500-fold selectivity compared to EGFR. Preclinical studies have demonstrated synergistic activity with ONT-380 plus chemotherapy or trastuzumab, as well as superior activity compared to lapatinib and neratinib in models of HER2+ CNS metastases. In a Phase 1 single agent study in HER2+ MBC, ONT-380 was well tolerated and provided clinical benefit with minimal EGFR-type toxicities, with an MTD of 600 mg BID using an API-in-capsule formulation. Based on the potential for dual blockade of HER2 plus a cytotoxic agent to lead to clinical benefit, ONT-380 is being evaluated in combination with capecitabine and/or trastuzumab in pts with HER2+ MBC previously treated with trastuzumab and ado-trastuzumab emtansine (T-DM1).

Methods: This parallel 3+3 dose escalation study administers ONT-380 in a new tablet formulation with either capecitabine (1000 mg/m2 PO BID for 14 days of a 21 day cycle) (Combo 1), trastuzumab (8 mg/kg IV loading dose; then 6 mg/kg IV once every 21 days) (Combo 2), or with both capecitabine and trastuzumab (Combo 3). Prior treatment with trastuzumab and T-DM1 for metastatic disease is required. Prior therapy with lapatinib or neratinib and asymptomatic brain metastases (treated or untreated) are allowed. Study assessments include safety, ONT-380 and capecitabine PK, tumor response by RECIST 1.1, and CNS response by both modified RECIST and volumetric criteria. Dose escalation will be followed by enrollment of expansion cohorts in patients with and without CNS disease.

Results: As of 21 May 2014, 8 pts have been treated with ONT-380 at 300 mg BID in Combo 1 (n=4) for 2–7 cycles and Combo 2 (n=4) for 1–2 cycles. All pts are still active. No dose limiting toxicities (DLT) have been seen for either combination, and most toxicities have been Grade 1 or 2. The most common toxicities regardless of attribution have been nausea, diarrhea, dyspepsia, headache, and palmar-plantar erythrodysesthesia (PPE). There has been no Grade 3 diarrhea. Two Grade 3 events have been reported, both in Combo 1: PPE and ALT/AST elevation. There have been no SAEs. ONT-380 was dose reduced in one pt (Combo 1) for persistent Grade 2 diarrhea as well as in the pt with Grade 3 ALT/AST. Capecitabine was dose reduced in three pts: diarrhea (n=2), Grade 3 PPE (n=1), and Grade 3 ALT/AST (n=1). Best response seen to date in 3 pts evaluable for response has been stable disease. Initial PK data indicate greater drug exposure is achieved with the new tablet formulation of ONT-380 compared to the earlier capsule formulation with no evidence of drug interaction with capecitabine.

Summary: ONT-380 in combination with either capecitabine or trastuzumab has been associated with an acceptable safety profile, with no DLTs seen with either combination. No Grade 3 diarrhea has been observed, including pts receiving both ONT-380 and capecitabine. Early evidence of disease control has been seen. Dose escalation continues, and evaluation of the three drug combinations is planned. Updated results will be presented for additional cohorts.
Title: Insulin-like growth factor receptor-1 (IGF-1R) expression is highly correlated with HER2 amplification on circulating epithelial tumor cells (CETCs) in breast cancer - this may be the reason for resistance to trastuzumab

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Body: Background: HER2 status has been the most extensively studied biomarker in circulating epithelial tumor cells of breast cancer patients. HER2 status can change during recurrence of disease and HER2 overexpression is a mandatory requirement to administer anti-HER2-directed drugs. Nevertheless, 70% of patients with HER2-positive breast cancers develop intrinsic or secondary resistance to trastuzumab. This resistance has been associated with the activation of an alternative signalling pathway such as the insulin-like growth factor (IGF) pathway. Therefore we investigated the expression of IGF-IR on the CETCs in addition to HER2 amplification in breast cancer patients to identify patients who might benefit from a combined targeted therapy against HER2 and IGF-IR.

Methods: CETCs were determined from blood of 30 breast cancer patients. The number of vital CETCs and the expression of IGF-IR were investigated using the maintrac® approach. Fluorescence in situ hybridisation was used for analysis of HER2 amplification in CETCs.

Results: CETCs could be detected in all breast cancer patients. The number of CETCs ranged from 4 to 163 in 100 µl of cell suspension. IGF-IR expression on the surface of CETCs was detected in all patients. Setting a cut-off 30% positive cells as HER2 positive in 75% of patients HER2 positive CETCs were observed irrespective of the status in the primary tumor. In contrast, only 6% of patients changed their HER2 status from positive tissue to negative CETCs. A statistically high correlation was found between the percentage of IGF-IR positive and HER2 positive CETCs. A statistically significant association was found between IGF-IR expression or HER2 amplification and ER/PR receptor status. The higher frequency of HER2 amplified circulating tumor cells might be due to the difference in preparation steps between tissue and blood borne cells and one of the reasons for trastuzumab resistance and for the response of some HER2 negative patients to trastuzumab. Re-evaluation of HER2 status in CETCs could be a valid strategy with potential clinical applications.

Conclusion: Our results demonstrate a parallel expression of IGF-IR and HER2 amplification in CETCs. IGF-IR may be involved in the development of resistance to trastuzumab and may be an important potential therapeutic target in breast cancers with HER2 positive circulating tumor cells. Combining targeting of IGF-IR and HER2 may be a rational approach to improve response to trastuzumab in the sub-group of CETCs that express both, HER2 and IGF-IR.
Skin infections associated with the addition of pertuzumab to trastuzumab-containing chemotherapy

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**Body: Objectives:** The addition of pertuzumab, to trastuzumab-based chemotherapy is currently considered first-line therapy for locally advanced and metastatic disease HER2 positive breast cancer and has also been suggested for use in the adjuvant setting. Over the past 12 months, we have observed an increase in the incidence of severe skin infections in patients receiving chemotherapy with pertuzumab and trastuzumab. We report the natural history of what we believe is a previously unrecognized toxicity of these regimens. **Methods:** Shortly after the FDA approval of pertuzumab, our clinical team appreciated an increase in invasive skin infections. We discussed this concern and identified new cases at our weekly research meeting, keeping a log of cases as they were identified. Infection control reviewed the individual patient and hospital data during this time period. **Results:** Eleven women were identified to have severe skin and/or nail infections; 6 after cycle 1; 2 after cycle 2, 1 after cycle 3 and 2 after cycle 6. The median age was 51 (Range 46-64); 9 received pertuzumab, trastuzumab, carboplatin, and docetaxel (PTCH) and 2 pertuzumab, trastuzumab, and docetaxel. Folliculitis of the scalp, abdomen, and/or buttocks were observed in 4 patients. Abscesses were observed in 5 patients, 4 of whom required incision and drainage. Severe paronychial infections involving one to 16 digits were observed in 3 (including one who also had folliculitis). 1 pt required surgical removal of 2 nails. Quantitative immunoglobulins were found to be low in 2 of 8 women tested; 1 patient had a total protein of 4.7 but did not have an assessment of quantitative immunoglobulins. All patients were initially treated with oral antibiotics, but 3 required hospitalization. Cultures were obtained on 6 patients, Staph aureus was identified in 2 and methicillin resistant Staph (MRSA) in 4. All patients resolved their infections and 9 of 10 were able to complete six cycles of chemotherapy. Infection control could identify no increase in Staph infections at our institution. Patients were treated at different locations and received different lot numbers of drug. **Conclusions:** We believe this is the first report of a substantial incidence of invasive skin and nail infections with the addition of pertuzumab to trastuzumab-based regimens not reported in the product label. Low levels of quantitative immunoglobulin in select patients suggest a possible mechanism.
Title: The ASCO/CAP guideline update for HER2 testing increases the number of breast cancer patients eligible for HER2-targeted therapy

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Body: Background: The American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) published, in Nov/2013, the interpretive guidelines for HER2 testing of breast cancer patients. This aimed to improve the accuracy of HER2 testing and its utility as a predictive marker in invasive breast cancer. The former version of these criteria was written in 2007. Objectives: to compare the HER2 immunohistochemical (IHC) analysis using the 2007 versus 2013 algorithms in a cohort of breast cancer cases diagnosed in a single institution in Southern Brazil. The cases were previously classified as HER2 1+ or 2+, using the 2007 criteria. Methods: the sample included 100 invasive breast cancer cases. The HercepTest (Dako, Denmark) was used for determination of HER2 expression. The HER2 testing was analyzed independently by two pathologists. The FISH analysis was done using a HER2/D17Z1 probe set. Preliminary Results: The HER2 IHC interpretation changed in 11/69 (15.9%) cases: 8.7% negative or equivocal cases by the 2007 guidelines were positive by the 2013 classification and 7.2% of HER2 1+ cases became equivocal (p<0.001; x² test). The FISH analyses are ongoing. Conclusion: The 2013 ASCO/CAP guidelines resulted in less negative cases and in more equivocal (requiring reflex testing) and positive tests. Applying the ASCO/CAP 2013 guidelines resulted in a significantly increase of breast cancer patients eligible for HER2-targeted therapies.
Title: Validation of a software based clinical decision support system for breast cancer treatment in a tertiary care cancer center in India

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Body: Introduction: Access to expert, evidence based clinical decision making is crucial in maximizing the outcome of women with breast cancer, but is a scarce resource, especially in developing countries. The Navya Expert System is a patented, software based clinical decision support system that exhaustively searches and assimilates relevant medical literature and guidelines to make specific therapeutic recommendations for individual patients based on their clinical data. This study is a retrospective validation of Navya Expert System’s output against tumor board decisions of a multidisciplinary group of expert breast cancer clinicians working in a tertiary care oncology center in India.

Methods: Women with non-metastatic breast cancer who had already completed their loco-regional and systemic therapy based on the recommendations of the tumor board were included in the study. The protocol specified clinical and pathology data of these women were retrospectively abstracted from their case charts and processed through the Navya Expert System. The output was classified into major (neo-adjuvant chemotherapy versus upfront surgery and need for adjuvant chemotherapy, endocrine therapy and radiation therapy, respectively) and minor (breast conservation versus mastectomy, taxane versus non-taxane adjuvant chemotherapy and need for nodal radiation therapy) therapeutic decisions. Decisions discordant between the tumor board and the Navya Expert System were adjudicated by an expert panel of breast cancer clinicians from the same institution. Navya Expert System decisions were classified as discordant with appropriate clinical practice if they were in disagreement with both the tumor board and expert panel. All other Navya Expert System decisions were classified as concordant. The primary outcome of the study was concordance between the Navya Expert System and the tumor board or expert panel for major and minor therapeutic decisions.

Results: A total of 76 patients involving 224 major and 224 minor therapeutic decisions were included in the study. Navya Expert System’s output was concordant with the tumor board or expert review in 224/224 major decisions (100%, 95% CI 99.6%-100%) and 221/224 minor decisions (98.6%, 95% CI 97.1%-100%). Navya Expert System’s output was concordant with the tumor board alone in 210/224 (93.75%, 95% CI 90.6%-96.9%) major decisions and 160/224 (71.4%, 95% CI 65.5%-77.3%) minor decisions. Most common reasons for discordance were non-prescription of HER2 targeted therapy by the tumor board due to financial constraints and non-use of nodal radiation for 1-3 node positive patients. Of the 64/224 Navya Expert System decisions discordant with the tumor board, only 3 were finally deemed discordant after review by the expert panel.

Conclusions: Navya Expert System treatment recommendations, only requiring the input of commonly available clinical data, are highly concordant with those of a tumor board comprised of breast cancer experts with high level expertise. If these results can be prospectively validated, Navya Expert System has the potential to increase global access to evidence based clinical decision making in breast cancer.
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Title: Attitudes of medical oncologists towards research breast biopsies in patients with newly diagnosed stage I-III breast cancer not enrolled in a clinical trial

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Body: Background:
Patients (pts) with breast cancer treated with neo-adjuvant therapy on clinical trials are often asked to consent to pre-treatment and on-treatment research biopsies. There is increasing interest in obtaining tissue samples at similar time points in pts treated with neo-adjuvant therapy outside of clinical trials. However, medical oncologists’ (MOs) attitudes towards approaching pts about research biopsies in this setting are unknown.

Methods:
Three hundred and nine academic breast MOs identified from websites of the National Cancer Institute (NCI) – designated cancer centers were asked to complete a survey either by paper or online. Eligible MOs (MOs who saw breast cancer pts and who saw pts >4 hours/week.) were asked to predict what proportion of their pts with newly diagnosed, non-metastatic breast cancer would consent to research purposes only biopsies (RPOBs) i.e., biopsies with no clinical benefit to pt. Median values are reported. Two-sided Fisher’s exact test was used to compare categorical variables using a \( \alpha \) level of .05.

Results:
Of 221 (101F,85M, 5 unknown) MOs who completed the survey, 30 MOs were ineligible (response rate=221/309,72%). Median age was 50 (Range 33-80). Median years of oncology experience was 15 (Range 1-45). MOs predicted that 14\%, 63\% and 21\% of their pts would definitely/probably, maybe, probably not/definitely not consent to a RPOB of the breast. Forty-one percent, 34\%, 19\%, 3\% of MOs were very comfortable, somewhat comfortable, somewhat uncomfortable, and very uncomfortable asking pts to consent to RPOBs respectively. The only factor associated with increased comfort discussing an RPOB was MOs’ years in practice. MOs with fewer years (<15 years) in practice were more comfortable in asking pts to participate in RPOBs compared to MOs with more years in practice (>15 years) (Adjusted RR=1.2, \( p =0.02 \)). Gender, number of pts enrolled onto clinical trials, and MOs with pts who had research biopsies in the last 3 months was not associated with increased comfort.

MOs who were more comfortable in approaching pts for RPOBs were associated with estimating a larger proportion of their pts as willing to undergo RPOB. For example, nearly one third of MOs who were very comfortable with approaching pts for RPOBs estimated that greater than 50\% of their pts would consent to research biopsies. In contrast, nearly all the MOs who were very uncomfortable with approaching pts for RPOBs estimated that less than 25\% of their pts would consent to research biopsies.

The 3 most common reasons why MOs were reluctant to consent pts for a RPOB include pain/discomfort of a biopsy (59\%), risk of a biopsy procedure complication, (44\%), and inconvenience to the pt (33\%).

Conclusions:
Academic breast MO’s predicted that fewer than 1 in 5 women with newly diagnosed, non-metastatic breast cancer would definitely or probably agree to a request for an RPOB outside of the context of a therapeutic trial, and approximately one-quarter of MOs expressed discomfort in approaching pts for such procedures. Our results have important implications regarding the feasibility of such research efforts, and identify potential barriers to target for intervention.
Title: Do participants in adjuvant breast cancer trials reflect the breast cancer patient population?

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Body: Background: To be clinically relevant, randomised clinical trials need to involve participants who reflect those in the community likely to receive the treatments under evaluation. However, as few as 5% of patients with chronic medical illnesses (asthma, diabetes) are documented as meeting therapeutic trial entry criteria. This study examined a population based cohort of women diagnosed with early breast cancer to compare the eligibility criteria of 12 influential adjuvant endocrine therapy, chemotherapy or radiotherapy trials with clinical eligibility and actual treatment at a single regional cancer centre.

Methods: 421 Phase III trials of adjuvant endocrine therapy, chemotherapy or radiotherapy treatment for early breast cancer referenced in 5 recent national guidelines were identified and shortlisted by breast cancer specialists of multiple disciplines at a national meeting to identify the twelve most influential trials: 5 endocrine (ATAC, BIG 1-98, IES, MA17, TEAM), 2 chemotherapy (NSABP B-28, TACT) and 5 radiotherapy trials (Whelan et al JNCI 2002, EORTC Bartelink NEJM 2001, START A, START B, TARGIT-A). Eligibility criteria were extracted from protocols and subsequently applied to a 16-year (1993 – 2008) community-based cohort of 4811 women who had received a diagnosis of breast cancer, with the proportion who met each criterion calculated. HER2 therapy trials were excluded since the cohort did not have HER2 testing prior to 2000. Clinical guideline-based criteria for the adjuvant treatments described within each trial were also applied to the cohort. Finally, the proportion of women actually prescribed adjuvant endocrine therapy meeting the relevant trial eligibility criteria, taking account of drug availability, was calculated using linkage to community pharmacy records.

Results: Of 4811 women in the cohort, 3535 (73%) were eligible for at least one trial. Eligibility ranged from 34% (1653 women) for the chemotherapy trials, through 47% (2247) for at least one endocrine therapy trial, to 71% (3419) for a radiotherapy trial. The proportion of women considered clinically eligible for the same treatment, was greater (MA17, Whelan et al JNCI 2002, TACT), similar (BIG1-98, IES, NSABP B-28) or less than (ATAC, EORTC Bartelink NEJM 2001, START A and B trials, TARGIT A) trial eligibility criteria, with no consistent pattern of exclusion. It was rare for more than 45% of women of the community cohort to be eligible for a given trial. The proportion of women who actually received an endocrine therapy in clinical practice, but would not have been eligible for the trial evaluating that therapy (ATAC, BIG 1-98, IES and MA17), ranged from 44% to 83%.

Interpretation: Trial entry criteria for early breast cancer are reassuringly more inclusive for adjuvant breast cancer treatments than for chronic medical conditions. However, the application of trial evidence in the clinic may be at variance with the trials and, for adjuvant endocrine therapy at least, a substantial proportion of women receive therapy who would not have been eligible for the relevant trial.
Title: Relationship between type of therapeutic intervention and funding source in randomized clinical trials (RCTs) in breast cancer

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Body: Background: Pharmaceutical companies play an important role in new drug development and approval in an environment where performing RCTs has become increasingly costly and complex. However, myriad questions about the impact of clinical, surgical and radiotherapy interventions equally require adequate hypothesis testing in RCTs. A potential lack of funding for RCTs not directly related to new drug development might lead to important gaps in clinical knowledge.

Methods: We searched PubMed for all RCTs published between 01/2009 and 12/2013 in breast cancer. All articles published in this period were manually screened for eligibility. We included only RCTs with clinical endpoints such as TTP, PFS, OS and response rate. Two investigators independently selected phase 2 and phase 3 RCTs with at least 50 patients published in English. We also searched National Cancer Institute (NCI) data for all active, therapeutic phase 3 trials that are currently enrolling patients (as of 05/05/2014). We classified eligible trials according to the type of intervention (drugs, radiotherapy or surgery) and the stated funding source (industry versus nonprofit).

Results: We retrieved 1,676 PubMed studies of which 247 (15%) were eligible. 218 (88%) of the RCTs evaluated drugs, 14 (6%) radiotherapy and 15 (6%) surgery. 183/247 (74%) RCTs were funded entirely or partially by industry (pharmaceutical and device companies) and 64/247 (26%) by nonprofit organizations (government, academic centers or foundations). There was a significant association between source of funding and type of intervention: 183/218 RCTs (83%) evaluating drugs were funded by industry, in comparison to none of surgical and radiotherapy RCTs (P<0.0001). From the NCI data we retrieved 144 RCTs. 116 (81%) of the RCTs studies drugs, 20 (14%) radiotherapy and 7 (5%) surgery. 55/116 (47%) trials evaluating drugs were funded by industry in comparison to none of surgical and radiotherapy trials. Eighty eight trials were not funded by industry. Of those, 23(26%) were performed in China, 16(18%) in the USA, 10 (11%) in France, 7 (8%) in India, 6 (7%) in the UK and the remaining 26 studies were performed in 16 other countries.

Conclusions: The vast majority of RCTs in oncology relates to drug development and is being funded by industry, while 100% of RCTs evaluating surgical or radiotherapy related questions are not industry funded. This scenario seems not be changing over the last years given that the comparison between published trials and active trials shows the same scenario. Even though new drug development is of paramount importance, the extent to which clinically relevant issues are not being properly addressed by RCTs, at least in part due to lack of funding, should be considered and further evaluated. The oncologic community, as well as academic and nonprofit organizations, including governments, need to work together to forge new and alternative forms of research funding in order to allow us to answer critical clinical questions that we, as oncologists, face in our daily practice.
Title: IBCSG BIG 1-98 study: The long-term follow-up experience

Anita Giobbie-Hurder¹, Beat Thürlimann¹, Bent Ejlertsen¹, Patrick Neven¹, Robert E Coleman¹, Ian Smith¹, Andrew M Wardley¹, István Láng¹, Marco Colleoni¹, Marc Debled¹, John F Forbes¹, Karen N Price¹, Meredith M Regan¹, Manuela Rabaglio¹, Aron Goldhirsch¹, Alan S Coates¹ and Richard D Gelber¹. 'BIG 1-98 Collaborative Group and International Breast Cancer Study Group.

Background

Industry-sponsored clinical trials often have duration of patient follow-up that is defined according to regulatory requirements. However, in diseases such as endocrine-responsive, early breast cancer, recurrences occur after protocol follow-up, and monitoring of long-term toxicity is important. It is challenging to continue patient follow-up after industry sponsorship ends. Transferring responsibility for additional follow-up to the participating academic centers is required. One such example is the long-term follow-up (LTFU) of patients in the Breast International Group (BIG) 1-98 Trial. We present the procedures and current status of the BIG 1-98 LTFU protocol.

Methods

In 2010, the BIG 1-98 trial embarked on a new LTFU protocol to gather data on patient outcomes for an additional five years after study completion (2011-2015). Industry sponsorship ceased at the end of 2010. The LTFU study is designed as an observational, non-interventional study to continue the collection of simplified and updated data on survival, disease status, and long-term adverse events from centers participating in the 4-arm option. The International Breast Cancer Study Group (IBCSG) is sponsoring BIG 1-98 LTFU, and per case reimbursement is available.

Results

The potential BIG 1-98 LTFU cohort consists of the 148 academic medical centers that participated in the 4-arm option with a maximum of 6843 patients enrolled to the parent study. In May 2014, approximately 3 years after initiation of the LTFU protocol, 96 centers had agreed to participate, of which 67 sites had activated the protocol and submitted LTFU data; 31 additional centers were not participating, and the status of 21 centers was unknown.

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</table>

Because the original BIG 1-98 informed consent indicated life-long follow-up, only three countries required patient re-consent in order to participate. At least one LTFU data submission has occurred for 73% of patients participating in the LTFU (May 2014).

Conclusion

Long-term follow-up for a large-scale clinical trial is feasible, but challenging. The methods used for BIG 1-98 LTFU will be described and the status will be updated at the meeting.
Pretargeted immuno-PET with an anti-carcinoembryonic antigen (CEA) bispecific antibody (BsMAb) and a $^{68}$Ga-labeled hapten-peptide compared to conventional imaging and FDG-PET in metastatic breast cancer patients (BC): First results

Objectives: Different imaging methods are available to detect BC recurrence, but several new noninvasive antibody imaging methods targeting membranous IGF-1R expression, HER2/neu or CD138 have been tested in BC pre-clinical trials. Today, a new generation anti-CEA x anti-HSG humanized trivalent TF2 BsMAb and $^{68}$Ga-IMP288 HSG peptide is available with good features for immuno-PET in preclinical studies. This study aimed to compare the sensitivity of anti-CEA immuno-PET/CT using pretargeted $^{68}$Ga-IMP288 to morphological imaging and FDG-PET/CT in metastatic BC patients.

Methods: Ten patients, enrolled in an optimization immuno-PET study underwent whole-body immuno-PET/CT recorded 1h and 2h after injection of 150 MBq of $^{68}$Ga-IMP288 pretargeted by 120 nmol of TF2 injected 24h to 30h before, in addition to thoracic-abdominal-pelvic CT and FDG-PET/CT. Bone (n=5) and brain MRI (n=2) were also performed in some cases to confirm abnormalities detected by other modalities. The gold standard was determined by follow-up and a lesion detected by at least 2 imaging modalities was considered as positive.

Results: Median CA15-3 was 264.1 kU/L (31.6 to 2448) and median CEA was 48.25 µg/L (9.5 to 1359.0). A total of 537 lesions were detected by immuno-PET/CT, 247 by CT, 160 by bone MRI, and 428 by FDG-PET/CT. To date, 524 lesions were confirmed as pathologic by the gold standard: 17 in nodes, 1 in lung, 83 in liver, 418 in bone, 1 in skin, and 4 in brain. Overall sensitivity of immuno-PET was 92.8%, with 100% sensitivity for bone, liver, skin, and brain, 92.8% for nodes, and 28.6% for lung. Overall sensitivity of CT and FDG-PET/CT were 74% and 95.2%, respectively. CT and FDG-PET/CT had 54.5 and 100% sensitivity for nodes, 90 and 100 % for liver, 100 and 85.7% for lung, and 68% and 94% for bone, respectively. Bone MRI had 92.3% sensitivity. Brain lesions were only detected by immuno-PET/CT and confirmed by MRI. Median tumor SUVpeak on immuno-PET at 1h and FDG-PET/CT were 5.05 (3.52 to 24.55) and 3.3 (0.47-10.95), respectively. A 2h, median tumor SUVpeak on immuno-PET was 5.23 (3.09-34.27), 5/9 patients showing an increased tumor uptake between 1 and 2h, with no lesion being detected only at 2h.

Conclusion: These results demonstrate the high accuracy of anti-CEA pretargeted immuno-PET/CT for staging BC patients, especially for bone, liver and brain evaluation. Immuno-PET allowed detection of bone lesions in eras not explored by MRI.

**Title:** Evaluation of apoptosis in breast cancer using the novel PET probe $[^{18}\text{F}]$ICMT-11 in patients treated with neoadjuvant FEC chemotherapy: Initial assessment of optimum imaging time and relation to caspase-3 immunostaining

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**Body:**

**Background:**

$[^{18}\text{F}]$ICMT-11 is an isatin analogue which has been developed by our group as a novel PET radiotracer for studies of apoptosis in vivo. Preclinical studies have demonstrated subnanomolar affinity to caspase 3, and validated the potential for imaging apoptosis in xenograft models. A first-in-man study showed that the agent was well tolerated with acceptable dosimetry. This is the first study of this agent to measure the effect of chemotherapy on radiotracer uptake in patients. As apoptosis is a dynamic process, one of the main objectives of the study was to determine the optimal time-point for imaging post-chemotherapy and compare the results with immunohistochemistry assessments at the same time-points. The study was approved by a regional ethics committee and ARSAC.

**Methods:**

7 patients with breast tumour lesions measuring 15mm or more, due to undergo neoadjuvant chemotherapy with FEC (5FU, Epirubicin, Cyclophosphamide) had dynamic PET scans for 66mins 30seconds following intravenous injection of $[^{18}\text{F}]$ICMT-11 with a mean activity of 340.82 Mbq±20.76 and Specific Activity range of 447.014-5128.34 Gbq/µmol prior to chemotherapy and 24h-2 weeks post-chemotherapy. A breast biopsy was also obtained within a few hours of the 2nd PET scan to correlate apoptosis in the breast tissue utilising TUNEL and Caspase 3 staining by immunohistochemistry. Volumes of interest were drawn manually and analyzed using Analyze software.

**Results:**

The scans were well tolerated in all patients. Uptake of $[^{18}\text{F}]$ICMT-11 was demonstrated in all tumor lesions. The tumours studied included ER positive and PR positive, HER2 positive and triple negative patients. The first cohort patients were imaged pre-chemotherapy and 24-48h post chemotherapy. Tumour to Breast ratio (TBR) showed an increase from 1.42±0.21(pre) to 1.71±0.33 (post). Tumour to muscle ratio (TMR) was not increased, 1.52±0.30(pre) and 1.22±0.09 (post). In addition, an increase the SUV was noted in the lymph nodes of patients, at both 24 and 48h. ($\text{SUV}_\text{av}$ 0.39±0.02 (pre), 0.45±0.03 (post), and $\text{SUV}_\text{max}$ 0.87± 0.02(pre) ,1.22±0.12 (post). The lymph nodes were however not sampled for immunohistochemistry. A further cohort of patients had the follow-up scan 2 weeks post chemotherapy, TBR and TMR were both increased in this cohort 1.50±0.22 (pre), 2.52±0.48 (post),and 1.82±0.10 (pre), 2.08±0.0.04 (post), respectively. Caspase and TUNEL labelling with immunohistochemistry also showed increased in apoptosis in the breast biopsies at 2 weeks compared to baseline in keeping with the PET data.

**Conclusion:**

These preliminary data suggest that $[^{18}\text{F}]$ICMT-11 is a promising marker for chemotherapy induced apoptosis in vivo, and correlates with findings in tumor biopsy using TUNEL and immunostaining for caspase 3. Further work is underway to study a larger cohort of patients, and identify the optimal PET pharmacokinetic parameter to describe $[^{18}\text{F}]$ICMT-11 uptake and retention.
Background/Objective:
Diagnostic specificity remains disappointingly low for ultrasound-based methodologies optimized to achieve near 100% sensitivity. Opto-acoustic (OA) imaging is a fusion of real time co-registered, interleaved laser optic and ultrasound imaging that shows fused functional findings (relative de-oxygenation of hemoglobin) and morphologic information (tumor angiogenesis) within and around breast masses using a hand-held duplex OA probe. We assess the improvement in sensitivities and specificities of nomograms (N) based on regression models to classify (CL) benign (B) vs. malignant (M) and to project probability of malignancy (POM) based on five feature-based metrics scored by 21 independent readers (IRs) and an expert reader. We examine OA feature separation and nomogram performance.

Methods:
Masses from a series of 100 subjects with 80 biopsies (42 B, 38 M) were blindly evaluated prior to both core biopsy and excision. The OA algorithm was locked. Three internal OA findings (density of vascularity [V], relative blood oxygen saturation [O], and hemoglobin [H]) and two external OA findings (boundary zone [Z] and peri-tumoral radiating vessels [R]) were assigned 0-5/6 ordinal scores. Feature distributions were compared using a two-sided Kruskal-Wallis test.

Results:
There were consistently significant differences between the feature distributions for benign vs. malignant: density of vascularity (14/21 IRs), relative blood oxygen saturation (11/21 IRs), hemoglobin (16/21 IRs), boundary zone (all IRs), and peri-tumoral radiating vessels (all IRs) always with lower scores for benign vs. malignant. The mean IR sensitivities were near 100% for all IRs. The mean specificities from the nomograms were 49% (POM), 43% (CL), and 48% (averaged).

Conclusions:
The study results indicate that OA findings can be independently and quickly mastered by practicing IRs to consistently differentiate masses. Nomograms offer further confidence to enhance decision making to differentiate. If confirmed in a larger series, OA findings might be useful in differentiation and thus sparing biopsies in addition to more customized surgeries.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-01-04
Average Grade: 4.75

Title: In vivo lymphatic imaging of a human inflammatory breast cancer model

Sunkuk Kwon¹, Germaine Agollah¹, Grace Wu¹ and Eva M Sevick-Muraca¹. ¹University of Texas Health Science Center, Houston, TX.

Body: Background: Inflammatory breast cancer (IBC) is the most aggressive but poorly understood type of breast cancer. IBC constitutes only approximately 5% of all newly diagnosed breast cancers, yet responsible for 8-10% of breast cancer-related deaths. Unfortunately, there are no definitive molecular or pathological, early diagnostic criteria for IBC. IBC grows in nests or sheets, spreading outward into the skin and eventually distant sites through dermal lymphatic vessels; therefore, one of the key pathological characteristics of IBC is dermal lymphatic invasion by tumor emboli, which can lead to obstruction of the lymphatic drainage possibly causing the clinical inflammatory features of diffuse erythema, rapid breast enlargement and edema as indicated by peau d'orange (orange peel) appearance covering at least a third of the breast surface. However, relatively little is known about the role of lymphatic function in IBC growth and metastasis. The purpose of this study was to non-invasively and longitudinally image changes of lymphatic drainage patterns in mice bearing the triple negative, human IBC SUM149 cells, which were stably transfected with iRFP gene reporter (iRFP-SUM149) in order to better monitor tumor growth and metastasis.

Methods: Eight to ten weeks old female non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice were housed and fed special sterilized pelleted food and sterilized water. Two weeks after feeding special diets, mice were subcutaneously injected with iRFP-SUM149 cells in the left hindlimb or orthotopically in the left inguinal mammary fat pad (MFP). Fluorescence images were acquired immediately after and for up to 10 mins after i.d. injection of indocyanine green (ICG) using a custom-built NIRF imaging system. To achieve a greater magnification, a macrolens was used.

Results: Our NIRF imaging data showed gradual changes of lymphatic vessels around the tumor. In mice with an orthotopic tumor, ICG accumulation was observed in the distal peritumoral region at up to 3 weeks p.i., indicative of the start of tumor obstruction of normal lymphatic drainage. As tumors grew, the greater extent of lymph flow, but not all, was obstructed by a tumor, resulting in dermal backflow and thus staining more lymphatic capillaries and eventually rerouting of lymphatic drainage was detected due to complete obstruction of lymph flow by a tumor. Similar to an orthotopic model, mice with a s.c. tumor also showed altered lymphatic drainage patterns during tumor growth. Interestingly, extravasation of ICG into iRFP-SUM149 was detected at 3 weeks p.i., due to leaky lymphatic vessels. A similar lymphatic phenotype has also been observed in IBC patients. Previously, we have imaged atypical tortuous lymphatics and altered lymphatic drainage in the arm and affected breast of a patient with IBC using clinical NIRF imaging developed in our team to image as deep as 3-5 cm with a microdose of ICG.

Conclusions: Longitudinal, non-invasive imaging of lymphatic functional and architectural changes provides a new method to dynamically monitor lymphatic response to IBC growth and metastasis.
Early optical tomography changes correlate with residual cancer burden scores in women receiving neoadjuvant chemotherapy

Emerson A Lim1,2, Jacqueline E Gunther3, Molly Flexman4, Hyun K Kim3, Hanina Hibshoosh2,5, Kevin Kalinsky1,2, Katherine Crew1,2,6, Matthew Maurer1,2, Sheldon Feldman2,7, Bret Taback2,7, Preya Ananthakrishnan2,7, Margaret Chen2,7, Susan Refice2, Andreas Hielscher3,8,9, and Dawn L Hershman1,2,6,1 Internal Medicine â–“ Columbia University Medical Center, New York, NY; 2Herbert Irving Comprehensive Cancer Center, New York, NY; 3BioMedical Engineering â–“ Columbia University, New York, NY; 4Philips Corporation, Tarrytown, NY; 5Pathology â–“ Columbia University, New York, NY; 6Mailman School of Public Health, New York, NY; 7Columbia University Medical Center, New York, NY; 8Electrical Engineering â–“ Columbia University, New York, NY and 9Radiology â–“ Columbia University Medical Center, New York, NY.

Background: The Residual Cancer Burden (RCB) score predicts survival in patients (pts) with breast cancer (BC) treated with neoadjuvant chemotherapy (NACT). Predicting tumor response early during NACT may allow for treatment optimization. Diffuse optical tomography (DOT) is an imaging modality that measures the distribution of water (H2O), oxy- (HbO), and deoxy-hemoglobin (Hb) concentrations as a surrogate for vascularity and architecture. We hypothesize that the 2-week change in DOT parameters will correlate with the RCB score. We also explored the association between DOT parameters and tissue biomarkers: Ki-67 change and microvessel density (MVD).

Methods: Women with stage II-IIIc invasive BC scheduled to receive NACT with 12 cycles of a weekly taxane followed by 4 cycles of doxorubicin with cyclophosphamide were enrolled. Treatment with biologic therapies was allowed. DOT assessments were made before NACT and after 2 weeks on treatment. DOT data were reconstructed into 3D images of the tumor region, from which HbO, Hb, and H2O concentrations were extracted. Final pathology specimens were scored for the RCB index (continuous), RCB class (0, 1, 2, 3), and a dichotomized RCB score (RCB class 0 or 1: responders; RCB class 2 or 3: non-responders). Ki-67 was measured on baseline tumor biopsies and surgical specimens. Correlation analysis, ANOVA testing, and two sample t-tests were used to evaluate the relationship between the 2-week changes in DOT parameters and the RCB score and Ki-67 change. Correlation was assessed between MVD and baseline DOT measures.

Results: Since July 2011, we have recruited 28 pts of a total planned accrual of 40. 25 pts have had surgery and complete data are available for 23. Of the 23 pts, 6 had a pCR (RCB 0), 2 had RCB 1, 10 had RCB 2, and 5 had RCB 3. The Pearson correlations (r) between the 2-week change in HbO, Hb, and H2O with the continuous RCB index were 0.65 (p=0.002), 0.70 (p=0.0006), and 0.70 (p=0.0006), respectively. There was a significant difference in the 2-week Hb change for pts with RCB 0 compared to pts with RCB 1, 2, or 3. There were significant differences in the 2-week change in H2O and HbO for pts with RCB 0 compared to pts with RCB 2. There were also significant differences between DOT parameters by the dichotomized RCB score (table 1). There was an association in Ki-67 change and 2-week H2O change (r=0.43 p=0.059). A subset of 15 pts had MVD assessments, but these did not correlate with baseline DOT parameters (r ≤0.18, p>0.5).

Conclusions: Two-week DOT change is an early predictor of response to NACT as measured by the RCB score. We found significant associations between the RCB index with 2-week changes in HbO, Hb, and H2O. Significantly different changes in DOT parameters were associated with the other RCB classifications. Ki-67 changes and baseline MVD were not statistically significantly associated with DOT parameters. We are analyzing static and dynamic DOT data on the remaining pts. Additional pts
are being recruited to evaluate DOT’s predictive ability by tumor subtype.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-01-06
Average Grade: 6.75

Title: Characterization of metastatic breast cancer lesions with ferumoxytol MRI and treatment response to MM-398, nanoliposomal irinotecan (nal-IRI)

Jasgit C Sachdev\(^2\), Ramesh K Ramanathan\(^3\), Natarajan Raghunand\(^3\), Jaeyeon Kim\(^1\), Stephan G Klinz\(^1\), Eliel Bayever\(^1\), Jonathan B Fitzgerald\(^1\) and Ronald L Korn\(^4\). \(^1\)Merrimack Pharmaceuticals, Inc, Cambridge, MA; \(^2\)Virginia G. Piper Cancer Center, Scottsdale Healthcare, Scottsdale, AZ; \(^3\)Translational Cancer Imaging, Arizona Cancer Center, Tucson, AZ and \(^4\)Imaging Endpoints, Scottsdale, AZ.

Body: Introduction
Irinotecan has known activity in metastatic breast cancer (MBC). MM-398, nanoliposomal irinotecan (nal-IRI), is designed to exploit leaky tumor vasculature for enhanced drug delivery to tumors. Tumor deposition of nal-IRI and subsequent conversion to SN-38 in both neoplastic cells and tumor associated macrophages (TAM) may positively correlate with activity. Predictive biomarkers to measure tumor deposition could identify patients likely to benefit from nal-IRI. Ferumoxytol (FMX), an iron-oxide superparamagnetic nanoparticle with MRI contrast properties, is taken up by TAMs with similar distribution patterns to nal-IRI in preclinical models. Our previous work has shown the feasibility of quantitative FMX MRI (Fe-MRI) of tumor lesions, and we developed a quantitative mechanistic PK model of FMX deposition (AACR 2014, abstract #CT224). Here we report nal-IRI activity and FMX levels in MBC patients on the study.

Patients and methods
Patients (n=15) with refractory solid tumors and at least two metastatic lesions >2 cm accessible for percutaneous biopsy were enrolled in a Phase 1 study. Fe-MRI scans were performed using T2* iron sensitive sequences prior to and following FMX infusion (1 h, 24 h, 72 h). T2* signal was used to calculate FMX levels in total lesions by comparison to a standard curve. Comparison of quantified FMX lesion uptake with a mechanistic PK model previously indicated that tissue permeability to FMX contributed to early Fe-MRI signals at 1 h and 24 h, while FMX binding contributed at 72 h. Patients then received nal-IRI (80 mg/m\(^2\) q2w) until progression. Core biopsies were obtained 72 h after both FMX and nal-IRI infusions. RECIST evaluation was done by CT every 8 weeks.

Results
FMX was well tolerated, and adverse events to nal-IRI were consistent with previous studies. Three of the 13 patients receiving nal-IRI had ER/PR+ MBC (median # of prior Rx: 8 compared to 4 for all study patients). Thirteen liver lesions (4-5/pt) were evaluated by FMX-MRI and CT for these 3 patients. Average lesion size: 26.9±11.2 mm diameter and 8.1±11.3 cm\(^3\) (median 4.7 cm\(^3\)). Time on treatment for the 3 patients was 57, 126 and 256 days (study median 57 days). Best overall response was 1 stable disease (SD) and 1 partial response (PR) in these 3 patients. The patient with a PR had an average lesion size reduction of 44.5%, while the patient with SD had an average lesion size increase of 12.5% at final evaluation. Lesions that shrank after nal-IRI showed higher early levels of FMX compared to the study median (median 39.6 vs. 32.6 mcg/mL at 1 h; median 37.7 vs. 34.5 mcg/mL at 24 h). This relationship between lesion response and FMX levels was consistent with the lesion behavior in the full data set (n=31 lesions/9 patients across 7 indications) of the study.

Conclusions
Clinical activity of nal-IRI was observed in a subset of heavily treated ER/PR+ MBC patients. The relationship between FMX levels in tumor lesions and nal-IRI activity suggests that lesion permeability to FMX may be a useful biomarker for nal-IRI deposition and tumor response in MBC and potentially other indications. A multi-institution expansion of this study in HER2-negative MBC is planned to confirm these findings.
Fidelity of FDG-PET in breast cancer: Reproducibility at multiple sites

Hannah M Linden¹, Jennifer Specht¹, Lanell Peterson¹, Brenda Kurland², Andrew Shields¹, Darrin Byrd¹, Alena Novakova¹, Rebecca Christofel¹, Mark Muzi¹, David Mankoff³ and Kinahan Paul¹. ¹University of Washington, Seattle, WA; ²University of Pittsburgh, Pittsburgh, PA and ³University of Pennsylvania, Philadelphia, PA.

Introduction
Breast cancer is a common, treatable malignancy, with frequent metastasis to bone. Patients with bone-dominant disease are often excluded from clinical trials due to a lack of RECIST "measurable" disease. FDG-PET can help quantitatively measure multiple tumor sites, and assay disease activity in bone. However, the reproducibility of FDG-PET at multiple sites is unproven. We sought to pilot alignment of clinical protocols prior to imaging, phantom testing and then repeat patient scans, in patients undergoing clinical FDG-PET to test the fidelity of quantitative FDG-PET imaging.

Methods
After determination and alignment of clinical protocols, and serial successful phantom imaging, ten female patients with metastatic breast cancer underwent paired FDG-PET/CT test-retest studies with no more than 15 days between scans and without interim change in treatment. Seven patients were studied in the same scanner and 3 patients were studied in 2 different scanners in our clinical network. Each PET/CT scanner’s quantitative performance was monitored with NIST-traceable reference sources to ensure proper calibration. Images were interpreted and SUV metrics were estimated at a central lab. Linear mixed models with a random intercept were fitted to compare test-retest differences in multiple lesions per patient.

Results
SUVmax was assessed in a total of 68 lesions (52 bone, 16 other sites). Average SUVmax ranged from 2.3 to 18.2 (mean±SD = 5.7±2.6) per patient. The median SUVmax difference was 0.25 (5%) for 35 lesions imaged twice in the same scanner, and was 0.01 (0.2%) for 33 lesions imaged in two different scanners. In a linear mixed-effects model with random patient effects, there was no difference in average percentage SUV difference for the same scanner versus different scanners (p=0.70). In the same model, the absolute percentage difference in SUVmax for bone lesions was estimated as 6 percentage points lower than for other sites (p=0.002, 95% confidence interval 2%-10%).

Conclusions
If PET/CT systems are carefully calibrated, and imaging protocols are consistent, then variability associated with FDG SUVmax between scans is similar to prior test-retest studies. Bone lesions appear to have tighter reproducibility than soft tissue lesions. Clinical trials that utilize quantitative PET/CT imaging throughout a network of calibrated PET/CT scanners could increase patient recruitment and improve confidence in trial results. Accrual is ongoing and results will be updated.

Research support
Supported by NIH grant U01-CA148131 and NCI-SAIC Contract 24XS036-004.
Title: Single dose acute toxicity and long-term biodistribution of perfluoropentane loaded iron doped silica nanoshells

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Body: Background: Our lab has been focusing on developing a better method of localizing non-palpable breast cancers without wire or seed localization. Perfluoropentane (PFP) loaded Fe-SiO2 nanoshells have been developed as a color Doppler ultrasound contrast imaging agent which can act as small volume (100 ul) injectable stationary guide-marker for breast tumor resection. Preliminary experiments have demonstrated that the nanoshells can provide robust contrast for periods extending past 10 days in vivo in Py8119 epithelial breast tumor bearing mice with no adverse affect to the mice. Short-term biodistribution over 72 hours of nanoshells using In111 labeled nanoshells demonstrated with gamma scintigraphy that intravenously dosed particles primarily accumulate in the liver but some radioactive signal can be seen in the bladder. The long imaging lifetime of these nanoshells necessitates the need to study long-term toxicity and biodistribution.

Materials and Methods: Fe-SiO2 nanoshells and Pure SiO2 nanoshells where synthesized via sol-gel method on polystyrene templates and then calcined to yield 500 nm hollow rigid nanoshells which were then filled with vaporized perfluoropentane. 100 ul of nanoshells at 4 mg/ml of the Fe-SiO2 nanoshells and at a dose of 2 mg/ml of pure SiO2 nanoshells were injected IV into healthy 8-week old Swiss white mice. The difference in mass dose was due to make the particle count between the two doses equivalent. Blood was collected weekly for serum chemistry and hematology. After 10 weeks mice were sacrificed, H&E was performed on organs of interest as well as inductively coupled plasma optical emission spectroscopy (ICP-OES) for trace silicon determination for long-term biodistribution.

Results: No significant effect due to the administration has yet been observed on the health of brain, lung, heart, kidney, liver, spleen or muscle tissue examined from these animals at a dose 4 mg/ml 100 ul of the Fe-SiO2 nanoshells and at a dose of 2 mg/ml of pure SiO2 nanoshells. Mouse weight steadily increased from 25.8 ± 2 grams to 30.7 ± 2.6 grams over the course of 10 weeks. Creatinine levels were detected at 0.2 ± 0.14 mg/dl indicating healthy renal function. Serum glutamic pyruvic transaminase (SGPT) was used as a measure of liver health, and SGPT values for both control (55.81 ± 6.31 U/L) and nanoshell injected mice (47.74 ± 11.04 U/L) are approximately the same over the course of 10 weeks indicating good liver health. Silicon content in mouse organs diminished over the course of 10 weeks by ICP-OES in both the Fe-SiO2 and pure SiO2 nanoshells. Conclusions: No indication of toxicity was observed from a 400 ug systemically administered dose of Fe-SiO2 nanoshells. Furthermore, the reduction of silicon content in the organs over the course of 10 weeks suggests a possible excretion pathway for silica or solid nanoparticulate materials. The efficacy in long term ultrasound contrast and high margin of safety indicates that this particle formulation is ready for phase 1 clinical trial in humans as a future method to localize nonpalpable breast cancers.
Title: Improving specificity and refining diagnostic accuracy of MRI in breast cancer with dedicated breast PET (dbPET)

Ines Dominguez¹, Michel Herranz¹, Sze Yiun Teo², Elena Brozos¹, Carmela Rodriguez¹, Jasper Chaal³, Juan Cueva¹, Jose Ramon Antúnez¹, Gabriel Gonzalez Pavón⁴, Marielle Fortier¹, Rafael López¹ and Alvaro Ruibal¹. ¹Complejo Hospitalario Universitario de Santiago de Compostela, Santiago de Compostela, Galicia, Spain; ²Kerbang Kerbau (KK) Women and Children’s Hospital, Singapore, Singapore; ³Clinical Imaging Research Centre, Singapore, Singapore and ⁴Oncovision, Valencia, Comunidad Valenciana, Spain.

Body: BACKGROUND.
Continued progress in the control of breast cancer will require sustained efforts to provide high-quality screening, diagnosis, and treatment to all segments of the population. MRI has the advantages of providing a 3D view of the breast with high sensitivity, using non-ionizing radiation. However, MRI has significant limitations including its moderate specificity that, in combination with high sensitivity, often leads to unnecessary biopsies. Recently, the MAMmography with Molecular Imaging (MAMMI) dedicated breast PET (dbPET) has emerged as an additional imaging tool for breast cancer diagnosis, clarification of complex lesions and therapy follow-up. It is well known that 18F-FDG-PET has high specificity by assessing metabolic activity, potentially reducing the number of false positive findings. To compare FDG-dbPET with the conventional magnetic resonance imaging (MRI) on breast lesion characterization, we analyzed sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) of 37 cases of patients with BC using both dbPET and MRI.

METHODS.
Thirty-seven women with known or suspected breast carcinoma (41 lesions: 36 invasive carcinomas, 2 noninvasive carcinomas, 1 case of ductal hyperplasia, and 2 benign lesions) were enrolled in this study. Both a prone dbPET Mammi scan, and routine breast MRI scans were performed. A joint reading of MRI and PET scans side-by-side by a nuclear medicine physician and a radiologist was performed. Sensitivity and specificity of MRI and dbPET scans were calculated on the basis of post-surgical pathology reports. Breast MRI examinations were performed in a 1.5-T or 3-T commercial imager (Siemens Medical Solutions) with the use of a dedicated breast coil. The imaging sequence included a sagittal T1-weighted localizing sequence followed by a sagittal T2-weighted sequence. A T1-weighted 3D, fat-suppressed fast spoiled-gradient-echo sequence was then performed with an injection of 0.1 mmol per kilogram body weight of gadolinium dimeglumine (Magnevist; Schering). A prone position high-resolution dedicated breast PET was performed 60 min after administration of 90-120 MBq of 18F-FDG.

RESULTS.
A total of 41 lesions were assessed. Lesion size range was 0.2 to 7.6 cm. In lesion-by-lesion analysis, sensitivity and specificity of MRI alone were 91% and 54%, respectively; while lesion-based sensitivity of dbPET was 93% and breast-based specificity was 100%. The positive predictive value and the negative predictive value for MRI alone were 69% and 85%, respectively; and for dbPET were 100% and 89%, respectively. In a significant number of cases, dbPET helped to clarify or disprove positive findings by MRI, and in four cases helped to define new positives that had gone unnoticed at MRI.

CONCLUSION:
Dedicated breast PET scans increase the specificity of MRI. False positives, one of the most challenging aspects of MRI in breast lesions, are reduced. The results of the current study show that FDG-dbPET is more effective than MRI in detecting true breast cancer positives. Its functional information may improve the likelihood of a successful excision, reduce costs from additional procedures, and minimize discomfort and anxiety for the patient.
Title: In vitro and in vivo imaging of Her2 with cyclic peptides derived from directed evolution

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Body: The implementation of personalized medicine for Her2-positive breast cancer treatment (Trastuzumab and Pertuzumab) has made a dramatic effect on overall patient outcome. Unfortunately, there are currently no FDA-approved imaging agents to monitor Her2-positive breast cancer leaving physicians to rely on invasive biopsies and anatomical imaging to monitor treatment with Her2-targeted therapeutics. There is also increasing evidence that women initially diagnosed with Her2-negative breast can present with Her2-positive disease upon recurrence. Noninvasive, whole-body visualization of Her2 would identify Her2-recurrent disease and serve as a powerful tool to monitor the effectiveness of new and emerging Her2-targeted therapeutics (e.g. TDM-1). While many Her2-antibody based imaging agents have been used in preclinical applications, their long circulating half-lives, high liver uptake, and poor synthetic accessibility pose a challenges for clinical translation. Here we describe the use of highly stable cyclic peptides derived from biological display as imaging agents for Her2-positive breast cancer. These peptides have antibody-like high affinity (<10 nM) for Her2-positive tumors and their size (<2 kDa) allows for higher rates of clearance, lower background signal, and consequently higher image sensitivity. Anti-Her2 peptides were pre-optimized for affinity, protease resistance and bio-stability prior to labeling with the Cy7.5 near-infrared dye. Her2-specificity was determined by in vitro assays with Her2-positive (BT474 and SKOV3), HER2-normal (MCF7) and Her2-negative (MDA-MB-231) cell lines. Sequences that showed superior affinity and selectivity were used to acquire fluorescent images of ectopic and orthotopic murine models of Her2-positive breast cancer. Our preliminary imaging data suggests that this scaffold has excellent translational potential for targeted molecular imaging of Her2-postive breast cancer.
Title: PEM tests will have a high accuracy detecting breast cancer, if an individualized dosage of F-18-FDG is used

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Body: Introduction: The diagnostic performance of the first European PEM (PET mammography) is based on individualized dosage of FDG evaluated in 108 females with 166 suspected breast lesions.

Methods: PEM imaging of the breast was performed in 108 consecutive females with 166 suspicious breast lesions or known breast cancer (BC) 90 min after i.v. application of 3.5 MBq/kg F-18-FDG per kg body weight. The maximum PEM uptake value (PUV) was derived from a ROI around the target lesion and was correlated with a corresponding non-target ROI in the contra lateral healthy breast to determine the target/non-target ratio (PUV-ratio). Images were analyzed by 2 experienced readers independently as compared to histopathology in all cases. The between group analyses for all malignant, benign and corresponding non-target lesions were calculated by paired Student t-Test. The mean target/non-target ratio in patients with BC compared to healthy patients was calculated by independent Student t-Test. Significance level was considered at p value <0.05. Receiver operating characteristic (ROC) analyses were employed to determine associations with PUV and PUV-ratio.

Results: A total of 27 out of 166 (16.2%) lesions were malignant. Mean of PUV was estimated to be 3.9±2.5 in malignant lesions and 1.2±0.4 for the contra-lateral healthy breast (p<0.001). The mean PUV-ratio in patients with BC of 3.4±1.5 was significantly higher as compared to benign lesions 1.2±0.3 (p<0.001). The area under the ROC curve was 0.997 (0.000-1.000) for PUV and 0.986 (0.965-1.000) for PUV-ratio.

PEM was true-positive in 27 cases of cancers and false positive in 3 cases (papilloma, mastopathia bds.) considering a PUVmax > 1.9, resulting in sensitivity of 100%, specificity of 97%, positive predictive value of 90%, and a negative predictive value of 100%.

Conclusion: The different European approach in PEM using personalized FDG-dosage enables the comparability of FDG-metabolism in patients, resulting in a high diagnostic accuracy of PEM tests.

Literature:
Title: Breast specific gamma imaging (BSGI) and breast magnetic resonance imaging (MRI): Comparison of sensitivity and specificity in women prior to breast biopsy with BIRADS 4 or 5 finding on mammography in a community setting

Alison K Conlin1, Nicole Moxon1, Helena Hoen1, Christina Gougoutas-Fox1, Maureen O Baxter1, Amy Weinstein1, Maritza Martel1, Tracy L Kelly1 and Walter J Urba1. 1Providence Cancer Center, Portland, OR.

Body: Background: Diagnostic imaging following a new diagnosis of breast cancer remains an active area of research balancing value and outcomes. Decisions about surgical options and neo-adjuvant therapy depend greatly on the accuracy of these pre-operative assessments. BMRI use has increased tenfold from 2000 to 2011 (Stout et al, JAMA 2013) and estimation of sensitivity has been high but specificity has varied between 30-80%(Bluemke et al, JAMA 2004). BSGI is a novel molecular imaging technique that uses a gamma camera to track the uptake of a radio tracer (technitium Tc99m sestamibi) by breast cancer cells and has been used interchangeably with BMRI without rigorous evidence of equivalency (Khalkhaulili, et al, J Nuc Med 2000).The majority of research into the sensitivity and specificity of these tests has been retrospective, only on women with known cancer, and potentially biased by post-biopsy changes to breast tissue.

Methods: Therefore we performed a prospective study employing both techniques to image women with BIRADS 4 or 5 lesions on diagnostic mammogram prior to their planned breast biopsy. The BSGI and BMRI were reviewed by one of three dedicated breast radiologists and the pathology was reviewed on the biopsy or any additional biopsy/excision by one pathologist. We compared the BSGI and BMRI against the final pathology for sensitivity and specificity. In addition, we surveyed the women for quality of life measures 3 months later.

Results: Between January 2012 and April 2014 we enrolled 74 women (ages 30-80) at 2 NAPBC accredited breast centers located in a community based setting in Portland, OR. The initial diagnostic mammographic studies resulted in 23 women (32%) with BIRADS 4A, 27 (37%) with BIRADS 4B, 8 women with 4C (11%) while 8 women (11%) had BIRADS 5 lesions prompting biopsy. All women were biopsied and 27 (37%) were found to have an invasive or in situ cancer while 5 (7%) had atypical hyperplasia or LCIS found. Sixteen women had additional biopsies performed, outside of the planned area, as a result of BMRI or BSGI, 11 (69%) were based on BMRI findings and 5 (31%) were areas seen on both BMRI and BSGI. In these additional biopsies 5 were in situ or invasive cancer and 2 were contra-lateral cancers, the rest were benign tissue. The sensitivity of BMRI was 84.0% and BSGI was 74.1%. The specificity was found to be 57.8% and 80.4% respectively. One patient withdrew and 3 women did not complete BMRI due to claustrophobia or body habitus.

Quality of life data is still being analyzed.

Conclusions: We report here the sensitivity and specificity of BMRI compared with BSGI in women with BIRADS 4 and 5 breast lesions on diagnostic mammography. Importantly imaging was done before biopsy and therefore not biased by any effect from that procedure. In this study we find BMRI appears to have better sensitivity but lower specificity than BSGI. We also observed that the use of BMRI and/or BSGI prompted 16 extra biopsies of which less than half were additional or contra-lateral cancer. The incorporation of these tests into the evaluation of suspected cancer should consider these findings as well as cost and quality of life.
**Title:** The spectroscopic feature of breast cancer

Hiroyuki Ogura¹, Nobuko Yoshizawa², Kenji Yoshimoto³, Hatsuko Nasu², Yumiko Taki¹, Youko Hosokawa¹, Ryouichi Matsunuma¹, Yoshimi Ide¹, Etsuko Yamaki³, Toshihiko Suzuki³, Motoki Oda¹, Yukio Ueda³, Yutaka Yamashita³ and Harumi Sakahara². ¹Breast Surgery, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; ²Central Research Laboratory, Hamamatsu Photonics K.K., Hamamatsu, Shizuoka, Japan and ³Radiology, Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan.

**Body:** Objectives: To examine optical properties of breast cancer by time-resolved spectroscopy.

Materials and Methods: We irradiated a pulsed laser of 760, 800, and 830 nm wave-length lights at multiple sites of both breasts including the site just above the cancer and detected the light transmitted through the breast with TRS-20SH (Hamamatsu Photonics K.K.). Absorption coefficient (\(\mu_a\)), reduced scattering coefficient (\(\mu_s'\)), total hemoglobin (tHb), and oxygen saturation (SO2) of the breast were calculated by photon diffusion equation. The clinical trial started in January 2007. A total of one hundred fifty-two breast cancer patients participated in the trial and written informed consent were obtained from all of the patients.

Results: The absorption coefficient (\(\mu_a\)) in 760, 800 and 830 nm wave-length of breast cancer tissue was significantly high compared with contra-lateral normal breast (760nm: cancer;0.078, normal;0.063, p<0.001. 800nm cancer:0.071, normal;0.050, p<0.001. 830nm: cancer;0.084, normal;0.063, p<0.001).

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<th>(\mu_a) ((/\text{cm}))</th>
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<tr>
<td>cancer</td>
<td>0.078</td>
<td>9.58</td>
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There was no difference in reduced scattering coefficient (\(\mu_s'\)) between breast cancer tissue and contra-lateral normal breast(760nm: cancer;9.58, normal;9.71, 800nm: cancer;9.23, normal;9.40. 830nm; cancer;9.07, normal;9.22). The tHb of breast cancer tissue was significantly high, compared with normal breast (cancer:32.3±14.6, normal breast:22.0±8.6, p=0.001). There was no difference in oxygen saturation (SO2) between breast cancer tissue and contra-lateral normal breast (cancer:73.2±4.3, normal breast;73.6±5.9, p=0.31).

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<td>tHb((\mu\text{M}))</td>
<td>32.3±14.6</td>
<td>22.0±8.6</td>
<td>p&lt;0.001</td>
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<td>SO2(%)</td>
<td>73.2±4.3</td>
<td>73.6±5.9</td>
<td>p=0.32</td>
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Conclusion: Absorption coefficient (\(\mu_a\)) and tHb increased in breast cancer, whereas reduced scattering coefficient (\(\mu_s'\)) and oxygen saturation did not.
Title: Visualisation of histologic proven breast cancer on the MAMMI-PET: A dedicated PET for hanging breast imaging

Suzana C Teixeira¹, José Ferrer Rebolleda², Bas B Koolen¹, Raúl Sánchez Jurado², Marcel P Stokkel¹, María del Puig Córzar Santiago², Emiel J Th Rutgers¹ and Renato A Valdés-Olmos¹. ¹Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands and ²ERESA, General University Hospital of Valencia, Valencia, Spain.

Body: Aim: The MAMMI–PET, a high–resolution full–ring system for dedicated hanging breast imaging was originally developed in the context of a EU–founded project to improve the detection of breast cancer. The aim of the present study was to evaluate the performance of the MAMMI–PET device in patients with at least one histologic confirmed primary breast cancer lesion (or index lesion), scanned in two European centres. All patients were included in the study after being scheduled to receive pre–operative chemotherapy (NAC) or radiotherapy.

Material and methods: From March 2011 to March 2014, we included 234 female patients (mean age 52 y, range 24–82y) with histologically confirmed breast cancer. All patients were scanned with the MAMMI–PET (Oncovision, Valencia, Spain) after giving informed consent. Scans were acquired 110 min after a dose of a mean dose of 197.12 MBq¹⁸F–FDG. In both centers the acquisitions, the reconstruction of the images and the data collection were performed using similar standardized methods. We tested the relation between visualization of the primary tumor and possible additional lesions on the MAMMI–PET as well as the influence of various variables; including age, weight, breast cancer subtypes and receptor status, breast length, maximal tumor diameter and affected breast quadrants.

Results: A total of 236 breasts were imaged and 211 (98.4%) of the index lesions (diameter 5–170 mm, mean 32 mm) were located within the MAMMI–PET scanning range. Of all index lesions within the scanning range 1.4% was not FDG avid on the MAMMI–PET images. Lesions that were FDG-avid were either clearly (86.3%) or moderately (12.3%) visible. The overall MAMMI–PET sensitivity increased from 88.6% to 98.6% after exclusion of lesions outside the scanning range. No significant differences in lesion visibility were found due to breast cancer subtypes or breast quadrant location. Of the 35 index lesions touching the pectoral muscle 62.9% reached into the scanning range. A total of 41 additional FDG-avid lesions were detected, not categorized as an index tumor.

Conclusions: The MAMMI–PET missed only a small percentage of malignant lesions located within the scanning range of the device. Lesions near the pectoral muscle were the subgroup less often visualized. No significant influence on the visualization of the FDG avid lesions was seen due to tumor subgroups, hormone receptor status, and breast quadrant location or tumor size.
Title: The uptake of 18F-fluorodeoxyglucose (18F-FDG) by normal breast tissues as measured by a dedicated breast positron emission tomography (PET) scanner: A preliminary study

Sze Yiun Teo¹, Jasper Chaal², Jung Ah Lee¹ and Michel Herranz³. ¹KK Women's and Children's Hospital, Singapore; ²Agency for Science, Technology and Research, Singapore and ³Complexo Hospitalario Universitario Santiago de Compostela, Spain.

Body: Introduction
Breast positron emission tomography (PET) is a molecular imaging method which identifies breast cancers with high sensitivity and specificity. Dedicated breast PET scanners have an improved spatial resolution compared to whole body PET scanners, and are able to differentiate between glandular and fat tissues within the breast. The aim of this study is to determine how normal breast tissues take up 18F-FDG with time.

Method
In this IRB-approved prospective study, patients with a newly diagnosed invasive breast cancer who desired breast conservation surgery were recruited. Participants underwent bilateral breast PET scans using a two-ring dedicated breast PET scanner (MAMMI breast PET, Oncovision, GEM Imaging S.A.) after receiving a standard 18F-FDG dose of 5 mCi. After an uptake phase of 60 minutes, all patients were scanned at 3 time points: at 60 minutes, 90 minutes and 120 minutes post-injection. Reconstructed images obtained on the MAMMI breast PET scanner were then read and processed using the OsiriX software. For each scan performed at each time point, a series of continuous axial images through the central portion of the breast containing the nipple as a landmark was identified. Two elliptical 1cm² regions of interest (ROI) were drawn, one on the central portion of the glandular parenchyma and a second on the fat. This was propagated through the included slices, after ensuring that the ROI lay entirely within the desired component of the breast in the same region for each breast. In the ipsilateral breast, the quadrant opposite the index tumor was used to avoid contamination. The mean SUV values for breast parenchyma and breast fat were measured, compared to the mammographic density of the breast tissues and tabulated against time.

Results
9 patients participated in the study, yielding a total of 54 breast PET scans for analysis (bilateral scans for each patient at 3 time points). The mean SUV measurements for breast parenchyma ranged from 0.7 to 2.8 and the mean SUV measurements for breast fat ranged from 0.1 to 0.8. The mean SUV of breast parenchyma was approximately 3 to 4 times that of the mean SUV of breast fat at each time point. There was a gradual increase in the mean SUV of breast parenchyma and breast fat with time, although the increase appeared more pronounced with breast parenchyma. Patients with dense breast tissues tended to have a higher parenchymal mean SUV value.

Conclusion
Normal breast parenchyma demonstrates a greater uptake of 18F-FDG compared to breast fat tissues at a ratio of approximately 3-4 to 1. There is accumulation of 18F-FDG with time in both parenchymal and fatty tissues within the breast, more so with the former. Knowledge of the background SUV measurement of parenchymal tissues is important as this affects the SUV measurement of the underlying breast cancer. Increasing accumulation of FDG with time may decrease the contrast resolution of a cancer, especially in patients with dense breast parenchyma.
Title: Diagnostic performance of preoperative 18F-FDG PET/CT in predicting pathologic tumor response after neoadjuvant chemotherapy for patients with breast cancer

Wesley P Andrade¹, Eduardo N Lima¹, Fernando A Soares¹, Cynthia B Toledo¹, Maurício Doi¹, Hirofumi Iyeyasu¹, Alessandro Lima¹, Renato Cagnacci¹, Tadeu F Paiva¹ and Glauco Baiocchi¹. ¹A.C.Camargo Cancer Center, São Paulo, Brazil.

Body: Introduction: Breast cancer is the most common malignant neoplasm in women, and its treatment is based on surgery even for advanced disease. The best strategy for patients with locally advanced breast cancer (LABC) and for those with unfavorable tumor/breast size index is to begin the treatment with neoadjuvant chemotherapy (NAC) with the objective of further breast conservative surgery, measure in vivo response to chemotherapy, and potentially treat micrometastatic disease. A 65% complete pathologic response rate (pCR) can be obtained after recent improvement in NAC efficacy based on new regimens. The progress in pCR rates may theoretically help to select a group of patients that do not require surgery, since their tumors have been completely eradicated by NAC. The present challenge is to develop of a diagnostic tool capable to precisely predict pCR after NAC and further omit surgical treatment. In this scenario, PET/CT is a nearly recent imaging tool that should be tested after NAC. Theoretically, a negative PET/CT after NAC should correspond also to pCR. Objective: The aim of our study was to analyze the role of PET/CT after NAC as a method to predict pathologic response for patients with breast cancer and correlate its result with the other pathologic variables in the surgical specimen. Patients and Methods: We performed a prospective study that included 73 patients with either LABC or unfavorable tumor/breast size index that were submitted to NAC followed by surgery. The surgical specimens were evaluated with TNM and RCB (Residual Cancer Burden) protocols. Results: Between February 2010 and June 2013, 73 patients entered the protocol. Median age was 41 years (range, 26-76) and median primary tumor size was 55mm (range, 21-200). According to TNM criteria, complete clinical complete response (cCR) was 45.2% (33/73), metabolic complete response (mCR) 61.6% (45/73), and pCR (ypT0 ypN0) was 27.4% (20/73). We correlated the PET/CT results with pCR (ypT0 ypN0) and still found a 62% rate of residual tumor in 45 patients that had mCR, with sensibility of 85%, specificity of 47%, positive predictive value (PPV) of 38%, negative predictive value (NPV) of 89%, and accuracy of 58% in predicting ypT0ypN0. Seventy patients were suitable to RCB protocol, and we found RCB 0 (ypT0-Tis ypN0) in 38.6% (27/70) of cases, RCB I in 4.3% (3/70), RCB II in 30% (21/70), and RCB 3 in 27.1% (19/70). We correlated the PET/CT results with RCB 0 and still found a 49% rate of invasive residual tumor in 43 patients that had mCR, with sensibility of 81%, specificity of 51%, PPV of 51%, NPV of 89%, and accuracy of 63%. Conclusion: PET/CT use after NAC in breast cancer treatment was not able to accurately predict the absence of residual tumor (complete pathological response). We found high false positive response rate, where even after a negative PET/CT performed after NAC, 62% patients still had residual tumor according to TNM criteria.
Title: Comparison of ESR1, PGR, HER2 and Ki67 expression by central IHC and MammaTyper® RT-qPCR kit in the FinHer trial

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Body: Background: Subtyping of breast cancer has become an integral part of standard evaluation of breast cancer patients1. However, assaying of ER, PgR and Her-2/neu by immunohistochemistry (IHC) carries an up to 20% risk of erroneous results2,3. Moreover, reliable assessment of Ki-67 by IHC in grade 2 breast cancer is challenging due to high intra- and interobserver variations4,5. Interobserver concordance for ESR1, PGR, HER2, and Ki67 determination on mRNA level using MammaTyper® reagents were 96.8%, 97.2%, 100%, 97.6%, respectively, based on predefined cutoffs (Laible et al., abstract submitted). Here we tested the clinical concordance and prognostic value of MammaTyper® determinations in the FinHer trial patient population.

Methods: RNA was extracted from formalin-fixed paraffin-embedded (FFPE) breast tumor tissue, and candidate gene expression was analyzed using the RNXtract® IVD and MammaTyper® IVD kits (BioNTech Diagnostics GmbH, Mainz) from 791 patients who participated in the FinHer trial. RNA levels of ESR1, PGR, HER2, and Ki67 mRNA expression were measured using RT-qPCR, normalized according to the 40-DDCT method and compared with IHC or CISH results. Associations with distant disease-free survival (DDFS) and overall survival (OS) were assessed using the log-rank test.

Results: ESR1, PGR, HER2, and Ki67 mRNA levels exhibited strong correlations with the respective clinical assays (each p-value <0.0001). The concordance rate of the mRNA assay and the clinical assay was 92% for ESR1, 92% for HER2, 83% for PGR, and 68% for Ki67 RNA. Using predefined cut-off values for ESR1, PGR, and Ki67, the mRNA levels were prognostic for DDFS (p=0.002, p=0.005, and p=0.0005) and OS (p<0.0001, p=0.0001, and p=0.0024, respectively), whereas HER2 mRNA expression was not (p=0.17 and 0.11, respectively). Unexpectedly, the HER2 mRNA expression distribution of the ER-negative cancers was bimodal with little overlap between the HER2-low and HER2-high subsets, while in ER-positive cancers HER2 mRNA distribution was almost unimodal and in between the two subpopulations of ER-negative cancers. When different cut-offs were used for ER-positive and ER-negative cancers, tumor HER2 mRNA was also significantly associated with DDFS (p=0.031) and OS (p=0.018). Interestingly, 17% of ESR1 mRNA-negative and HER2 mRNA-negative patients exhibited high mRNA expression of PGR, and such patients had high 5-yr DDFS and OS (>95%).

Conclusions: Determination of ESR1, PGR, HER2, and Ki67 without macrodissection of routine whole tissue FFPE specimens results in highly concordant results when compared to clinical assays. A significant minority of HER2 negative breast cancers expressed PGR mRNA despite low ESR1 mRNA levels and had superior outcome. HER2 mRNA levels differed substantially between ER-positive and ER negative tumors. This may explain why HER2 determination using a single cut-off for HER2 mRNA with the Oncotype DX assay frequently results in a false negative finding6. The MammaTyper-defined ESR1, PGR, HER2, and Ki67 expression showed strong correlations with the corresponding clinical assays and survival.

1) Goldhirsch et al., Annals of Oncology 2013
2) Hammond et al., JCO 2010
3) Wolff et al., JCO 2007
4) Varga et al., PloS 2012
5) Polley et al., JNCI 2013.
Title: Central testing of ER, PR, HER2, and Ki67, routinely analyzed at 27 pathology departments in Sweden – Potential consequences for the choice of adjuvant therapy

Maria Ekholm\textsuperscript{1,2}, Dorthe Grabau\textsuperscript{2,3}, Per-Ola Bendahl\textsuperscript{2}, Göran Elmberger\textsuperscript{4}, Hans Olsson\textsuperscript{5}, Leila Russo\textsuperscript{6}, Guiseppe Viale\textsuperscript{6} and Mårten Fernö\textsuperscript{2}.\textsuperscript{1}Ryhov County Hospital, Jönköping, Sweden; \textsuperscript{2}Division of Oncology and Pathology, Lund University, Lund, Sweden; \textsuperscript{3}Skåne University Hospital, Lund, Lund, Sweden; \textsuperscript{4}Örebro University Hospital, Örebro, Sweden; \textsuperscript{5}Linköping University Hospital, Linköping, Sweden and \textsuperscript{6}European Institute of Oncology, University of Milan, Milan, Italy.

Body: Background

Immunohistochemical (IHC) techniques are still gold standard for measurement of estrogen receptors (ER), progesterone receptors (PR), the proliferation marker Ki67, and human epidermal growth factor receptor 2 (HER2) (confirmation with in situ hybridization for 2+) in routine breast cancer pathology. These biomarkers are also included in the St Gallen 2013 four-marker panel, as a surrogate for the intrinsic subtype classification; Luminal A-like, Luminal B-like (HER2-), Luminal B-like (HER2+), HER2+ (non-luminal), and Triple negative. Correct analyses are of outmost importance since these biomarkers are underlying treatment decisions in breast cancer. It is mandatory for departments performing breast cancer pathology to participate in quality assurance programs.

Aims

In 2013, SweQA-breast cancer (Swedish Quality Assurance) performed a study to investigate the agreement of ER-, PR-, Ki67-, and HER2-scoring between pathology departments in Sweden and a central reference laboratory (European Institute of Oncology (EIO), Milan, Italy). To explore the clinical consequences, the aim was also to investigate to what extent the central testing would potentially have altered the adjuvant medical therapy.

Methods

Twenty-seven of the 31 pathology departments in Sweden participated by collecting the first breast carcinoma diagnosed after the 15th each month during 2012, except for July and December (n=270). Paraffin embedded tumor tissue were collected and sent to EIO, where new sections, stainings, and evaluations were performed. These results were compared with the results from the routine analyzes performed in Sweden. Expression scores of ER, PR, Ki67, and HER2 were dichotomized according to predefined thresholds from the St Gallen consensus statement of 2013 (Goldhirsch et al. Ann Oncol 24(9);2206-13;2013).

Results

The pairwise agreement figures for ER, PR, and Ki67 were; 99\% (kappa-value ($\kappa$)=0.95), 95\% ($\kappa$=0.84), and 85\% ($\kappa$=0.70) respectively. Eight of the PR discrepant cases were locally positive but centrally negative, whereas six showed the opposite pattern. In 34 of the 39 discrepant cases for Ki67, one or both assessments were located near cut-off (15-25\%). When categorizing HER2 as 0/1+ vs. 2+/3+, agreement was 86\% ($\kappa$=0.65). The HER2-IHC 2+/3+ cases in Sweden were further analyzed with in situ hybridization as part of clinical routine, and when these results were used to obtain the final HER2 status, only 1 discrepant case of 265 evaluable cases was observed. When using the St Gallen 2013 surrogate definition for the five intrinsic subtypes, the overall pairwise agreement between Sweden and Italy, for all tumors with complete data (n=256), was 86\% ($\kappa$=0.78).

Conclusions

The agreement was very good ($\kappa$>0.80) or good ($\kappa$ 0.61-0.80) for all four biomarkers (ER, PR, Ki67, and HER2). Almost 90\% of the discrepant cases for Ki67 were explained by values near cut-off. When strictly applying the St Gallen 2013 surrogate definition of the five intrinsic subtypes, without regard to TNM stage, the results of the central testing would potentially have altered the medical adjuvant treatment for 37 of the 256 patients (14\%). For 28 of these 37 patients, the discordance was explained by Ki67.
**Title:** A comparison of the hot spot and the average cancer cell counting methods and the optimal cut-off point of the Ki-67 index for luminal type breast cancer with or without recurrence – A case control study for prognostic factors

Nobuyuki Arima¹, Reiki Nishimura¹, Tomofumi Osako¹, Yasuyuki Nishiyama¹, Yasuhiro Okumura¹, Masahiro Nakano¹, Mamiko Fujisue¹, Rumiko Tashima¹ and Yasuo Toyozumi¹. ¹Kumamoto City Hospital, Kumamoto, Japan.

**Body: Background:** Uncontrolled proliferation is a key factor of malignant tumors. The Ki-67 index is known to be a significant prognostic factor in terms of disease-free and overall survival in breast cancer. However, there are several limitations in the use of this biomarker, the biggest being a difference in opinion among breast cancer specialist on how to create an international standard for the Ki-67 index. Therefore, the aim of this case control study was to investigate the most suitable area to count and to determine the optimal cut-off point of the Ki-67 index for a more accurate prognosis.

**Patients and Methods:**
Thirty recurrent cases (< 5 years after initial treatment) were selected among hormone receptor (HR)-positive/HER2-negative breast cancer patients. As a control, 90 non-recurrent cases (>5 years after initial treatment) were randomly selected by allotting 3 controls to each recurrent case based on the following predetermined criteria; age, nodal status and tumor size. All patients were treated with adjuvant endocrine therapy alone. Both the hot spot and the average area of the tumor were evaluated on a Ki-67 immunostaining slide and then photographs were taken. The Ki-67 index was automatically scored using the CountoCell (Seiko Tech., Fukuoka, Japan). Moreover, the difference in the Ki-67 index values (ΔKi-67) between the hot spot and the average area were calculated. The Chi-square and Fisher's exact tests were used for inter-group comparison. Paired t-test and Wilcoxon's (non-parametric) test were used to compare the mean value for the Ki-67 index values. Logistic regression analysis was used to calculate the odds ratio of the Ki-67 index related to recurrence.

**Results:**
1) A higher Ki-67 index value at the hot spot was more significantly associated with recurrence than the average area, and the Ki-67 index value of 20% at the hot spot was the most suitable cut-off point for predicting recurrence. Irrespective of the area counted, the Ki-67 index value was significantly higher in all of the recurrent cases (p<0.0001). The next step was to determine the most suitable cut-off point for the Ki-67 index. Out of all the cut-off points used in this study, the most significant difference was found in the values 20, 25 and 30% at only the hot spot (each p<0.0001). Furthermore, logistic regression analysis demonstrated that the highest odds ratio was 20% at the hot spot.

2. The ΔKi-67 index value was significantly correlated with recurrence and the ΔKi-67 index value of 10% was the most suitable cut-off point.
The ΔKi-67 index values ranged from 2 to 54% (median: 10%) and significantly correlated with recurrence and the Ki-67 value at hot spot (p<0.0001). Logistic regression analysis revealed that a ΔKi-67 index cut-off point of 10%.

**Conclusion:**
A higher Ki-67 index value at the hot spot was strongly correlated with recurrence, and the most suitable cut-off point was 20%. ΔKi-67 index value also significantly correlated with recurrence, and the most suitable cut-off point was 10%. Finally, the hot spot counting method is strongly related to tumor biology and prognosis in HR-positive/HER2-negative breast cancer.
Title: Tumor heterogeneity impairs robustness of Ki67 scoring in breast cancer

Matthijs V Nijenhuis1, Emilie Groen1, Tim J Dekker2, Caroline A Drukker1, J Sanders1, V T Smit2, S Linn1, E J Rutgers1 and J Wesseling1. 1Antoni van Leeuwenhoek and 2Leiden University Medical Center, Leiden, Netherlands.

Body: Background
A study by Cheang et al. reported that for ER-positive, HER2-negative breast cancer a Ki67 score $\geq$14% distinguishes Luminal B from Luminal A tumors. The (neo)adjuvant treatment of these Luminal B tumors consists of endocrine treatment and chemotherapy while chemotherapy can be omitted for Luminal A tumors. For the determination of these subtypes, resection specimens and particularly core biopsies in the neoadjuvant setting are used. Since Ki67 scoring is advocated by some to be a marker in deciding if systemic chemotherapy will be given or not, its assessment should be highly reproducible and reliable. I.e, Ki67 assessment should not vary significantly between different laboratories, but also not within the tumor. To test this, we analyzed (1) interlaboratory agreement using whole slides and (2) agreement within a particular tumor using tissue microarrays (TMA) as well as whole slides from surgical specimens.

Material and methods
The first 100 patients from the microarray prognoSTics-in-breast-cancER (RASTER) study (n=427) were selected. From each patient clinicopathological characteristics and tumor blocks were available. From each tumor block 2 whole slides were cut for staining in laboratory 1 and 2. Also six single cores were taken to construct 2 tissue microarrays (core 1-3 and core 4-6). Intrinsic subtypes were defined as follows: Luminal A, Luminal B/HER2-, Luminal B/HER2+, HER2-overexpressing, and Basal-like. Experienced pathologists (EG, JS, VTHMBS, JW) performed the scoring.

Results
There were 99 whole slides suitable for analysis. Substantial agreement ($\kappa = 0.723$, P<0.001) was demonstrated in discrimination for Ki67 low and high between whole slides stained in laboratory 1 and laboratory 2 with a discordance rate of 13.1% (Table 1). Moderate agreement ($\kappa = 0.595$, $\kappa = 0.584$, P<0.001) was demonstrated in discrimination of Ki67 low and high between whole slide and core 1-3 and core 4-6 with a discordance rate of 20.3% and 20.8% respectively (Table 2). Substantial agreement ($\kappa = 0.666$) was demonstrated for Ki67 expression between core 1-3 and core 4-6.

Table 1. Discordance between laboratories for whole slide Ki67 result

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>Laboratory 1</td>
<td>&lt;14%</td>
<td>$\geq$14%</td>
<td>Discordance rate %</td>
<td>Kappa</td>
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<td></td>
<td>&lt;14%</td>
<td>56</td>
<td>0</td>
<td>13.1</td>
<td>0.723</td>
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<tr>
<td></td>
<td>$\geq$14%</td>
<td>13</td>
<td>30</td>
<td></td>
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</tbody>
</table>

Table 2. Discordance between whole slide and core biopsies 1-3 and 4-6 for Ki67 result

<table>
<thead>
<tr>
<th></th>
<th>Whole slide</th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Core 1-3</td>
<td>&lt;14%</td>
<td>$\geq$14%</td>
<td>Discordance rate %</td>
<td>Kappa</td>
</tr>
<tr>
<td></td>
<td>&lt;14%</td>
<td>33</td>
<td>9</td>
<td>20.3</td>
<td>0.595</td>
</tr>
<tr>
<td></td>
<td>$\geq$14%</td>
<td>7</td>
<td>30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Core 4-6</td>
<td>&lt;14%</td>
<td>$\geq$14%</td>
<td>Discordance rate %</td>
<td>Kappa</td>
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<tr>
<td></td>
<td>&lt;14%</td>
<td>31</td>
<td>8</td>
<td>20.8</td>
<td>0.584</td>
</tr>
</tbody>
</table>


**Conclusion**

Tumor heterogeneity results in substantial variation of Ki67 scores between TMA cores and whole slides that may result in considerable differences in distinguishing luminal A from luminal B ER-positive, HER2-negative tumors. This may have far reaching consequences for the choice for (neo)adjuvant treatment.
Title: Assessment of HER2 status in invasive breast cancer with increased centromere 17 copy number by fluorescence in situ hybridization

Min Hye Jang¹, Eun Joo Kim¹, Hyun Jeong Kim¹ and So Yeon Park¹,². ¹Seoul National University Bundang Hospital, Korea and ²Seoul National University College of Medicine, Seoul, Korea.

Body: Background: A subset of breast cancers can show increased copy numbers of chromosome 17 centromere (CEP17) by in situ hybridization (ISH). However, recent studies have revealed that true polysomy 17 is a rare event in breast cancer, and increased copy number of CEP17 represents amplification or coly number gain in the centeromeric region. In this situation, the utility of CEP17 in ISH is very limited and thus, alternative methods for precise assessment of HER2 status are necessary. ISH using probes of other genes on chromosome 17 as additional reference genes has been proposed by 2013 ASCO/CAP guideline and several previous studies. In this study, we applied this method to breast cancers with increased CEP17 copy number (≥2.6), and compared it with conventional method based on ASCO/CAP guideline 2007.

Methods: After reviewing all HER2 fluorescence in situ hybridization (FISH) reports based on HER2/CEP17 ratio, documented from June 2004 to December 2011 at our institution, we identified 300 cases (29.6%) with ≥2.6 CEP17 copy number from 1013 breast cancers. We performed FISH with probes for RARA, SMS, TP53 genes on 243 breast cancers with available tissue blacks, using tissue microarrays. If one or more genes showed < 2.6 copy number, the largest number of them was chosen for alternative to CEP17 and re-graded the HER2 status based on HER2: alternative gene ratio.

Results: Of 243 breast cancers with ≥2.6 CEP17 copy number, 2 cases showed ≥2.6 copy numbers in all RARA, SMS and TP53 genes. Of 151 breast cancers classified as non-amplified based on HER2:CEP17 ratio, 42 (27.8%) were re-graded as amplified after additional FISH studies, and 7 of 8 cases which had been classified as equivocal were re-graded as amplified. Of the 47 cases with mean HER2 copy number of 4 to 6, 30 (63.8%) were upgraded from non-amplified to amplified, and 5 (10.6%) were upgraded from equivocal to amplified. Two (1.9%) of 75 cases with <4 mean HER2 copy number were upgraded from non-amplified to amplified, and 24 (23.8%) were changed from non-amplified to equivocal. After re-grading, equivocal cases were significantly increased, compared to the original reports (14.1% vs. 3.3%). And the concordance between FISH and immunohistochemistry became poorer (kappa value: 0.390 vs. 0.624).

Conclusion: Using additional reference genes can be an effective way for accurate HER2 evaluation in breast cancer with increased CEP17 copy number. However, it still has some limitations. It can lead to over-grading of HER2 status and increase of the equivocal cases, when tumor has loss of reference genes. Additional study for searching most stable genes that barely show copy number alteration is needed to simplify the procedure and to increase accuracy.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-02-06
Average Grade: 4.60

Title: Digital quantification of estrogen receptor expression in normal breast in post-menopausal women with breast cancer and association with tumor subtypes

H Evin Gulbahce1,2, Cindy Blair3, Carol Sweeney4, Rachel Factor1,2 and Mohamed Salama1,2. 1University of Utah School of Medicine, Salt Lake City, UT; 2ARUP Laboratories, Salt Lake City, UT; 3University of Minnesota, Minneapolis, MN and 4University of Utah School of Medicine, Salt Lake City, UT.

Body: Background: ER expression in normal breast epithelium (NBR) is higher in women with a history of breast cancer (BC) compared to controls. In adjuvant setting, metanalysis showed effective Tamoxifen treatment was restricted to ER positive cancers. However, it is also known prophylactic oophorectomy (a form of estrogen suppression) significantly reduces the incidence of BC in BRCA1 carriers. This is in contrast with the 80% rate of ER negative tumors in BRCA1 patients. The aim of this study is to quantify ER expression in NBR away from tumor in women with BC and to correlate it with BC subtypes.

Methods: 204 consecutive patients were identified for whom NBR away from tumor was available. 27 reduction mammoplasty (RM) tissues from women with no history of BC were used as controls. Tissue microarrays were constructed and slides were stained with ER and scanned using Aperio XT Scan Scope. Normal terminal duct lobular epithelium was manually circled on scanned images and annotations were recorded in separate digital layers. ER staining was quantitated in marked areas of the electronic image using an optimized scoring nuclear IHC algorithm (Aperio technologies, Inc., Vista, CA). Clinical information and tumor characteristics (menopausal status, ER, HER2 expression, grade, size, number of positive nodes, stage) were recorded based on pathology reports and tumor registry data.

Results: The mean ER positivity in NBR was 16 ± 12.4 % (range: 0-5-5.7%) for all patients with BC, 20.8±13.9% for postmenopausal (n=74 ) and 13.7 ±10.9% for peri+ premenopausal (n=170) subgroups. ER positivity was higher in patients with BC compared to those undergoing RM with no history of BC. ER positivity in NBR did not vary by tumor size, positive lymph nodes status, tumor grade, or stage in post-menopausal, and pre + perimenopausal women. Older age at diagnosis was significantly associated (p<0.0001) with ER in NBR. In post- menopausal women ER expression in NBR was significantly higher in patients with ER negative or triple negative tumors.

<table>
<thead>
<tr>
<th>Tumor ER Status</th>
<th>Postmenopausal (N=74)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Tumor ER Status</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
</tr>
<tr>
<td>Positive</td>
<td>60</td>
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<tr>
<td>Tumor HER2 Status</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>57</td>
</tr>
<tr>
<td>Positive</td>
<td>8</td>
</tr>
<tr>
<td>Tumor Triple Negative</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>53</td>
</tr>
<tr>
<td>Yes</td>
<td>12</td>
</tr>
</tbody>
</table>

HER2 status was not known in some cases and was excluded from statistical calculations.

Conclusion: This computer assisted image analysis study confirms ER expression in NBR increases with age and menopausal status in women with BC. We report, for the first time, a significant association between ER expression in normal breast...
epithelium with ER negative and triple negative cancers in post-menopausal women. Our study suggests that ER expression in normal epithelium may play a role in development of hormone receptor negative breast cancers.
Title: Effect of the new 2013 ASCO / CAP guidelines on HER2 reporting

H Evin Gulbahce¹,², Rachel E Factor¹,², Erinn Downs-Kelly¹,², Margaret Coppin² and Katherine B Geiersbach¹,². ¹University of Utah School of Medicine, Salt Lake City, UT and ²ARUP Laboratories, Salt Lake City, UT.

Body: Introduction: In 2013 ASCO/CAP published new guidelines for HER2 testing to decrease false negatives. We retrospectively classified cases submitted to a national reference laboratory for HER2 testing using the new guidelines in order to 1) see the overall effect of the 2013 vs 2007 guidelines and 2) predict shifts in interpretation of different HER2 testing methods in the future.

M&M: Our laboratory offers HER2 immunohistochemistry (IHC) by HercepTest (Dako) or 4B5 (Ventana) and HER2 dual probe FISH (Abbott Molecular). HER2 IHC and/or FISH tests performed between 1/2010-8/2013, originally scored with the 2007 guidelines, were reclassified with the 2013 guidelines. For IHC, the guideline scores of 0, 1+, 2+ and 3+ were recorded along with intensity (weak, moderate, strong) and % circumferential staining. For FISH, the HER2 and CEP 17 copy number per cell, and HER2:CEP 17 ratio were recorded.

Results: 2358 samples submitted for HER2 FISH were available for review. Using the 2007 guidelines, 246 (10.4%) were amplified (Amp), 62 (2.7%) equivocal (Eq), and 2050 (86.9%) were non-amplified (NA). 29/62 (46.8%) FISH Eq cases had HER2:CEP17 ratio between 2.0-2.2, 1 (1.6%) had HER2 copy number >6 per cell. Therefore 30/2358 (1.3%) of all FISH tests previously classified as Eq would be reclassified as Amp following new guidelines increasing the overall FISH Amp rate by 12.5% to 11.7%. Over the same time period, 4043 HER2 IHC tests were performed using old guidelines 1097/4043 (27.1%) of which were Eq (2+). 731/1097 (66.6%) had reflex FISH requested on 2+ IHC cases. 24/731 (3.3%) did not have an interpretable FISH result. Using the 2007 guidelines, 49 (6.9%) of the 2+ cases were FISH Amp, 23 (3.3%) remained Eq, 635 (89.8%) were NA. Using the new guidelines, an additional 8 (1.1%) of these 707 reflex FISH would be classified as Amp (7 cases due to HER2:CEP17 ratio 2.0 to 2.2 and one case for HER2 copy number >6). Also 8 NA cases would be reclassified as Eq due to a HER2 copy number between 4 and 5.9. With the new guidelines, the overall FISH Amp rate identified from reflex 2+ IHC is 8.1%. 507/707 (71.7%) of HER2 Eq (2+) cases reflexed to FISH had 10-30% circumferential staining (8 strong; 136 moderate; 363 weak). HER2 Amp rates for these are shown in Table 1.

Conclusion: 2013 guidelines will identify a higher % (1.1% for FISH) of eligible patients for targeted therapy. In this series, 2+ IHC cases with reflex FISH testing also had higher Amp rates by 2013 guidelines (8.1% vs 6.9%). Evaluating these rates over time will help assess the effect of the change in the scoring criteria. Amp rates for Eq (2+) IHC cases with 10-30% strong and moderate membrane staining are similar, although only the former group is classified as positive (3+) following new guidelines and would not be retested by FISH. Cases with less than 30% membrane staining may represent a biological spectrum that is not well represented by current IHC testing guidelines, and FISH confirmation on these cases may be justified.
Title: Incidence of misattributed specimen provenance among surgical breast biopsies

Arthur G Lerner¹, Arla L Bush², Andrew S Kenler³, David D Dorfman⁴, Travis A Morgan², Beth Boyd¹, William E Burak¹ and Richard E Fine¹. ¹Advanced Breast Technologies Consulting LLC, Palm City, FL; ²Strand Diagnostics LLC, Indianapolis, IN; ³Bridgeport Hospital, Yale New Haven Health, Bridgeport, CT and ⁴Zwanger-Pesiri Radiology, Lindenhurst, NY.

Body: Background:
The medical literature reports on the diagnostic challenges posed by tissue contamination and transposition among surgical biopsy specimens. These specimen provenance complications (SPCs) can lead to a misdiagnosis of cancer, resulting in unnecessary surgery and a potential delayed diagnosis. The histopathology process involves many manual steps during which specimens must be estranged from their identification, and provenance errors are often invisible absent DNA analysis. Prostate biopsy is the setting in which specimen provenance has been studied, with complication rates reported in over 0.9% of positive diagnoses despite best efforts to minimize errors. Because the processing workflow is virtually identical for histopathology specimens of all types, it is expected that error rates among breast biopsy specimens are similar to prostate.

Methods:
We analyzed over 4200 patients diagnosed with breast cancer between February 2011 and April 2014. All biopsies were collected using a uniform best-practice protocol including forensic chain of custody principles, bar-coding of specimen containers, and collection of the patient’s reference DNA sample via buccal swab. After a pathologic diagnosis of breast cancer a portion of the diagnostic specimen was forwarded to an independent DNA laboratory (Strand Diagnostics, Indianapolis, IN) where genetic STR profiles were compared to the patient’s reference DNA to rule out the presence of undetected SPCs prior to therapy.

Results:
3,545 breast cancer cases from 7 practices contributing 100 or more cases each were examined (Fig.1). DNA testing revealed occult provenance complications in 16 cases (0.45%), of which 6 (0.17%) were a complete transposition with another patient and 10 (0.28%) reflected contamination of the specimen by tissue from one or more unidentified individuals. Four (57%) of the practices experienced at least one provenance error during the study period, with the highest error rate being 1.41% at one practice. Pathology was performed by 14 different laboratories, 6 (43%) of which were implicated in occult SPCs (Fig. 2). Finally, patients seen by 8 (13%) of the 61 physicians performing surgical biopsies in the cohort were subjects of occult specimen provenance errors.

Conclusions:
These data suggest that the incidence of SPCs among breast biopsies is comparable to that reported for prostate biopsies. The errors are distributed broadly across laboratories, practices, and physicians. Due to the potential clinical consequences for patients with undetected SPCs, and the medical malpractice implications, further study of the nature and economics of provenance complications in the breast biopsy setting is warranted.

Fig 1

<table>
<thead>
<tr>
<th>Practice</th>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Errors</td>
</tr>
<tr>
<td>Practice A</td>
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<td>4</td>
</tr>
<tr>
<td>Practice B</td>
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<td></td>
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<tr>
<td>Practice C</td>
<td>173</td>
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<td>Practice D</td>
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<tr>
<td>Practice F</td>
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<tr>
<td>Practice G</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>Type I Cases</td>
<td>Type I Errors</td>
</tr>
<tr>
<td>------------------</td>
<td>--------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Total</td>
<td>3,545</td>
<td>6</td>
</tr>
</tbody>
</table>

**Figure 1. SPCs by Practice**

Type I=Transpositions,
Type II=Contaminations

**Figure 2. SPCs by Pathology Laboratory**

Type I=Transpositions,
Type II=Contaminations

<table>
<thead>
<tr>
<th>Location</th>
<th>Cases</th>
<th>Type I Errors</th>
<th>Type II Errors</th>
<th>SPC Rate</th>
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<td>4</td>
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<tr>
<td>Lab B</td>
<td>967</td>
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<td>3</td>
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<tr>
<td>Lab C</td>
<td>430</td>
<td></td>
<td></td>
<td>0.00%</td>
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<td>Lab D</td>
<td>260</td>
<td>2</td>
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</tr>
<tr>
<td>Lab E</td>
<td>182</td>
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<tr>
<td>Lab F</td>
<td>84</td>
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<td>Lab G</td>
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<td>Lab H</td>
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</tr>
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<td>Lab I</td>
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<td>Lab J</td>
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</tr>
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<td>Lab K</td>
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<td>Lab M</td>
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<td></td>
<td>0.00%</td>
</tr>
<tr>
<td>Total</td>
<td>3,545</td>
<td>6</td>
<td>10</td>
<td>0.45%</td>
</tr>
</tbody>
</table>
Title: Ki67-labelling index of luminal breast cancers: Agreement of core biopsy and surgical specimen results

Cornelia M Focke¹ and Thomas Decker¹. ¹Dietrich Bonhoeffer Medical Center, Neubrandenburg, Germany.

Body: Objective: To highlight the agreement between Ki67-Labelling indices (LIs) of core biopsies (CB) and related surgical specimens (SSP) in luminal breast cancers (BCs) adopting the recommendations of the St Gallen Consensus Conference 2013 and the International Ki67 In Breast Cancer Working Group.

Methods: We investigated CBs and the related SSPs of 220 luminal Her2 negative BCs. To assess the Ki67-LI a total of 510 tumour cells including hot spot, cold spot and an area of intermediate proliferation was counted by one observer. Every positive nucleus was counted, independent of the staining intensity. A Ki67-LI cut-off <20% as recommended by the St Gallen Consensus was used to define luminal A BCs. Agreement between CB and SSP, rates of under- and overestimation, and positive predictive values (PPV) of CB based subtyping (luminal A vs. B) were calculated.

Results: In SSP 140/220 BCs (64%) were luminal A, 80 (36%) were luminal B. The agreement between CB and OP was 77% (169/220). Overestimation of proliferation in CB occurred in 10 (4%) and underestimation in 41 (19%) cases. The PPVs of CB based Ki67-LI for luminal A and luminal B were 0.76 (130/171) and 0.79 (39/49), respectively.

Conclusion: The agreement of CB and SSP based subtyping of luminal BCs is generally high. However, underestimation of proliferation in CB may result in subtype misclassification in almost one fifth of luminal Her2 negative luminal tumours.
Title: Factors influencing agreement of Ki67 labeling index between core needle biopsy and surgical resection specimens

Zsuzsanna Bago-Horvath¹, Fabian Roessler¹, Philipp Wimmer², Martina Mittlboeck², Nicolas Kozakowski¹, Katja Pinker-Domenig³, Rupert Bartsch¹, Peter Dubsky⁵, Martin Filipits⁶ and Margaretha Rudas¹. ¹Clinical Institute of Pathology, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; ²Informatics and Intelligent Systems, Section for Clinical Biometrics, Medical University of Vienna, Vienna, Austria; ³Division of Molecular and Gender Imaging, Medical University of Vienna, Vienna, Austria; ⁴Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria; ⁵Comprehensive Cancer Center, Medical University Vienna, Vienna, Austria and ⁶Institute of Cancer Research, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria.

Body: Reliable determination of Ki67 labeling index (Ki67 LI) on core needle biopsy specimens (CNB) is essential for determining breast cancer intrinsic subtype (IST), preoperative treatment decisions and short-term treatment response during neoadjuvant therapy. However, analyses investigating robustness of Ki67 LI assessment upon repeated tumor biopsies are scarce and results of studies investigating agreement of Ki67 LI between CNB and surgical resection (SR) specimens are conflicting. In the present study, we analysed the role of clinical and pathological factors in influencing concordance of Ki67 LI between CNB and SR specimens.

502 matched pairs of CNB and SR specimens of patients with invasive ER-positive, HER2-negative breast cancer were included in our study. Ki67 LI was determined according to recent recommendations. Luminal B disease was defined by Ki67 LI > 20% according to SR. Ki67 LI values were considered concordant by a divergence of 10% points. Agreement was calculated by Cohen’s kappa. Associations with clinicopathological factors were analyzed by Chi square test and logistic regression. Factors investigated included age, menopausal status, CNB method, BIRADS assessment category of imaging abnormality, time between CNB and SR, extent of surgery, histological grade (including individual grading score components according to Elston and Ellis), tumor size, lymph node stage, estrogen- (ER), progesterone- (PR) and HER2-receptor status.

A cutoff value of 20% for Ki67 LI in SR demonstrated a sensitivity of 90% and a specificity of 60% for identifying luminal B breast cancer in CNB. Upon investigating measurement agreement, we found substantial agreement of Ki67 LI between CNB and SR specimens with a weighted kappa value of 0.837. IST assessment in CNB and SR showed only moderate concordance with a kappa value of 0.587. Concordant diagnosis of IST in CNB and SR was significantly associated with PR expression and histological grade (p>0.05). Agreement of Ki67 LI was higher in tumors expressing low and high levels of PR compared to tumors with intermediate PR score. 27% and 22% of low and intermediate grade breast cancers showed discordant IST in CNB and SR, respectively. In contrast, only 3% of high grade breast cancers differed regarding IST upon repeated measurements. Interestingly, concordance of IST was significantly associated with all separate grading score components in CNB samples such as extent of glandular differentiation (p=0.015), nuclear polymorphism (p=0.005) and number of mitotic figures (p>0.001).

We conclude that agreement of breast cancer IST according to Ki67 LI between CNB and SR specimens is significantly influenced by histological tumor grade and PR status. These factors, including all three grading score components are likely to mirror tumor heterogeneity that might compromise obtaining a CNB sample representative of the entire tumor. Our results cast a doubt upon robustness of single CNB-driven measurements of prognostic indicators and outcome predictors in estrogen-receptor positive breast cancer of low or intermediate histological grade. Whether molecular testing of CNB specimens improves classification of luminal breast cancer and helps resolve diagnostic disparities remains to be determined.
Title: Triple negative breast cancer: The role of classic histological and prognostic factors on disease free survival

Ahmed Elkhanany¹, Vera J Suman¹, Victoria Cafourek¹, Judith A Gilbert¹, James N Ingle¹, Fergus Couch¹, Daniel W Vissscher¹ and Matthew P Goetz². ¹Mayo Clinic, Rochester, MN.

Body: Background: Triple negative breast cancer (TNBC) represents 15% of all breast cancers, and is characterized by an aggressive clinical course. While efforts are ongoing to characterize the molecular basis for variation in TNBC, there are conflicting data regarding the impact of prognostic factors such as age and Ki-67 typically used for treatment decisions in other breast cancer subtypes. We identified a retrospective cohort of women with ER- breast cancer with long term follow-up, and performed central confirmation of ER, PR, HER2, Ki-67 and histological classification to assess the association of Ki-67 and histologic subtype on clinical outcome in TNBC patients (pts).

Methods: 9,836 women who underwent breast cancer surgery at Mayo Clinic Rochester from 1985-2005 were identified. Pts were excluded due to: ER+ disease (7363); prior cancer (553); non-invasive disease (465); bilateral breast cancer (167); metastatic disease [at diagnosis or within 60 days of surgery (110)]; ER- disease treated with neoadjuvant chemotherapy (121) or adjuvant hormonal therapy (112); or tumor block exhausted (94). For all others, centralized ER/PR/HER2 identified the following for exclusion, >1% ER staining (n=76), > 1% PR (n=14) and HER2+ (n=144) by IHC (3+) or FISH amplification. 225 cases are still undergoing review. For the centrally confirmed pts with TNBC (n=392), Ki-67 and histological characterization (WHO subtypes) were assessed.

Results: Patient characteristics are listed in the table. The median age for all TNBC’s was 45 (range 29-88) and nearly all tumors were grade 3 (91%) with high proliferation (63% with Ki-67 > 30%). The median follow-up was >10 years, where 139 pts had a disease event: progression (86) or second primary (53) and 53 died without a disease event. The 10 yr DFS rate was 55.8% (95%CI: 48.8-63.9%) among the 238 pts who did not receive adjuvant chemotherapy (AdjCT) and 58.9% (95% CI: 48.9-70.9%) among the 102 pts administered AdjCT. In pts without adjCT, DFS was found to differ with respect to number of positive LNs (0 vs. 1-4 vs. 4+: log rank p=0.003) and medullary histology (log rank p=0.015) but not with age (< 50 vs. ≥ 50), tumor size (< 2.0 vs. 2-5 vs. ≥ 5.0 cm) or Ki-67 (0-15% vs. 15.1-30% vs. >30 %).

Conclusions: Our findings confirm the poor prognosis of TNBC. The medullary histological subtype is associated with significantly better prognosis than other TNBC subtypes (10 year DFS rate of 78.5%; 95%CI: 59.1-99.9% in pts not treated with adjCT). After central confirmation, neither age nor Ki-67 provide additional prognostic information.

<table>
<thead>
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<th>Patient characteristics</th>
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<th>(29-88)</th>
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<tbody>
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<tr>
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Title: Concordance between semiquantitative immunohistochemical assay and oncotype DX RT-PCR assay for estrogen and progesterone receptors

Patricia Novas¹, Elena Galve¹, Sara Fernández¹, Maria Ángeles Sala¹, Alberto Arévalo¹, Juan Arango¹, Borja López de San Vicente¹, Seila Fernández¹, Ane Zumárraga¹ and Purificación Martínez del Prado¹. 'Basurto University Hospital, Bilbao, Vizcaya, Spain.

Body: Introduction: Estrogen receptor (ER) and progesterone receptor (PR) expression are generally determined by semiquantitative immunohistochemistry (IHC) as recommended in the clinical practice guidelines of the American Society of Clinical Oncologists. This method may be subject to variability because of differences in fixation, ER antibody clones, as well as to immunostain interpretation and use of arbitrary cut-off points. Materials and methods: We identified 72 breast cancer cases at Basurto University Hospital from 1 September 2012 to 31 May 2014 that have been analyzed by Oncotype DX with reporting on ER/PR expression levels by RT-PCR (Genomic Health Inc, Redwood City, CA). According to the company’s report, a tumor is considered as ER positive with expression units of >=6.5 and PR positive with expression units of >=5.5. Oncotype DX is a commercial assay that predicts tumor recurrence in node-negative ER-positive breast cancers. It is a reverse transcription-polymerase chain reaction (RT-PCR) based assay that analyzes the expression of 21 genes (16 cancer-related and 5 control genes) to provide a distant disease recurrence score ranging from 0 to 100. At our institution, hormone receptors by IHC were analyzed on corresponding surgery breast specimens at the time of initial diagnosis. They were evaluated by IHC on formalin-fixed paraffin-embedded tissue, between 8-72 hours. ER was assessed using Roche antibody clone SP1 and PR was assessed using antibody clone 1E2. Percentage of positive nuclei was determined by visual microscopic estimation: <1 negative and ≥1 positive. We compared our semiquantitative method of reporting ER and PR status based on IHC to the RT-PCR hormone receptor results from Genomic Health. Results: There was 100% concordance between IHC and the RT-PCR assay for estrogen receptor status. PR findings between IHC and Oncotype Dx revealed a lower concordance (82%) than for ER. RT-PCR was negative in 10 (14%) cases in which IHC was positive. Pearson correlation coefficient for PR was 0.313. Although our results demonstrated high concordance between IHC and Oncotype Dx for ER, our data showed poor concordance for PR.
Title: Triple negative breast cancer, the impact of isotype-specific progesterone receptor antibodies on the diagnosis results

Jacques Bonneterre¹, Jacques Bosq², Charline Alleaume³, Erard Gilles⁴, Philippe Jamme¹ and Alexander Zukiwski⁵. ¹Centre Oscar Lambret, Lille, France; ²Gustave Roussy Institute, Villejuif, France; ³Biodoxis, Romainville, France; ⁴Invivis Pharmaceuticals, Bridgewater, NJ and ⁵Arno Therapeutics, Flemmington, NJ.

Body: Background: Given the poor therapeutic outcomes for triple negative breast cancer (TNBC), diagnostic accuracy is vital. In routine IHC testing, the progesterone receptor (PR) is determined using bispecific antibodies (Ab) that recognize epitopes common to PRA and PRB. PRA and PRB expression can be imbalanced in BC. Relative expression of the PR isotypes appears to be prognostic in BC evaluated with a bispecific Ab (Hopp 2004). Tumors can express PRA or PRB on different cells in the same tumor (Mote 2008), which differs from those expressing ERα (Zukiwski 2013). A true TN phenotype might escape detection with the use of one single Ab to detect both PRA and B epitopes, depending on sensitivity/specificity. This study evaluated the use of two isotype-specific PR Abs to fully characterize the PR status.

Methods: 83 dual ERα and HER2 negative archived BC specimens with clinical data were obtained from the Oscar Lambret Cancer Center, Lille, FR. IHC was performed using anti-PRA, anti-PRB, and the bispecific anti-PR Pg636 antibodies. PR tumor positivity was explored using 2 cut-offs, ≥1% or ≥5% stained tumor cells. PR positive tumors were defined as either PRA or PRB positive.

Results: For PR positive tumors with ≥1% positive cells, average PRA positivity was 41%, PRB was 38% (PRA vs PRB p = NS), and PRAB 3% (PRA vs PRAB = 0.001, PRB vs PR AB p = 0.0001). Using 1% as positivity cut off PRA and PRB were discordant in 14% of the cases, PRA and PR AB in 12%. PR B and PR AB were discordant in 2%. Discordance between PR positivity (either A or B) and PRAB positivity was 7% with no PR negative PRAB positive tumors i.e. no positivity was missed using two PR A and B antibodies while all 7% cases were missed by the PRAB antibody. Using 5% as a cut off, the discordance rate was 8% between PRA and PRB, 25% between PRA and PRAB and no PRA negative and PRAB positive case were found, and 26% between PRB and PRAB with no PRB negative PRAB positive cases either. PR positivity (A or B) was missed in 30% of the cases with a PRAB. Patients with tumors identified as PR positive (≥5%) using the isotype specific antibodies and PR negative with the PRAB antibody have a better prognosis (DFS).

Conclusion: In TNBC, there is a different staining pattern when using isotype-specific vs bispecific anti-PR anti-bodies. The average percent of positive tumors cells is substantially reduced when using a bispecific Ab as compared to isotype-specific AB. This translates into potential false negative PRAB testing which varies from 7% to 30% depending of the cut off criteria. TNBC reclassified by the use of isotype-specific anti-PR antibodies may be appropriate for investigation with anti-progestins.
Title: Breast cancer biology varies dramatically by method of detection and may account for overdiagnosis

Brandon Hayse¹, Prathima Kanumuri¹, Brigid K Killelea¹, Nina R Horowitz¹, Anees B Chagpar¹ and Donald R Lannin¹. ¹Yale University School of Medicine, New Haven, CT.

Body: Background: Epidemiological and clinical trial data suggest that 20%-30% of breast cancers diagnosed by screening represent overdiagnosis; that is cancers that, if left untreated, would never present symptomatically nor cause any harm to the patient later in life. The biology and presentation of these overdiagnosed cancers, however, is not well understood.

Methods: A retrospective review was performed of a prospectively collected database of breast cancers diagnosed at a tertiary academic medical center from 2004-2013. The mode of initial presentation was categorized into five separate groups according to the abnormality that first precipitated a breast workup: screening mammogram, screening MRI, screening ultrasound, self-detected masses, and physician detected masses. The relationship between tumor characteristics and mode of initial presentation was evaluated using bivariate analysis and multivariate logistic regression.

Results: The table shows data for a total of 2,714 cases. As expected, screen-detected cancers were significantly smaller than cancers found by the patient or the physician, and included a higher percent of T1 cancers, whereas palpable cancers had a larger percent of T2 and T3 cancers (p< 0.001). Also not surprisingly, screening modalities detected a higher rate of DCIS compared to symptomatic presentation (p < 0.001). However in addition to a simple stage shift, screen-detected cancers also had a higher proportion of luminal and low-grade cancers, whereas symptomatic cancers had a higher incidence of high-grade and triple-negative cancers (p < 0.001 for each). In a multivariate logistic regression model adjusted for age, race, and tumor size, cancers detected by screening had a lower odds of being triple-negative (OR 0.51, 95% CI 0.35-0.75), and a lower odds of being high-grade (OR 0.43, 95% CI 0.29-0.64) compared to cancers found by the patient.

Conclusion: In addition to a stage shift, screening detects cancers with a much more indolent biology and this may account for the observed rate of overdiagnosis. With the increasing use of MRI and ultrasound for screening, the rate of overdiagnosis is likely to increase further.

Tumor Characteristics by Method of Detection

<table>
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<tr>
<th></th>
<th>Screening mammogram</th>
<th>Screening MRI</th>
<th>Screening ultrasound</th>
<th>Self-detected mass</th>
<th>Physician physical exam</th>
<th>p value</th>
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<td></td>
<td></td>
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<tr>
<td>% DCIS</td>
<td>33% (556/1669)</td>
<td>36% (36/101)</td>
<td>13% (6/46)</td>
<td>4% (33/786)</td>
<td>4% (5/112)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>INVASIVE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER/PR+, Her2-</td>
<td>79% (718/907)</td>
<td>85% (45/53)</td>
<td>85% (33/39)</td>
<td>61% (393/641)</td>
<td>75% (63/84)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ER/PR+, Her2+</td>
<td>7% (63/907)</td>
<td>2% (1/53)</td>
<td>5% (2/39)</td>
<td>11% (67/641)</td>
<td>7% (6/84)</td>
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<tr>
<td>ER/PR-, Her2+</td>
<td>5% (43/907)</td>
<td>4% (2/53)</td>
<td>0</td>
<td>6% (40/641)</td>
<td>6% (5/84)</td>
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<tr>
<td>ER/PR-, Her2-</td>
<td>9% (83/907)</td>
<td>9% (5/53)</td>
<td>10% (4/39)</td>
<td>22% (141/641)</td>
<td>12% (10/84)</td>
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<tr>
<td>Grade</td>
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<td></td>
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<td></td>
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<tr>
<td>1</td>
<td>28% (280/983)</td>
<td>45% (25/56)</td>
<td>42% (15/36)</td>
<td>12% (73/633)</td>
<td>20% (17/87)</td>
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<tr>
<td>2</td>
<td>53% (517/983)</td>
<td>46% (26/56)</td>
<td>42% (15/36)</td>
<td>49% (313/633)</td>
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<td>19% (186/983)</td>
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<td>Tumor size</td>
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<td>T3 (30/1113)</td>
<td>T4 (4/1113)</td>
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<td></td>
<td>78% (874/1113)</td>
<td>81% (53/65)</td>
<td>77% (31/40)</td>
<td>38% (284/753)</td>
<td>49% (52/107)</td>
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<td>18% (205/1113)</td>
<td>14% (9/65)</td>
<td>20% (8/40)</td>
<td>47% (354/753)</td>
<td>41% (44/107)</td>
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<td>3% (30/1113)</td>
<td>5% (3/65)</td>
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<td>12% (89/753)</td>
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Title: What is the optimal diagnostic approach for small breast lesions? Fine needle aspiration cytology vs. Vacuum-assisted core needle biopsy

Satoko Nakano¹, Masahiko Otsuka¹, Akemi Mibu² and Toshinori Oinuma³. ¹Kawaguchi Municipal Medical Center, Kawaguchi, Saitama, Japan; ²Kawaguchi Municipal Medical Center, Kawaguchi, Saitama, Japan and ³Kawaguchi Municipal Medical Center, Kawaguchi, Saitama, Japan.

Body: [Background] Although advanced imaging technology enables us to detect small breast lesions, it remains a challenge whether it is a benign or malignant tumor with imaging findings alone. A definitive diagnosis is required with cytological or pathological diagnosis under ultrasonography guidance. Excessive examinations and malpractice are also concerned with increased examinations. The purpose of the study was to identify the optimal diagnostic approach for small breast lesions.

[Patients and Methods] We reviewed a total of 1532 cases of ultrasonography-guided vacuum-assisted core needle biopsy (VAB) performed at a single institution between June 1996 and December 2013. There were 519 small breast lesions (274 non-palpable lesions and 245 non-mass lesions). Ultrasonographic examinations were performed on a LOGIC 500 (GE Healthcare, Waukesha, WI, USA) using an 11 MHz linear transducer before November 2011, and on Apio MX (TOSHIBA, Minato, Tokyo, Japan) using an 8 MHz linear transducer since then. We performed VAB under ultrasonography guidance using 11-gauge probes (Mammotome Biopsy system; Biopsys Medical Inc., Irvine, CA, USA) for diagnosis and 8-gauge probes for excision of the lesion.

[Results] The mean age of the 519 patients was 52.7 years. Ultrasonography-guided fine needle aspiration cytology (FNAC) was performed before VAB in 269 cases (51.8%). The FNAC revealed 76 malignant, 23 suspected malignant, 92 indeterminate, 47 benign or normal, and 31 inadequate cases. Of these FNAC results, the final pathological diagnosis was benign in 2 of 76 malignant and 6 of 21 suspected malignant cases, and malignant in 4 of 47 benign cases. Accordingly, the true positive, true negative, false positive, and false negative rates for the 146 cases (excluding the indeterminate and inadequate cases) by FNAC were 95.8%, 84.3%, 15.7%, and 4.2%, respectively. The pathological results of the VAB specimens were malignant in 229 and benign in 290 cases. Of the 290 cases classified as benign by VAB, we performed post-VAB excisional biopsy in 20 cases. The reasons for this second pathological examination were malignant or suspicious findings for malignancy by FNAC (8 cases), inconsistent imaging and FNAC findings (5 cases), and others (7 cases). Excisional biopsy revealed malignancy in 3 of these 20 cases. Furthermore, we re-performed VAB in 9 out of the original 290 cases, and these were all diagnosed benign as with the first VAB. Excision of the lesion was the primary reason for the second VAB (6 cases), followed by inconsistencies with the imaging findings (2 cases), and suspected malignancy by FNAC (1 case). The true positive, true negative, false positive and false negative rates of VAB were 98.7%, 100%, 0%, and 1.3%, respectively. The mean follow-up duration was 43 months.

[Conclusion] To prevent the excessive examinations, FNAC should be excluded from the initial diagnostic approach for small breast lesions. VAB is a highly reliable technique as the initial diagnosis for small breast lesions with high true positive and true negative rates and a very low false positive rate. The optimal strategy for diagnostic procedures should be adopted in consideration of reducing stress and anxiety in patients and costs.
Title: Value of follow-up control 4 to 6 months after a benign breast biopsy

Johanna Daroles¹, Isabelle Borget¹, Marie Christine Mathieu¹, Sandra Canale¹, Chafika Mazouni¹, Suzette Delaloge¹ and Corinne Balleyguier¹. ¹Institut Gustave Roussy, Villejuif, France, Metropolitan.

Body: Introduction: A radiologic follow-up 4-6 months after a breast biopsy of FNA with a benign result remains mandatory. Since the current trend is to limit imaging investigations, irradiation and to decrease costs, the relevance of this approach is raised. The two objectives of this study were to evaluate the value at this follow-up control in terms of number of cancers detected, as well as to estimate its cost.

Methods: We retrospectively assessed all consecutive patients (pts) seen at a one-stop breast clinic (OSBC) for a suspicious breast lesion between 2004 and 2012, who had received a benign histological or cytological result, and who underwent a radiological follow-up examination (FUE) 4 to 6 months after OSBC. We evaluated the number of cancers detected by radiological techniques at this FUE, as well as the mean cost of the diagnostic procedures and examinations per patient, from the perspective of the French National health insurance system.

Results: Among the 10,833 patients seen at the OSC over the period, 1,398 patients with initial lesion classified as benign after FNA, US-guided biopsy or image-guided macrobiopsy had a FUE at an average of 6.4 interval of months from OSBC. Mean age was 53 years old. Mean size of initial breast lesion was 12.9 mm and BI-RADS categories were 2, 3 and 4 in 3%, 28% and 69% of cases, respectively. Pts underwent 1 FUE in 735 cases, 2 FUE in 591 cases and > 3 in 71 cases. At FUE, 13 breast cancers (10 on the same side and 3 on the other side) were diagnosed, with a cancer detection rate of 0.93%. Mean cost of FUE was estimated at 140 â”… +/− 320 per patient.

Conclusion: The rate of cancers detected at FUE after a benign breast biopsy was high (0.93%, which is higher than that of the French organised screening). The mean cost per patients appeared acceptable. This suggests that a systematic radiologic follow-up after a benign breast lesion remains recommended.
Title: The role of lung biopsy in the management of lung nodules in breast cancer patients

Kazuo Matsuura\textsuperscript{1}, Takayuki Kadoya\textsuperscript{2}, Norio Masumoto\textsuperscript{2}, Hideo Shigematsu\textsuperscript{2}, Akiko Emi\textsuperscript{2}, Keiko Kajitani\textsuperscript{2}, Morihito Okada\textsuperscript{2}, Tsuyoshi Kataoka\textsuperscript{2}, Rumi Haruta\textsuperscript{2}, Koji Arihiro\textsuperscript{2}, Midori Noma\textsuperscript{1} and Toshiyuki Itamoto\textsuperscript{1}. \textsuperscript{1}Hiroshima Prefectural Hospital, Hiroshima, Japan and \textsuperscript{2}Hiroshima University Hospital, Hiroshima, Japan.

Body: Background: A biopsy of lung nodules in patients, who had received previous surgery for breast cancer, can be performed with three aims: to confirm that the lesion is lung metastasis, to confirm the diagnosis of other diseases including primary lung cancer, and to reassess tumor’s characteristics. Discordance in estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) status between primary breast cancer and metastatic lesions has been reported. The aim of this study was to assess the role of lung biopsy in the diagnosis and to determine the changes in hormonal receptor and HER2 status of the metastatic lesions.

Methods: A total of 38 consecutive patients who underwent surgery in 31 or transbronchial lung biopsy (TBLB) in 7 for lung nodules between 1997 and 2014 after curative operation for breast cancer were reviewed.

Results: Eighteen patients (47\%) had a solitary lung nodule. The pathologic diagnoses of lung nodules were lung metastases of breast cancer in 20 patients, primary lung tumor in 14 (Adenocarcinoma in 10; Large cell carcinoma in 2; Small cell carcinoma and Carcinoid tumor in 1 each), and other diagnoses in 4 (Inflammation and organizing pneumonia in 2 each). Median follow up duration were 118.8 months in metastatic breast cancer patients and 105.3 months in other histology patients (p=0.392). The average disease-free interval from the surgery for primary breast cancer were 68.6 months in metastatic breast cancer patients and 66.3 months in other histology patients (p=0.897). The 10-year survival rate after the surgery for primary breast cancer was significantly longer in other histology patients (92.3\%) than in metastatic breast cancer patients (55.1\%) (p=0.0308). In 20 cases of metastatic breast cancer, rates of discordance were 5\%, 10\% and 10\% for ER gain, PgR gain and HER2 loss, respectively. Sixteen of patients maintained the same tumor phenotype, whereas 4 changed during progression. Especially, 2 cases of ER, PgR gain could receive endocrine therapy instead of chemotherapy.

Conclusion: As lung nodules that appear in breast cancer patients are not always lung metastases, the pathologic diagnosis should be confirmed, and surgery is an option for the pathologic confirmation. Furthermore, discordance in biomarker status between primary breast cancer and the lung metastasis occurred in 20\% of cases. It is necessary for clinicians to check biomarker status in recurrent breast cancer patients as it may assist a shift in the treatment plan.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P5-04-01  
**Average Grade:** 4.20

**Title:** Breast cancer related immune suppression in the sentinel lymph node can be effectively countered by combined CpG-B administration and JAK2/STAT3 inhibition

Kim M van Pul¹, Ronald JCLM Vuylsteke², Sinéad M Lougheed¹, Lisette EA te Velde³, Petrousjka van den Tol³, Emiel J Th Rutgers⁴, Hein BAC Stockmann² and Tanja D de Gruijl¹. ¹VU Medical Center, Amsterdam, Noord-Holland, Netherlands; ²Kennemer Gasthuis, Haarlem, Netherlands; ³VU Medical Center, Amsterdam, Noord-Holland, Netherlands and ⁴Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands.

**Body:**

**Background**

Increasing evidence suggests that immune regulated pathways influence both breast cancer (BrC) development and response to (neo) adjuvant chemotherapeutic treatment. The sentinel lymph node (SLN) is the first site of BrC induced suppression of immune effector subsets, rendering them unable to mount an effective anti-tumor response. Since detailed knowledge of the functional status of these immune effector subsets is lacking, we compared the immune status of BrC SLN with healthy lymph nodes (HLN) using viable cell samples and correlated our findings to several clinico-pathological characteristics. Additionally we investigated if ex vivo conditioning with the Toll-like receptor-9 ligand CpG-B with or without simultaneous inhibition of JAK2/STAT3 signaling (a downstream signaling pathway implicated as the ‘master switch’ in tumor induced immune suppression) could overcome the observed immune suppression in BrC SLN.

**Methods**

Viable SLN cells were obtained from 40 clinically node negative BrC patients. Axillary HLN were obtained from 17 BRCA-1 or -2 patients undergoing a prophylactic mastectomy. Frequencies and activation state of dendritic cell (DC) subsets and regulatory T cells (Treg) were determined by extensive multi-color flowcytometric analyses. Additionally, SLN cells from 12 BrC patients were cultured in absence and presence of CpG-B (PF-3512676) and the combination of CpG-B with a JAK2-STAT3 inhibitor (AG-490).

**Results**

Of the 40 BrC SLN 11 contained metastasis. We found clear evidence of BrC-related immune suppression, as significantly higher Treg frequencies were observed in metastasis negative BrC SLN as compared to HLN. These frequencies further increased in metastasis positive BrC SLN. Activation state (by expression of the co-stimulatory molecules CD40, CD83 and CD86) of lymph node resident (LNR)-DC subsets (both CD11c⁺ myeloid DC and CD123⁺BDCA2⁺ plasmacytoid DC), but not of CD1a⁺ migratory subsets, was significantly lower in BrC SLN as compared to HLN. Additionally, in BrC SLN, activation state of these LNR-DC subsets was further reduced upon metastatic involvement and in non-luminal BrC subtypes (triple negative and HER-2⁺) as compared to luminal BrC subtypes (ER and/or PR positive). We previously showed in early-stage melanoma patients that local treatment with CpG-B led to the preferential activation of LNR-DC subsets. Similarly, ex vivo targeting of BrC SLN with CpG and CpG + JAK2/STAT3 inhibition resulted primarily in significantly enhanced activation of LNR-DC subsets, with superior effects of combined CpG and JAK2/STAT3 targeting evidenced by significantly higher CD83 expression.

**Conclusion**

BrC induced immune suppression in SLN seems to be primarily mediated by hampered activation of LNR-DC subsets, which was most effectively overcome (ex-vivo) with the immune activating compound CpG-B in combination with a small-molecule inhibitor of the immune suppressive JAK2/STAT3 signaling pathway (AG490). This combined immune targeting strategy might be of clinical benefit in enhancing effectiveness of conventional (neo)adjuvant chemotherapeutic treatment, especially in -more immune suppressed- non-luminal BrC subtypes with known poor prognosis.
Title: Hyaluronan (HA) depletion sensitizes HA\textsuperscript{high} tumors to antibody-dependent cell-mediated cytotoxicity

Netai C Singha\textsuperscript{1}, Tara Nekoroski\textsuperscript{1}, Chunmei Zhao\textsuperscript{1}, Rebecca Symons\textsuperscript{1}, Ping Jiang\textsuperscript{1}, Gregory Frost\textsuperscript{2}, Zhongdong Huang\textsuperscript{1} and H Michael Shepard\textsuperscript{1}. \textsuperscript{1}Halozyme Therapeutics, San Diego, CA and \textsuperscript{2}Intrexon Corporation, San Diego, CA.

Body: Therapeutic efficacy of monoclonal antibodies (M Abs) against solid tumor targets, like trastuzumab (anti-HER2) and cetuximab (anti-EGFR), have been used successfully to treat cancer, despite the many physical barriers impeding their access to the malignant cell surface\textsuperscript{1}. For example, only about 50\% of HER2\textsuperscript{3+} patients have a durable response to therapy with trastuzumab\textsuperscript{2}. Extracellular matrix (ECM)-mediated inhibition may be among the mechanisms of resistance to M Ab therapy of solid tumors. Aberrant accumulation of hyaluronan (HA), a major component of the ECM in many tumors, is associated with poor prognosis and treatment-resistance in multiple malignancies\textsuperscript{3-5}. We investigated HA-dependent pericellular matrix-mediated inhibition to ADCC in HA\textsuperscript{high} human cancer cells \textit{in vitro} and \textit{in vivo}. We observed high levels of tumor associated HA (HA\textsuperscript{3+}) in >50\% of HER2\textsuperscript{3+} breast adenocarcinoma and ∼40\% of EGFR\textsuperscript{+} head and neck squamous cell carcinoma (HNSCC) primary tumors. Human hyaluronan synthase 2 (HAS2)-overexpressing breast cancer cells formed an HA\textsuperscript{high} pericellular matrix, which inhibited both natural killer (NK) cell access to tumor cells and ADCC \textit{in vitro}. Hyaluronan depletion by PEGPH20, a pegylated recombinant human PH20 hyaluronidase currently in clinical study for pancreatic cancer, increased NK cell access to HAS2-overexpressing breast cancer cells and greatly enhanced trastuzumab- or cetuximab-dependent ADCC. Trastuzumab and NK cell accessibility to HAS2-overexpressing tumors was enhanced following HA-depletion by PEGPH20. In \textit{in vivo} ADCC-based efficacy study, PEGPH20 treatment in combination with trastuzumab and NK cells enhanced tumor growth inhibition. This work describes a novel tumor microenvironment (TME)-dependent mechanism of inherent resistance to therapeutic antibody-mediated ADCC \textit{in vitro} and \textit{in vivo}, and furthermore shows that ADCC can be enhanced by hyaluronan depletion. These results may help to explain as to why tumors with high levels of HA are more aggressive, and suggest potential benefits of PEGPH20-mediated HA depletion in combination with therapeutic antibodies like trastuzumab or cetuximab in the treatment of HA\textsuperscript{high} solid tumors.

References:
Deconvoluting immune cell populations using ‘in silico flow cytometry’ with CIBERSORT: Association with neoadjuvant therapy response and genomic instability in TNBC

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Background: Increased tumor infiltrating lymphocytes (TILs) are prognostic and predictive of therapy response in TNBC. CIBERSORT, a highly novel ‘in silico flow cytometry’ gene expression-based method, can assess the overall immune content and relative levels of distinct leukocyte subsets in tumors.

Methods: We applied CIBERSORT to PrECOG 0105, a neoadjuvant trial of carboplatin, gemcitabine and iniparib for patients with clinical stage I-IIIA TN or BRCA1/2 mutation-associated BC. H&E tumor sections from pre-therapy biopsies were evaluated for density of stromal (sTILs) and intratumoral (iTILs) lymphocytes. Pathologic response was assessed at definitive surgery. Overall immune content and fractions of 23 distinct leukocyte subsets were derived from Affymetrix U133 plus 2.0 arrays, using CIBERSORT. Using an input matrix of reference gene expression signatures of 23 purified leukocytes, this methodology can infer an unknown fraction of each cell type from tissue expression profiling, yielding a p value for deconvolution. All patients had BRCA1/2 genotyping and levels of tumor genomic instability were assessed by the homologous recombination deficiency (HRD) assay.

Results: 57 pts had gene expression and TILs assessment. TILs, sTILs, and iTILs were significantly associated with a measure of absolute immune content determined by CIBERSORT, termed an ‘immune score’ (R 0.73, p<0.0001; R 0.70, p<0.0001; R 0.57, p<0.0001 respectively). Both iTILs and the CIBERSORT immune score were significantly associated with pathologic complete response (pCR; ypT0/is N0) in independent models. Specific leukocyte subsets significantly associated with pCR, included activated memory CD4 T cells, CD8 T cells, and M1 macrophages (all p<0.05). Regarding genomic instability measures, BRCA1/2 germline mutation status was not associated with TILs or immune score, whereas a high HRD assay score was significantly associated with the CIBERSORT immune score (p=0.038). Individual leukocyte subsets significantly associated with a high HRD assay score, included plasma cells, activated memory CD4 T cells, M1 macrophages, and unstimulated mast cells (all p<0.05).

Conclusions: Neoadjuvant platinum-based therapy response significantly associates with both iTILs and CIBERSORT immune score. A measure of global genomic instability (HRD score) significantly associates with immune score alone. Enumeration of 23 leukocyte subsets in therapy-naïve TNBC by CIBERSORT revealed three distinct cell types that significantly predict pCR, two of which also associate with genomic instability. These results suggest an intimate interplay between genomic instability and immune infiltration, potentially shaping adaptive anti-tumor humoral immune responses, and thereby affecting neoadjuvant response in TNBC.
Title: Co-stimulation through 4-1BB/CD137 improves expansion and function of tumor-infiltrating T lymphocytes from primary and metastatic triple-negative breast cancer and inflammatory breast cancer

Michiko Harao¹, Hui Gao¹, Jie Qing Chen¹, Elizabeth A Mittendorf¹, Gildy V Babiera¹, Sarah M DeSnyder¹, Korrene F Rockwood¹, Savitri Krishnamurthy¹, Huiming Sun¹, Jie S Willey¹, Naoto T Ueno¹, James M Reuben¹ and Laszlo G Radvanyi¹,². ¹University of Texas MD Anderson Cancer Center, Houston, TX and ²Lion Biotechnologies, Woodland Hills, CA.

Body: Background: Increased CD8+ tumor-infiltrating lymphocytes (TIL) is associated with improved prognosis in triple-negative breast cancer (TNBC) suggesting that T-cell responses at the tumor site can be harnessed for autologous T-cell therapy using TIL expanded ex vivo. Although TIL therapy has been developed for solid tumors such as melanoma, cervical, and ovarian cancer. Moreover, methods facilitating CD8+ TIL expansion from TNBC are desirable given their cytotoxic potential against tumor cells. One approach to address this need is to provide agonist signals through the 4-1BB/CD137 pathway during TIL expansion selectively costimulating CD8+ T-cell activation. In this study, we established a method of expanding TIL from surgical specimens and core biopsies from primary TNBC patients and compared the phenotype and function of these TIL to lymphocytes from peripheral blood.

Patients and methods: Eight primary human TNBC tumor samples were obtained by surgical resection or core biopsy after neo-adjuvant chemotherapy. Small (4-6 mm²) tumor fragments were cultured for 28 days in 24-well plates in medium containing 3000 IU/ml IL-2 alone or in combination with 10 µg/ml agonistic anti-4-1BB IgG4 (BMS663513) added at the start of culture. Viable cell numbers and the expression of CD3, CD8, CD4, CD27, CD28, CD56, CD16, Granzyme B, and Perforin were determined by flow cytometry on day 28 after culture. Cytotoxic function of the TIL was evaluated by measuring Caspase 3 cleavage in target cells. Blood samples collected at surgery were immunophenotyped for subsets of T cells and NK cells, and assessed for ability of T cells to synthesize Th1/Th2 cytokines following activation through the T-cell receptor (TCR), and potential of NK cells to kill K562 targets.

Results: TIL were successfully expanded from tumor fragments in 6/8 of the cases, with addition of anti-4-1BB greatly increasing the percentage and yield of CD8+CD3+ T cells. CD8+ TIL isolated from cultures receiving 4-1BB costimulation however had decreased CD27 and CD28 expression together with increased cytotoxic T-cell activity. Gene expression analysis also found that TIL from these 4-1BB costimulated cultures had a more differentiated CD8+ T-cell gene profile. Peripheral blood CD3+, CD8+, and CD4+ T cells were lower than those of healthy controls, but TCR-activated cytokine synthesis was not significantly different. Peripheral blood NK cells expressed normal Granzyme A/B and Perforin levels and exhibited normal IFN-γ secretion and CD107a-release following exposure to IL-12/IL-18 or with K562 targets.

Conclusions: TIL can be reproducibly expanded from TNBC tumors with IL-2 after neo-adjuvant therapy, with CD8+ TIL outgrowth and effector activity increased by provision of 4-1BB/CD137 costimulatory signals during culture initiation. These CD8+ TIL however had a more differentiated phenotype with higher cytotoxic activity. Peripheral blood T-cell and NK cell function was comparable to those of healthy donors. Our results support further development of an autologous TIL expansion protocol after neo-adjuvant therapy for use in an adoptive cell therapy approach to treat TNBC recurrence or metastasis.
Title: Oral immunomodulatory agents prevent tumor growth and increase tumor CD8 T cell infiltrate; bexarotene further improves tumor response to conventional chemotherapy in breast tumors

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Body: The tumor immune environment is important in breast cancer with greater than 50% immune infiltrate (LPBC) prior to neoadjuvant chemotherapy predicting improved pathologic complete response, and LPBC and CD8 infiltrate prior to adjuvant therapy predicting improved survival. Unfortunately, the majority of breast cancers do not have LPBC or robust CD8+ infiltrate. Evidence has emerged that conventional chemotherapy can increase CD8+ T cells therefore discovering ways to boost this response should further enhance the anti-tumor function. Three oral agents have modest anti-tumor function: metformin (oral biguanide), bexarotene (retinoic receptor agonist), and celecoxib (COX2 inhibitor). In vitro data suggest a role for these agents in increasing Th1 immunity: metformin was shown to increase MHC class I expression on tumor cells, bexarotene was shown to decrease CD8+ T cell apoptosis, and celecoxib has been shown to decrease MDSC cells. The goal of this study was to demonstrate whether addition of these oral agents to conventional chemotherapy enhanced the anti-tumor function of chemotherapy, possibly by modifying the immune environment in the transgenic mouse mammary tumor model TgMMTV-neu (genetically similar to luminal breast cancer).

Two active chemotherapies in human breast cancer, doxorubicin and paclitaxel, inhibited tumor growth and increased CD8+ T cell tumor infiltrate in transgenic mice. Treatment of mice with 100 mm3 tumors with doxorubicin (5 mg/kg weekly for four weeks) showed a 32% increase in CD8+ T cells and 85% decrease in tumor growth as compared to control mice (p=0.0001) and treatment with paclitaxel (10 mg/kg weekly for four weeks) showed a 40% increase in CD8+ tumor infiltrate (p=0.0068) and 60% decrease in tumor growth as compared to control treated mice (p=0.0026). A third chemotherapy cyclophosphamide (100 mg/kg weekly for four weeks) increased CD8+ tumor infiltrate by 45% (p=0.011) but did not show a significant decrease in tumor volume (p=0.57). Metformin and bexarotene also demonstrated increased CD8+ tumor infiltration and decreased breast tumor growth but celecoxib did not. Metformin treated mice had a 46% increase in CD8+ tumor infiltrate (p=0.001) and a 52% decrease in mean tumor volume (p=0.011) as compared to controls (75 mg/m² of metformin for four weeks). Bexarotene treated mice had 44% increase in CD8+ tumor infiltrate (p=0.05) and 60% decrease in mean tumor growth (p=0.03) compared to control (treated with 50 mg/m² bexarotene for four weeks). Of all three oral therapies, bexarotene was most effective inhibiting spontaneous tumor growth in the mice. Furthermore, addition of bexarotene to chemotherapy was superior to chemotherapy alone. Adding bexarotene to weekly doxorubicin decreased tumor growth by 98% (p=0.008 compared to doxorubicin alone), adding bexarotene to weekly paclitaxel decreased tumor growth by 86% (p=0.02 compared to paclitaxel alone), and adding bexarotene to weekly cyclophosphamide inhibited tumor growth by 82% (p=0.04 compared to cyclophosphamide alone). These results suggest that addition of bexarotene, a well-tolerated oral agent, to chemotherapy may improve tumor response in breast cancer.
Title: Multispectral imaging allows visualization and quantification of multiple immunologic cell types in breast tumor tissues

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**Body:** Background: Multiple studies have identified tumor infiltrating lymphocytes (TIL) in breast cancer and shown them to have prognostic and predictive significance. TIL are comprised of CD8+ and CD4+ T cells, regulatory T cells (Treg) and B cells. To date, the majority of studies have identified TIL using H&E staining. Studies utilizing IHC to stain for TIL generally have been limited to evaluating a single immune cell phenotype such as CD8+ T cells, while studies evaluating multiple immune cell types have generated single cell suspensions from tumors for analysis by flow cytometry to determine relative percentages of specific immune cell populations. Although these studies have confirmed that immune infiltrates in tumors are heterogeneous, they have not addressed the spatial relationship between tumor and immune cells, which could be critical to anti-tumor immune effects. This study was undertaken to demonstrate the feasibility of multispectral imaging and multiplexed immunofluorescence to visualize and quantify specific immune infiltrates in the tumor and surrounding stroma on single FFPE tissue sections.

**Methods:** Single FFPE slides from 9 HER2+ breast cancer patients receiving neoadjuvant chemotherapy were stained with primary antibodies targeting cytokeratin, CD8, CD4, FoxP3 (Treg), CD20 (B-cells) and PD-L1 (T cell inhibitory molecule). Tyramide signal amplification was used to improve signal and specificity, as well as to reduce background. The Vectra multispectral imaging instrument was used for image acquisition and InForm software was used to quantitate the density (number of cells per square millimeter) of specific cell types in the tumor and in the surrounding stroma.

**Results:** Multispectral imaging successfully captured and quantified multiple immune cell types in all tissues (images to be shown at time of presentation). CD8+ and CD4+ T cell densities as well as PD-L1 expression in the tumor and surrounding stroma are shown.

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For patients not achieving a pathologic complete response (pCR), the density of both the CD8 (p=.03) and CD4 (p=.05) infiltrates in the stroma were significantly greater than in the tumor. For patients achieving a pCR, there was no significant difference in the densities of stromal and intratumoral CD8 (p=.11) or CD4 (p=.75) infiltrates suggesting that T cell infiltration into the tumor from the stroma is critical.

**Conclusion:** Multispectral imaging allows different immune cell phenotypes to be visualized and quantified simultaneously in the same tissue section enabling further study of the relationships and distribution of these cells within the tumor and tumor microenvironment, and their spatial distribution and proximity to the tumor cells. This technology will enable improved...
understanding of the immune infiltrate in breast tumors thereby facilitating the rational design and use of immunotherapeutic agents in combination with standard systemic therapies.
Title: Expression of novel immunotherapeutic targets in luminal breast cancer patients

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¹Caris Life Sciences, Phoenix, AZ; ²Mayo Clinic, Scottsdale, AZ and ³Arizona State University, Tempe, AZ.

Body: Background: The development of novel chemotherapeutic agents has significantly improved the prognosis and survival of patients with breast cancer. ER positive or luminal tumors represent around two thirds of all breast cancers and these cancers are comprised of different histologies including differing gene expression and mutational profiles. This study examined biomarkers involved in immune evasion including PD-L1 and its association with other biological pathways as potential treatment options for luminal breast cancer patients.

Methods: We analyzed 1311 breast samples using a multiplatform approach including whole genome mRNA expression (HumanHT-12 v4 BeadChip Illumina Inc., San Diego, CA), protein expression (immunohistochemistry), gene copy number changes (in situ hybridization) and gene sequencing and an additional 304 breast samples were tested for PD-1 and PD-L1 by IHC. The mRNA expression data was based on whole tumor and represents cell type heterogeneity. Heat map analysis was done to look at differential gene expression between the PD-L1 high vs low luminal breast cancers.

Results: Based on expression of ER, PR and HER2 by IHC, we subdivided the data into sub- cohorts. Elevated mRNA expression of immune markers including PD-L1, CTLA4, B7H-3, and IDO1 was noted in the ER+HER2- luminal population including ER+ PR- and ER+PR+ cohort. Positive correlation was found between PD-L1 and other immune regulators including CTLA-4, B7-H3 and IDO1 (Spearman correlations of 0.49, 0.36 and 0.46). Protein expression of PD-L1 was found to be specific to the HER2 negative cohort with no expression in the HER2 positive cohort regardless of ER status. Within the ER+HER2- cohort, PD-L1 expression was 5% (ER+PR+ was 5% (4/81) and the ER+PR- was 6% (2/34)). In contrast, PD-L1 expression was higher in the triple negative cohort, 17% (13/75). The expression of PD-1 on the other hand was present throughout the different cohorts. PD-1 expression ranged from 43% in the ER+HER2- cohort {41% (14/34) in ER+PR-HER2-; 43% (35/81) in ER+PR+HER2-} to 33% in the ER+HER2+ cohort {44% (4/9) in ER+PR+Her2+; and 17% (1/6) in ER+PR+HER2+}. In the ER-HER2+ cohort PD-L1 expression was 75%(6/8 in ER-PR-HER2+ and 0/0 in ER-PR+HER+) and 63%(47/75) in the triple negative cohort. Pathway analysis of the PD-L1 negative vs positive luminal population identified 127 genes involved in various cancer pathways including EGFR and VEGF signaling network (P=7.47e-09).

Conclusions: The expression of immune regulatory targets in the breast cancer population suggests that immune- targeted therapies with anti PD-1/PD-L1, CTLA4, B7-H3 and IDO1 may be effective especially in the luminal cohort. Our study further shows that ER+HER2- breast cancer patients may be better candidates for immunotherapy with checkpoint inhibitors as compared to ER+HER2+ due to lack of PD-L1 expression in the HER2 positive cohort. Further validation of our findings is ongoing.
Characterization of neutrophil elastase receptor in breast cancer: Implication for immunotherapy

Celine Kerros¹, Satyendra C Tripathi², Anne V Philips³, Gheath Al-Atrash¹, Kathryn E Ruisaard¹, Karen C Dwyer¹, Elizabeth A Mittendorf², Samir M Hanash² and Jeffrey J Molldrem¹. ¹University of Texas MD Anderson Cancer Center, Houston, TX; ²University of Texas MD Anderson Cancer Center, Houston, TX and ³University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Neutrophil Elastase (NE), a serine protease released by tumor-associated neutrophils in the tumor microenvironment induces invasion and metastasis. We have demonstrated that NE is not endogenously expressed in breast cancer (BrCA), but is taken up by BrCA cells. NE uptake results in the expression of the HLA-A2-bound peptides CCNE1 and PR1, derived from cyclin E and NE, respectively, on the surface of triple-negative (TN) BrCA cells (MDA-MB-231). Expression of these peptide/HLA-A2 molecules induces BrCA cell susceptibility to cytolysis by CCNE1- and PR1-specific cytotoxic T lymphocytes (CTLs), and to 8F4, a monoclonal antibody that binds specifically to the PR1/HLA-A2 complex. We hypothesize that NE uptake is a receptor-mediated process that results in cross-presentation of NE-derived peptides on HLA molecules of BrCA cells.

Here, we found that NE uptake is specific, time- and dose-dependent, and saturable, suggesting a receptor-mediated uptake mechanism. We showed that MDA-MB-231 did not take up cathepsin G, a related serine protease, suggesting specificity of receptor uptake. NE internalization was partially blocked by chlorpromazine and by wortmannin, suggesting clathrin-dependent uptake and PI3Kinase-dependence, respectively. Confocal microscopy showed that NE was colocalized with the early endosome marker, EEA-1, as early as 10 min after uptake. Flow cytometry indicated that surface-bound NE on MDA-MB-231 cells decreased after 5 minutes, and both flow cytometry and Western blot showed a simultaneous decrease of phospho-Erk and loss of IRS-1 signaling. Inhibition of NE enzyme activity by elafin or PMSF potentiated NE uptake in BrCA cells, thus enzyme activity is not required for uptake. Conclusion: The results support a novel mechanism of rapid receptor-mediated uptake of soluble exogenous NE by TN BrCA. NE uptake is efficient, PI3 kinase-dependent, sensitive to clathrin inhibition, and associated with down-regulation of Erk phosphorylation and IRS-1. In addition, uptake does not require NE enzyme activity. Following uptake, NE colocalizes to an early endosomal compartment, an organelle associated with peptide loading of MHC-I molecules for expression of the cell surface. We previously showed that the NE-derived peptide PR1 is cross-presented on MDA-MB-231 cells after NE uptake, leading to susceptibility to immunotherapies that target PR1/HLA-A2. Taken together, our results demonstrate receptor-mediated NE uptake, which is a potentially novel paradigm that could vastly expand the number of tumor-associated antigens that could be targeted on BrCA. An understanding of the mechanism that mediates NE uptake will help us develop strategies to increase expression of immunogenic peptides on cancer cells and to guide the design of targeted immunotherapies against CCNE1 and PR1 as new clinical therapies for BrCA.
Title: Characterization of the immune infiltrate in HER2+ breast cancer

Shridar Ganesan², Gabriela Alexe¹, Kim M Hirshfield², Ming Yao², Gyan Bhanot² and Toppmeyer Deborah². ¹Broad Institute of MIT and Harvard, Boston, MA and ²Rutgers Cancer Institute of New Jersey, New Brunswick, NJ.

Body: A subset of HER2+ breast cancer is characterized by high expression of lymphocyte-associated genes and the presence of a robust, histologically apparent, lymphocytic infiltrate. The presence of an immune signature and lymphocytic infiltration is associated with improved outcome, and may be a marker of improved response to trastuzumab-based therapy. The relationship of the immune signature in these cancers to the biology and functional status of the lymphocyte subsets present in the tumor microenvironment remain unclear. To better characterize the immune microenvironment of HER2+ cancers, we have analyzed paired RNA sequencing data and histologic image data from The Cancer Genome Atlas. We have confirmed the presence of a strong immune signature in a subset of HER2+ breast cancers that is correlated with the presence of a robust lymphocytic infiltrate. Immuno-phenotyping of the lymphocytic infiltrate was performed on an independent set of HER2+ breast cancers from the Rutgers Cancer Institute of New Jersey. CD8+ T-cells and macrophages are a significant component of the immune infiltrate in these cancers. Interestingly, CD20+ B-cells were also present in the tumor stroma as large aggregates whose organization is suggestive of the presence of ectopic germinal centers. The relationship of lymphocyte subsets present in HER2+ breast cancer, immunoglobulin subtypes expressed and correlation with immune checkpoint protein expression will be presented. The composition of the immune infiltrate and its organization into ectopic germinal centers may give new insight into the role of the immune microenvironment in the development of HER2+ breast cancer and the response to trastuzumab.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-04-11
Average Grade: 0

Title: Tumor educated T cells guide RANKL+ neutrophils in the bone marrow: Cooperation in the pre-metastatic niche

Triciana Gonçalves-Silva¹, Suelen Martins Perobelli¹, Ana Carolina Mercadante¹, Ana Carolina Monteiro², Wallace de Mello², Alex Bauduino² and Adriana Bonomo¹. ¹Federal University of Rio de Janeiro â–“ UFRJ, Rio de Janeiro, Brazil and ²Oswaldo Cruz Foundation â–“ FIOCRUZ, Rio de Janeiro, Brazil.

Body: Breast cancer is the most common malignancy among women, may give rise to bone metastases, occurring in 70% of patients with metastases. Normally bone metabolism is tightly regulated, allowing the bone remodeling without excessive degradation or deposition of bone matrix. However in bone metastasis this balance is disrupted, triggering a excessive bone degradation, which releasing growth factors that will nourish tumor cells establishing a positive feedback between bone degradation and growth tumor, known as the vicious cycle of bone metastasis. Our group added one more step in vicious cycle. In the murine model we showed that Th17 RANKL+ cells are essential to induce the ideal microenvironment to establishment bone metastases, being responsible for induction of osteolytic disease in pre-metastatic niche. In our model we observed early bone loss, in 6 days after inoculum of tumor cells we observed a 50% decrease in bone trabecular mass. But, T cells represent a 3% of total population in marrow, and it’s hard to believe that this small population alone are responsible for this aggressive bone loss. Recently, it has been describe that neutrophils in inflammatory conditions express RANKL and are able to induce formation of osteoclasts in vitro. In our model, RANKL+ lymphocytes secrete IL17, that is close related to neutrophils and this cells represent a 35% in total bone marrow. Thereby, we asked if this RANKL+ neutrophils (RANKL+neut) could be the cells working on a amplification loop for the observed phenomenon. First we asked if RANKL+neut were present in bones in physiological conditions. To this we divided bones into trabecular and cortical regions and we observed that total neutrophils has no difference in evaluatated regions, but RANKL+neut are preferentially in trabecular bones. Next we investigated if RANKL+neut was present in our model. To this we used a singeneic mouse breast cancer, where tumor cell line 4T1 were inoculated ortotopically in 4th mammary gland in BALB/c females. In accordance to our prediction we observed a significant increase in RANKL+ neut in the bone marrow in 3d tumor-bearing animals, which became even greater on the 6th day. Furthermore, in naive mice RANKL is expressed in all stages of maturation and tumor-bearing mice there are a with significant increase in RANKL expression in mature cells. RANKL+neut are able to induce osteoclasts differentiation in a RANKL dependent manner.Furthermore RANKL+ neut from naive mice expresses pro and anti-inflammatory cytokines and in 6d tumor-bearing mice this profile is altered, with decreased expression of IL10 and increased of IL17A and TNFα. We also demonstrated that RANKL+neut express molecules related to interaction with T cells, in 6d tumor-bearing mice these cells increase CD40 expression. Furthermore, in vitro assays and in T cells transfer to NUDEs, we observed that, unlike naive T cells, tumor primed T cells induced increase in RANKL+ neut. Taken together, our data suggest that RANKL+neut are present mainly in regions of trabecular bone and increase during tumor progression before the appearance of bone metastases. Indicating that RANKL+neut are potentially involved in bone remodelling and this phenomena can be regulated by primed T cells.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-04-12
Average Grade: 7.00

Title: Targeting of phosphatidylserine by monoclonal antibodies enhances activity of immune checkpoint inhibitors in breast tumors

Bruce Freimark¹, Jian Gong¹, Van Nguyen¹, Shen Yin¹, Rich Archer¹ and Jeff Hutchins¹. ¹Peregrine Pharmaceuticals, Tustin, CA.

Body: Phosphatidylserine (PS) is a phospholipid normally residing in the inner leaflet of the plasma membrane and becomes exposed on tumor vascular endothelial cells (ECs) and tumor cells. PS exposure becomes enhanced in response to chemotherapy, irradiation, and oxidative stresses in the tumor microenvironment. PS exposure in tumors promotes an immunosuppressive microenvironment which includes the recruitment of myeloid derived suppressor cells (MDSCs), immature dendritic cells, and M2-like macrophages as well as the production of anti-inflammatory cytokines. Binding of PS targeting antibodies on tumor endothelial cells, tumor cells and their secreted microparticles triggers an Fc-FcR mediated pro-inflammatory cellular and cytokine response that reverses the immunosuppressive PS mediated checkpoint, thereby enhancing anti-tumor immunity. A chimeric PS-targeting antibody, bavituximab, is being used in combination with chemotherapy to treat patients with solid tumors in multiple late-stage clinical trials. Using breast tumors in immune competent mice, we demonstrate PS targeting antibodies enhance the anti-tumor activity of anti-CTLA-4 and anti-PD-1 antibodies in the presence and absence of conventional chemotherapy. Tumor growth inhibition correlates with infiltration of immune cells in tumors and induction of adaptive immunity. The combination of these mechanisms promotes strong, localized, anti-tumor responses without the side-effects of systemic immune activation.
Title: Toll like receptor-9 and CD73 may act on the same pathway to induce immunosuppression in triple negative breast cancer cells

Katri S Selander¹, Johanna M Tuomela², Mikko Mella³, Joonas Kauppila³, Jouko Sandholm², Gennady Yegutkin², Peeter Karihtala³, Arja Jukkola-Vuorinen³, Kirsi-Maria Haapasaari³, Katri S Vuopala⁴ and Kevin W Harris¹. ¹University of Alabama, Birmingham, AL; ²University of Turku, Finland; ³University of Oulu, Finland and ⁴Lapland Central Hospital, Rovaniemi, Finland.

Body: Toll like-receptor-9 (TLR9) is an innate immune system DNA-receptor which is also widely expressed in breast cancer cell lines and in clinical breast cancer specimens. Although TLR9 ligands (such as bacterial DNA and synthetic, CpG-sequence containing oligonucleotides) induce TLR9-mediated invasion in breast cancer cells in vitro, the contribution of this protein to breast cancer pathophysiology remains unclear. We showed previously that tumor TLR9 expression is a highly significant prognostic factor in breast cancer, but only in tumors that are triple negative (TNBC). Specifically, low tumor TLR9 expression in TNBC is associated with a significantly shortened disease-specific survival. Our published, preclinical results further suggest that tumor TLR9 expression may be an important determinant of tumor immunophenotype and that patients with low-TLR9 TNBC may not gain the immunogenic benefit from chemotherapy. A possible mechanistic explanation for the change in the immunophenotype of low TLR9-TNBC tumors involves CD73, which is a 5’ectonucleotidase that converts extracellular adenosine monophosphate (AMP) into the highly immunosuppressive adenosine. CD73 is also expressed in breast cancer cells and in clinical specimens. High CD73 expression has been associated with poor prognosis, but similar with TLR9 expression, the prognostic significance was specific only for TNBC. There are no previous reports on TLR9 regulation of CD73 in any cancer. Interestingly, however, TLR9 deficiency was shown to promote CD73 expression of T-cells in a mouse model of diabetes. To begin to test the hypothesis that TLR9 affects CD73 expression in TNBC, we studied CD73 mRNA and protein expression in control and TLR9 siRNA TNBC cells in vitro. We discovered that TLR9 siRNA TNBC cells express significantly higher CD73 mRNA concentrations than control siRNA cells specifically in hypoxia. Similar findings were observed with immunofluorescence. Our results suggest that the lack or TLR9 in TNBC may allow high CD73 expression and through this mechanism result in an immunosuppressive phenotype of the tumors. The immunosuppression then would explain the poor prognosis associated with low TLR9-TNBC tumors. We are currently studying this with clinical TNBC specimens.
Title: Decreased functions of natural killer cells in peripheral blood of advanced triple negative breast cancer patients

In Hae Park1,2, Sun-Young Kong3,4, Joo Hyun Kang2, Hye Jin Mo2, Tae Sik Kim4, Keun Seok Lee1 and Jungsil Ro1,2. 1Center for Breast Cancer, National Cancer Center; 2Breast & Endocrine Cancer Branch of Research Institute, National Cancer Center; 3Hospital, National Cancer Center and 4Translational Epidemiology Branch, Research Institute, National Cancer Center.

Body: Background: Natural killer (NK) cells are a major player in innate immune response. Two distinct NK cell subsets with different functions as the strongest cytotoxic activity (CD56dimCD16+) or cytokine production (CD56brightCD16+/-) have been known. In this study, we evaluated the phenotypic and functional difference of NK cells in peripheral blood (pB) from the patients who had advanced triple negative (TN) breast cancer compared to healthy controls.

Methods: We enrolled advanced triple negative (TN) breast cancer patients treated at the National Cancer Center, Korea between March 2012 and March 2014. Healthy controls who visited for health screening were recruited at the same time period and peripheral blood (pB) samples were collected. In patient groups, pB was sampled either prior to the initiation or during the courses of chemotherapy. Then, patients were retrospectively classified into two groups as follows; non-responders (the group A) and responders (the group B) to the treatment. NK cells were isolated from peripheral blood mononuclear cells (PBMC) and analyzed based on their expression of CD56 and CD16. We looked at cytotoxic activity of pB NK cells against K562 cell line and measured CD107a expression as a parameter of NK cell activation.

Results: The absolute numbers of lymphocytes and NK cells per cubic millimeter of pB were similar among healthy controls (N=24), the group A (N=16), and the group B (N=21). There was no significant difference in the proportion of pB NK cell subsets among three groups except CD56brightCD16+- subset which was significantly higher in the group B compared to healthy controls (P=0.0014). Even in the presence of similar proportions of NK subsets, the activities of CD56dimCD16+ NK cells measured by CD107a expression were significantly lower both in the group A (P=0.0023) and in the group B (P=0.0127) compared with those of control group. The activities of CD56dimCD16+ NK cells were not different between group A and B. In the case of CD56brightCD16+/- NK cells, CD107a positivity was significantly lower in the group A compared with controls (P=0.0068) and group B (P=0.0465).

Conclusions: Our data suggested that cytolytic functions of NK cells were significantly decreased in advanced TN breast cancer patients compared with those of healthy controls despite a similar proportion of NK cell subsets in pB.
Title: Prognostic value of HC10 expression in Russian women with stage I breast cancer stratified for adjuvant systemic therapy

Irina Vladimirovna Kolyadina¹, Peter Kuppen², Cornelis van de Velde², Irina Vladimirovna Poddubnaya¹, Geeske Dekker-Ensink², Esther Bastiaannet², Bianca Prinse², Charla Engels², Apollon Karseladze¹, Georgii Frank¹, Dmitrii Komov¹ and Valeria Ermilova¹.
¹Russian Medical Academy of Postgraduate Education, Russian Cancer Research Center, Moscow, Russian Federation and ²Leiden University Medical Center, Leiden, Netherlands.

Body: Background: Some previous studies suggested a predictive role of immune markers for systemic therapy in patients with breast cancer; we studied the prognostic and predictive value of classical HLA class I (HC10-expression) in breast cancer stage I in women that used adjuvant systemic therapy (endocrine therapy, chemotherapy or both) and compared results with those from patients not systemically treated.

Material and Methods: our study included Russian (n=315) women with breast cancer stage I, treated in RCRC, RMAPE (1985-2009) by surgery ± adjuvant therapy. A Tissue Micro Array taken from tumor blocks of all women was constructed and analyzed in LUMC; sections were immunohistochemically stained for ER, PR, HER2, Ki67 (assessed by standard morphological criteria) and immune marker HC10 scored by the percentage positive tumor cells (0-100); expression between 0-5% (26 cases, 8,5%) was evaluated as negative; >5% (281 cases, 91,5%) as positive. We studied clinical prognostic value of HC10-expression for relapse-free survival (RFS) stratified for systemic therapy using SPSS 20.0.

Results: the total rate of relapses in Russian women that were not systemically treated was 48/105 cases (46%), but in women that received adjuvant systemic therapy it was significantly lower (32/202 women, 16%, p<0,0001). The rate of 5- and 10-years RFS was significant lower in patients that did not use any adjuvant systemic therapy (64% and 54% respectively) as compared to systemically treated women (5- and 10-years RFS: 89% and 82% respectively, p<0,0001). HC10 expression was clinically prognostic in the patient group that was not systemically treated for the rate of relapses (HC10-positive, 44%; HC10-negative, 67%, p=0,04) and for RFS too (HR 0.426; 95% CI 0.180-1.009, p=0.05). In contrast to this, in women that received any adjuvant system therapy, HC10 expression did not correlate with RFS (HR 1.425; 95% CI 0.341-5.967, p=0.628). The data did not retain significance in multivariate analyses (p>0,05) in both groups (systemically treated or not). We suggest to study larger number of observations to confirm our data.

Conclusion: immune marker HC10 is prognostic for tumor relapse in breast cancer. Furthermore, HC10 expression is a good predictable marker for RFS in women that were not systemically treated.
Title: Targeting the mevalonate pathway to overcome acquired anti-HER2 treatment resistance

Huizhong Hu1, Lukas M Simon1, Agostina Nardone1, Chad A Shaw1, Gary C Chamness1, Laura M Heiser2, Nicholas Wang2, C Kent Osborne1 and Rachel Schiff1. 1Baylor College of Medicine, Houston, TX and 2Oregon Health & Science University, Portland, OR.

Body: Background: Compelling preclinical and clinical evidence suggests that a more complete blockade of the HER receptor layer and its signaling, by combining anti-HER2 drugs, such as Trastuzumab (T) and Lapatinib (L), is highly effective. However, resistance is still common and remains a challenge. To understand resistance mechanisms and further to identify novel therapeutic strategies, we established a broad panel of L, T, and L+T resistant cell line models. Initial mRNA expression profiling identified upregulation or restoration of the mevalonate (MVA) pathway in some models where HER signaling is completely and sustainably blocked. The MVA pathway is commonly considered as a biosynthetic process primarily for cholesterol and isoprenoid intermediates, particularly farnesyl and geranylgeranyl pyrophosphates (FPP and GGPP, respectively). Statins, widely-used cholesterol-lowering drugs, block this pathway via inhibition of the rate-limiting enzyme, HMG-CoA reductase. While accumulating evidence also suggests a role of the MVA pathway in tumor initiation and progression, its role in anti-HER2 resistance remains elusive.

Methods: SKBR3, AU565, and UACC812 parental HER2+ cells and their T, L, and L+T resistant (TR, LR, and LTR respectively) derivatives were used in this study. Cell growth after treatment with statins in the presence or absence of MVA, cholesterol, squalene, FPP, or GGPP was measured by methylene blue staining. Apoptosis was determined by Annexin V staining and the protein level of cleaved PARP. Parallel analysis of molecular signaling was done by western blotting.

Results: Blocking the MVA pathway with lipophilic statins, simvastatin or atorvastatin, led to a marked growth inhibition or apoptosis in LR/LTR models, in which the HER signaling remains sustainably inhibited, while cognate parental cells and TR cells, in which HER is (re)activated, were only slightly inhibited. Interestingly, only lipophilic statins (which can be taken up by cancer cells), but not hydrophilic statins such as pravastatin (whose primary target is liver cells), conveyed the inhibitory effect. Prevention of statin-induced apoptosis by adding exogenous MVA indicated that the cell death caused by statin treatment was via its specific blockade of the MVA pathway. Cholesterol or its precursor squalene could not rescue growth inhibition. In contrast, both FPP and GGPP reversed the growth inhibition or apoptosis in SKBR3 and AU565 LR/LTR models, while in the UACC812LTR model only GGPP rescued. Interestingly, mTOR was identified as the downstream signaling target of the MVA pathway in SKBR3 and AU565LTR models, while in the UACC812LTR model, the growth inhibition by statin was due to substantial estrogen receptor (ER) protein reduction.

Conclusion: The MVA pathway plays a key role as an escape pathway by activating alternative signaling, including mTOR and ER pathways, in acquired resistance to potent HER2 inhibition in a cholesterol-independent but FPP/GGPP-dependent manner. Targeting the MVA pathway or its downstream effectors could provide a novel therapeutic strategy to overcome anti-HER2 resistance.
Yukari Hato, Yumi Endo, Nobuyasu Yoshimoto, Tomoko Asano, Mina Yamaguchi, Satoru Takahashi and Tatsuya Toyama.

1Nagoya City University Graduate School of Medical Sciences.

Body: Background: Selective estrogen receptor modulators (SERMs) can reduce the occurrence of breast cancer in high-risk women by 50%. Recently, a genome-wide association study identified SNPs in or near the ZNF423 (rs8060157) and CTSO (rs10030044) genes that were associated with breast cancer risk during SERM therapy and these SNPs were reported to be involved in estrogen-dependent induction of BRCA1 expression (Ingle JN. et al. Cancer Discovery 2013).

Materials and methods: A total of 588 breast carcinomas collected between 1983 and 2003 were available for polymorphism assay. TaqMan pre-designed SNP genotyping assays for ZNF423 rs8060157 and CTSO rs10030044 were used. We investigated whether these SNPs are associated with prognosis in breast cancer patients. The effects of several variables on survival were tested by Cox proportional hazards regression analysis.

Results: Estrogen receptor (ER)-positive breast cancer patients receiving adjuvant endocrine therapy with the genotype GG at CTSO rs10030044 showed significantly shorter disease-free survival (DFS) and overall survival (OS) (\( P = 0.0024 \) and \( P = 0.0003 \), respectively). On the other hand, this genotype were not associated with prognosis in ER-negative breast cancer patients. Multivariate Cox regression analysis revealed that the GG genotype at CTSO rs10030044 was an independent poor prognostic factor in ER-positive breast cancer patients receiving adjuvant endocrine therapy (OS: RR = 1.86; 95%CI, 1.18 to 2.85).

Univariate and multivariate Cox regression analysis of factors associated with overall survival

<table>
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<tr>
<th>Variables</th>
<th>n (%)</th>
<th>Univariate (P value)</th>
<th>Multivariate (P value)</th>
<th>Multivariate (RR :95% CI)</th>
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<tr>
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<tr>
<td>≤2cm</td>
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<td>&lt;.0001</td>
<td>2.268 (1.615-3.181)</td>
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<td>&gt;2cm</td>
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<td>&lt;.0001</td>
<td>&lt;.0001</td>
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<tr>
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<td>1 (reference)</td>
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<td></td>
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<tr>
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<tr>
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<td>TT+GT</td>
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<tr>
<td>GG</td>
<td>109 (19)</td>
<td>0.0282</td>
<td>0.0082</td>
<td>1,860 (1.181-2.853)</td>
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</table>

The SNP, ZNF423 rs8060157, was not associated with prognosis in this study.

Conclusion: We show that the genotype GG at CTSO rs10030044 is an independent factor indicating poor prognosis in ER-positive breast cancer patients receiving adjuvant endocrine therapy.
2014 San Antonio Breast Cancer Symposium

**Publication Number:** P5-05-03  
**Average Grade:** 5.20

**Title:** Clonal evolution of the HER2 L755S mutation leads to acquired HER-targeted therapy resistance that can be reversed by the irreversible HER1/2 inhibitor afatinib

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**Body:** Background: Targeting HER2 with lapatinib (L), trastuzumab (T), or the LT combination, is effective in HER2+ breast cancer (BC), but acquired resistance commonly occurs. In our 12-week neoadjuvant trial (TBCRC006) of LT without chemotherapy in HER2+ BC, the overall pathologic complete response rate (pCR) was 27%. To investigate resistance mechanisms our lab developed 10 HER2+ BC cell lines resistant (R) to these drugs (LR/TR/LTR). To discover potential predictive markers/therapeutic targets to circumvent resistance, we completed genomic profiling of the cell line panel and a subset of pre-treatment baseline specimens from TBCRC006.

Methods: Parental (P) lines and LR/TR/LTR derivatives of 9 HER2+ BC cell line models were profiled with whole exome and RNA sequencing. Mutations detected in R lines but not in same-model P lines were identified. cDNAs were assessed by targeted Sanger sequencing. Single cells of the BT474AZ-LR line were cloned and their cDNAs were sequenced. Mutant-specific Q-PCR was designed to sensitively quantify mutations. Whole exome sequencing (minimum depth 100X) of 17 baseline tumor/normal pairs from TBCRC006 were performed on Illumina HiSeq.

Results: We found and validated the HER2 L755S mutation in the BT474ATCC-LTR line and the BT474AZ-LR line (∼30% of DNA/RNA/cDNA in BT474AZ-LR), in which the HER pathway was reactivated to cause resistance. Overexpression of this mutation was previously shown to induce L resistance in HER2-negative BC cell lines, suggesting a role as an acquired L/LT resistance driver in HER2+ BC. Sanger sequencing of BT474AZ-LR single cell clones found the HER2 L755S mutation in every clone but only in ∼30% of the HER2 copies. Using sensitive mutant-specific Q-PCR, we found statistically higher levels of HER2 L755S expression in BT474ATCC-P and BT474AZ-P compared to parentals of other HER2+ BC cell lines (UACC812/AU565/SKBR3/SUM190). These data suggest that this mutation exists subclonally within BT474 parental lines and was selected to become the more dominant population in the two resistant lines. The HER1/2 irreversible tyrosine kinase inhibitor (TKI) afatinib (Afa) robustly inhibited growth of both BT474ATCC-LTR/AZ-LR cells (IC50: Afa 0.02µM vs. L 3 µM). Western blots confirmed inhibition of the HER and downstream Akt and MAPK signaling in the LR cells by Afa. Sequencing of TBCRC006 baseline samples found the HER2 L755S mutation in 1/17 subjects. This patient did not achieve pCR after neoadjuvant LT treatment. The variant was present in 2% of the reads, indicating it as a subclonal event in this patient’s baseline tumor.

Conclusion: Acquired resistance in two of our BT474 LR/LTR lines is due to selection of HER2 L755S subclones present in the parental cell population. The higher HER2 L755S levels detected in BT474 parents compared with other HER2+ BC parental lines, and detection of its subclonal presence in a pre-treatment HER2+ BC patient, suggest that sensitive mutation detection methods will be needed to identify patients with potentially actionable HER family mutations in primary tumor. Treating this patient group with an irreversible TKI like Afa may prevent resistance and improve clinical outcome of this subset of HER2+ BC.
Title: Acquired resistance to everolimus occurs independently of mTORC1 inhibition in preclinical in vivo models of ER+ breast cancer

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1Division of Hematology/Oncology, David Geffen School of Medicine at UCLA, Los Angeles, CA; 2Novartis Pharmaceuticals Corporation, Cambridge, MA and 3Novartis Pharmaceuticals Corporation, East Hanover, NJ.

Body: Background: The addition of the selective mTORC1 inhibitor everolimus (RAD001/Afinitor®), to exemestane significantly prolongs progression free survival in patients with advanced hormone receptor (ER+) positive breast cancer. However, despite these improved outcomes, the majority of patients that achieve an initial clinical benefit will eventually go on to develop progressive disease. In order to prevent or delay the onset of this acquired resistance, it is essential to identify the mechanisms by which continued proliferation is being driven in these resistant tumors. In this study, we used reverse phase protein array (RPPA) technology to compare cell signaling in ER+ breast cancer cell line xenografts that were responsive to everolimus versus those that had acquired resistance to everolimus.

Materials and Methods: Xenograft models were established from three ER+ breast cancer cell lines containing different molecular alterations that confer activated PI3K/mTOR signaling; MCF7 and KPL-1 (both PIK3CA mutant) and ZR75-1 (PTEN-null). Mice were treated daily with either vehicle or 10 mg/kg everolimus until progression. Snap frozen tissue samples (n = 3) were collected from vehicle control tumors, from everolimus resistant tumors and from everolimus responsive tumors. Whole cell lysates were prepared from tumor tissues and processed for RPPA analysis. A customized panel of 27 known cancer/PI3K pathway associated signaling proteins were selected for the RPPA analysis.

Results: In the vehicle controls, significantly higher levels of pAKT, pPRAS40 and p4EBP1 were detected in the PTEN-null ZR75-1 tumors compared to the PIK3CA mutant tumors, indicating potential variations in intensity of PI3K/AKT pathway signaling depending on the initiating molecular alteration. Complete inhibition of tumor proliferation was observed in response to everolimus in all 3 xenograft models for up to 7-weeks of treatment, until tumors showed signs of progression. Significantly lower S6 phosphorylation was observed in tumors responding to everolimus, which was accompanied by feedback activation of pAKT. Significant inhibition of S6 phosphorylation was also observed in each of the ZR75-1 and MCF7 tumors that were progressing on everolimus, indicating that although the tumor cells have acquired resistance to the anti-proliferative activity of everolimus, mTORC1 signaling is still being successfully blocked. Feedback activation of pAKT was observed in the ZR75-1 resistant model, however no feedback activation of AKT occurred in the MCF7 resistant model. The KPL-1 model showed the expected loss of S6 pathway inhibition upon resistance. No significant signaling changes were observed in any of the other signaling proteins measured, including ER, HER2, EGFR, MEK and ERK. Ongoing studies to measure the mRNA expression changes in these tumors using mRNA microarrays may identify signaling pathways that are driving the proliferation of these resistant tumors.

Discussion: These preclinical data show that multiple pathways of resistance can develop in response to long-term everolimus treatment and resistance can occur despite continued inhibition of PI3K/mTOR signaling. Data from mRNA microarray experiments will be presented at the time of the meeting.
Title: Low molecular weight cyclin E regulates response to aromatase inhibitors in post-menopausal breast cancer patients

Iman Doostan¹, Stacy L Moulder², Kelly K Hunt³ and Khandan Keyomarsi¹. ¹University of Texas, Graduate School of BioMedical Sciences, MD Anderson Cancer Center, Houston, TX; ²University of Texas MD Anderson Cancer Center, Houston, TX and ³MD Anderson Cancer Center, Houston, TX.

Body: Almost seventy percent of all breast cancer patients have estrogen receptor (ER) positive tumors requiring hormonal therapy. Aromatase inhibitors (AIs) are considered as the first line hormonal therapy for ER+ post-menopausal patients. However, resistance to these drugs remains a major challenge in clinic and the biology of such resistance is not clear. Previous studies have shown that cyclin E pathway, a key regulator in the G1 to S transition of cell cycle, is deregulated in breast cancer. Full-length cyclin E is abnormally cleaved into low molecular weight isoforms (LMW-E) that renders patients to poor survival. Here we hypothesize that cyclin E deregulation can confer resistance to AIs. To address this, we engineered aromatase overexpressing MCF7 cells to overexpress LMW-E under doxycycline inducible promoter. Full-length cyclin E, GFP and empty vector transfected cells were also generated and used as controls. Our results indicated that AIs inhibited proliferation by arresting the cells at G1 phase of the cell cycle. However, LMW-E expression significantly enhanced proliferation of the cells when treated with AIs. In addition, LMW-E bypassed G1 arrest following AI treatment. At the molecular level, AIs decreased CDK2, pCDK2, and Rb levels and attenuated Rb phosphorylation. However, these effects were completely rescued only when LMW-E was expressed. Moreover, using an in vitro kinase assay we indicated that AIs decreased CDK2 kinase activity while LMW-E expression reversed this effect by increasing CDK2 enzymatic activity. Taken together, these results suggest that LMW-E inactivates Rb protein as a tumor suppressor and renders the cells to bypass G1 checkpoint following AI treatment. In addition, this study provides early evidence that CDK2 inhibitors could be beneficial in combination with AIs for LMW-E expressing tumors. Currently we are investigating whether LMW-E can bypass the activity of AIs using inducible breast cancer cell line xenograft. We are also examining the correlation between cyclin E status and response to treatment in a cohort of patients who received AIs in the neo-adjuvant setting.
Title: Proteomics studies reveal important pathway and phosphoprotein changes contributing to cancer stem cell properties and drug resistance of triple negative breast cancer cells cultured in attached and suspension conditions

Xinyu Deng¹, Joe Capri², Huan Ming Hsu¹, Morris Kohanfars¹, William Luo¹, Puneet Souda², Julian P Whitelegge² and Helena R Chang¹. 'Gonda/UCLA Breast Cancer Research Laboratory and the Revlon/UCLA Breast Center, Los Angeles, CA and ²Pasarow Mass Spectrometry Laboratory, NPI-Semel Institute, University of California, Los Angeles, CA.

Body: Breast tumors without ER, PR and HER2 expression are referred to as triple-negative breast cancer (TNBC) and have been associated with a higher rate of recurrence and distant metastasis compared to other types of breast cancers. Because they lack a clear therapeutic target, chemotherapy is the only systemic treatment option for TNBC patients. Previous works suggested that the chemotherapy-resistant residual tumor cells may contribute to the high rate of recurrence and metastasis of TNBC. Recently cancer stem cells and circulating tumor cells have been reported to have a close relationship with cancer metastasis. Culturing tumor cells in suspension conditions was also reported to be an efficacious way to enrich cancer stem cells and to mimic the living conditions for circulating tumor cells. In the current study, four TNBC cell lines HCC1937, HCC1187, MDA-MB-468 and MDA-MB-231 were cultured in both attached and non-attached suspension conditions. HCC1937 and MDA-MB-231 cells formed tumorspheres in suspension condition and became more resistant to docetaxel comparing to those in the attached condition. Although MDA-MB-468 cells were unable to form tumorspheres in suspension conditions they formed clusters of aggregated cells that were also more resistant to docetaxel in suspension. HCC1187 cells could not form either tumorspheres or clusters in suspension conditions and were found to be more sensitive to docetaxel. Toward uncovering the signaling changes in these cell lines in different conditions, we performed a proteomics study to compare the phosphoproteomes of these cells in both culturing conditions. Totally 2,027 phosphorylated protein groups with no decoy, 5,474 unique phosphopeptides and 5,711 unique phosphosites were identified by a LTQ-Orbitrap LC-MS/MS system. Pathway analysis showed that MAPK signaling pathway, Focal adhesion pathway, p53 signaling pathway and MicroRNA pathways were dramatically changed in HCC1937, MDA-MB-231 and MDA-MB-468 cells, suggesting that these pathways may contribute to the stem cell properties and drug resistance of these cells. To further find out why HCC1187 behaved differently from the three other cell lines, we analyzed the phosphoprotein changes between the two groups in both culturing conditions. Interestingly, in the suspension condition, 17 phosphoproteins down-regulated in the three cell lines were found to be up-regulated in HCC1187 and one phosphoprotein up-regulated in the three cell lines was found to be down-regulated in HCC1187. More detailed studies of these functional changes to the phosphoproteome may further elucidate the roles of cancer stem cells and circulating tumor cells in drug resistance and relapse in TNBC.
Title: Elucidating molecular resistance to trastuzumab using next generation sequencing in isogenic cell models

Abde M Abukhdeir¹, Matthew Najor¹, Sanja Turturro¹, Melissa R Pergande¹, Jeffrey A Borgia¹, Hanif G Khalak² and Melody Cobleigh¹. ¹Rush University Medical Center, Chicago, IL and ²Weill Cornell Medical College, Doha, Qatar.

Body: A minority of all breast cancers will express increased levels of the ERBB2 protein. They are eligible for trastuzumab-based therapy. Some will respond, but all will progress. Thus, the problem of resistance to trastuzumab has generated an urgent need to determine the underlying mechanisms of that resistance.

Cancer is a genetic disease and the mechanism of trastuzumab resistance is likely also genetic in nature. However, the significant genomic heterogeneity between and within patient tumors greatly complicates the identification of a genetic mechanism of resistance for trastuzumab. In order to overcome some of these challenges, we looked to an isogenic model of trastuzumab resistance. We acquired the trastuzumab-sensitive breast cancer cell line, BT474 and two clones of this cell line that were conditioned to exhibit trastuzumab resistance.

To investigate a possible genetic mechanism of trastuzumab-resistance, we performed whole exome sequencing using Ampliseq chemistry on the Ion Torrent platform from Life Technologies and paired-end RNA-sequencing on the Illumina HiSeq platform. Next-generation sequencing data was bioinformatically analyzed using tools that allowed us to filter relevant variants based on statistical and functional significance. Variants of interest were those that that arose during drug treatment, which were identified as those in each of the resistant clones, which were novel compared to the parent clone. Proteins from whole cell lysates were resolved in two dimensions using 3-10 nonlinear strips for isoelectric focusing followed by resolution via 4-20% SDS-PAGE. Proteins were visualized via Gelcode blue and cored with a biopsy punch, trypsinized, and submitted for protein ID on an LTQ XL mass spectrometer. We performed functional validation of genetic alterations through the use of somatic cell gene targeting of an ERBB2-expressing clone of the MCF-10A cell lines, a non-tumorigenic model of breast cancer, which is sensitive to trastuzumab. Exome sequencing initially yielded more than 10,000 unique DNA variants across the three clones, which after bioinformatic analysis resulted in ~1000 variants of interest. Two-dimensional gel electrophoresis revealed 25-30 differentially expressed proteins per sample. Correlation between the sequencing and proteomics data provided us with a candidate gene list of less than 100 genes and several cellular pathways related to growth signaling and immunity. Genetic alterations were tested for their ability to cause trastuzumab resistance in MCF-10A clones.

We describe herein a detailed molecular analysis for a model of trastuzumab resistance. Validated genetic alterations will be investigated in a unique collection of archival specimens, which we hope will open the path towards the development of novel agents to augment the effects of trastuzumab.
Title: Sensitivity to c-Met inhibition is increased in dasatinib resistant TNBC cells

Patricia Gaule¹, Brendan Corkery¹, John Crown², Micheal J Duffy² and Norma O’Donovan¹. ¹National Institute for Cellular Biotechnology, Dublin City University, Dublin, Ireland and ²St Vincent's University Hospital, Dublin, Ireland.

Body: Pre-clinical models TNBC cells have demonstrated sensitivity to the multi-targeted Src kinase inhibitor dasatinib, however clinical trials with single agent dasatinib showed limited efficacy in unselected populations. Trials of dasatinib in combination with chemotherapy are ongoing. In order to study potential mechanisms of resistance to dasatinib in TNBC we established a cell line model of acquired dasatinib resistance (231-DasB).

The dasatinib resistant cell line (231-DasB) was developed by constant exposure to incrementally increasing concentrations of dasatinib, from 200 nM to 500 nM over a period of 13 weeks. Cell proliferation was measured by acid phosphatase assay after 5 day treatment with SRC inhibitors (dasatinib, PD180970), EGFR inhibitors (gefitinib and neratinib), chemotherapy drugs (carboplatin, docetaxel and doxorubicin) and a c-Met inhibitor (CpdA, Amgen). P values were calculated using the Student’s T-Test (2 tailed with unequal variance). Expression and phosphorylation of c-Met and Src was measured by immunoblotting and multiplex magnetic bead assays carried out on a MAGPIX® Instrument.

Following approximately 3 months exposure to dasatinib, 231-DasB cells were resistant to dasatinib with IC50 > 5 µM compared to 0.04 ± 0.001 µM in MDA-MB-231. 231-DasB cells also showed resistance (2.2-fold) to the Src kinase inhibitor PD180970 [table 1]. 231-DasB cells showed small but statistically significantly increases in sensitivity to docetaxel and doxorubicin.

<table>
<thead>
<tr>
<th>Drug</th>
<th>MDA-MB-231</th>
<th>231DasB</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>PD180970 IC50 (µM)</td>
<td>0.40 ± 0.04</td>
<td>0.87 ± 0.07</td>
<td>0.003</td>
</tr>
<tr>
<td>CpdA IC50 (µM)</td>
<td>&gt;10</td>
<td>2.1 ± 0.1</td>
<td>0.0001</td>
</tr>
<tr>
<td>Docetaxel IC50 (nM)</td>
<td>1.9 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>0.003</td>
</tr>
<tr>
<td>Doxorubicin IC50 (nM)</td>
<td>138.6 ±1.0</td>
<td>97.2 ±6.6</td>
<td>0.007</td>
</tr>
<tr>
<td>Carboplatin IC50 (µM)</td>
<td>13.2 ± 0.8</td>
<td>11.5 ± 1.3</td>
<td>0.147</td>
</tr>
<tr>
<td>Gefitinib IC50 (µM)</td>
<td>23.1 ± 2.2</td>
<td>20.9 ± 2.1</td>
<td>0.289</td>
</tr>
<tr>
<td>Neratinib (% growth @ 10µM)</td>
<td>94.5 ± 2.0</td>
<td>95.9 ± 7.6</td>
<td>0.834</td>
</tr>
</tbody>
</table>

No significant change in sensitivity to carboplatin or to the EGFR inhibitors gefitinib and neratinib was observed. However, the 231-DasB cells demonstrated a significant increase in sensitivity to the c-Met inhibitor, CpdA, with an IC50 value of 2.1 ± 0.1 µM compared to an IC50 greater than 10 µM in the parental MDA-MB-231 cells. Treatment of MDA-MB-231 cells with dasatinib (100 nM) blocked phosphorylation of Src kinase. In contrast, dasatinib treatment (100 nM) did not decrease p-Src levels in the 231-DasB cells. p-Met levels were significantly increased in 231-DasB cells relative to MDA-MB-231. Treatment with 2 µM CpdA decreased p-Met and p-Src in both 231-DasB and MDA-MB-231 cells. Other key receptor tyrosine kinases (EGFR, HER2, HER3, HER4, IGFIR and IR) show no significant changes in phosphorylation in 231-DasB cells compared to MDA-MB-231. Constitutive activation of p-Src through increased c-Met signalling may be a potential mechanism of resistance and suggests that combined treatment with dasatinib and a c-Met inhibitor may block the development of acquired resistance.
**Title:** Autophagy in three dimensional cultures provides survival advantage against trastuzumab in HER2+ mammary adenocarcinoma cells

Cristina E Rodríguez¹, Sara Reidel¹, Elisa D Bal de Kier Joffé¹, María A Jasnis¹ and Gabriel L Fiszman¹. ¹Institute of Oncology Angel H. Roffo, Ciudad Autónoma de Buenos Aires, Ciudad de Buenos Aires, Argentina.

**Body:** HER2 is overexpressed in 20-25% invasive breast tumors, and correlates with low free disease survival. Trastuzumab (Tz), monoclonal antibody anti HER2, is used to treat HER2+ tumors; however more than half of them are resistant or acquire resistance during treatment. Autophagy has been proposed as a tumoral escape mechanism. Multicellular tumor spheroids (MCS) are a model of cell growth in 3D that mimics the structure of in vivo avascular tumors. We have previously demonstrated that MCS present different subpopulations, with a gradient of proliferative, quiescent and apoptotic cells towards the center of the spheroid and these characteristics makes it more resistant to Tz than monolayers. The aim of this study was to analyze the role of autophagy in MCS growth and its relevance on the resistance of breast cancer cells to Tz. MCS of overexpressing HER2+ human mammary tumor cells (BT474 cell line) were cultured as cell suspensions on agar, one per well, and experiments were conducted when MCS reached 550 um initial size. When the autophagy marker LC3 was analyzed in MCS by Western blot, both LC3-I and LC3-II were significantly up-regulated compared with cells cultured as monolayers. The functional autophagic flux was confirmed by immunoblots of LC3 and p62 (SQSTM1/sequestosome1) in cells treated with Bafilomycin A1 (5 nM). MCS were fixed and included in paraffin to analyze the expression of LC3 and observed a differential localization of autophagic cells, increasing towards the center of the spheroid, correlating with the hypoxic population previously described. Upon Tz addition at a concentration of 50 ug/ml, a higher and uniform expression of LC3 was found in all the living cells. These observations were further supported by the finding that p62 was down-regulated in Tz treated spheroids in opposition to controls (commercial IgG).

In 2D, Tz (1 ug/ml) also exerted LC3-II conversion and increase in autophagosomes formation. Autophagy inhibition by 3-methiladenine (3-MA) used at 1 mM, in combination with Tz decreased two fold vs Tz alone in monolayers (49% vs 76% cell viability respectively, p<0.05). In 3D, the reduction in size induced by the autophagy inhibition plus Tz was 13% vs 10% Tz alone. 3-MA alone did not elicited citotoxicity in 2D or 3D. To investigate the link between apoptosis and autophagy, we expose monolayers and MCS to Tz during 6h with 1h of pre-incubation with 3-MA and analyze by Annexin V/propidim iodide. In monolayers, the combination of 3-MA with Tz enhanced Tz sensitivity since they induced an increase in total cell apoptosis 35% compared to Tz alone (15% vs 11% early + 21% vs 13% late apoptosis, p<0.05). Surprisingly in 3D, Tz had an opposite effect and decreased by 30% late apoptosis; however, this Tz-protection against apoptosis was fully reversed by the inhibition of autophagy. We conclude that Tz exerted a differential effect in BT474 breast cancer cells cultured as MCS, inhibiting apoptosis and generating a smaller spheroid composed only of remaining Tz-resistant living cells which are autophagic addicted. We propose that this model could be useful to study the mechanisms involved in resistance to Tz.
2014 San Antonio Breast Cancer Symposium

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Average Grade: 6.17

Title: Leptin peptide receptor antagonist linked to nanoparticles: A novel adjuvant therapy for triple negative breast cancer

Tia L Harmon¹, Adriana Harbuzariu¹, Courtney D Dill¹, Lily Yang² and Ruben R Gonzalez-Perez¹. ¹Morehouse School of Medicine, Atlanta, GA and ²Emory University, Atlanta, GA.

Body: Background: Obesity and high leptin levels are strongly associated with breast cancer, relapse, drug resistance, and poor patient outcomes. Over expression of leptin and its receptor, Ob-R, induce cell proliferation, angiogenesis, and metastasis in Triple Negative Breast Cancer (TNBC). This aggressive form of the disease has no targeted therapy and chemotherapeutics show several undesirable side effects. We have created a Leptin Peptide Receptor Antagonist, LPrA2, which has been shown to effectively prevent leptin signaling. LPrA2 was coupled to iron oxide nanoparticles (IONPs) and used to determine its potential use as an adjuvant to chemotherapeutics.

Methods: IONPs, bound to LPrA2, were confirmed by Western Blot. TNBC cells were then treated with IONP-LPrA2 plus Cisplatin, Doxorubicin, Paclitaxel, Cyclophosphamide, and Sunitinib. Subsequently, the TNBC cells were analyzed for proliferation, cell cycle progression, and apoptosis with the Cellometer Vision Image Cytometer®.

Results: IONP-LPrA2 was found to cause a greater decrease in DNA synthesis during the S phase of the cell cycle in TNBC cells than LPrA2 alone. Additionally, IONP-LPrA2 when combined with chemotherapeutics or anti-angiogenic drugs showed synergistic effects on cell proliferation and apoptosis.

Conclusion: These findings indicate that IONP-LPrA2 may be useful in the prevention and treatment of TNBC. Further IONP-LPrA2 treatment may increase the efficiency of chemotherapeutics. These results could be particularly relevant for obese patients, whom show high incidence and the poorest outcome of TNBC.

Acknowledgements: This work was partially supported by the National Institutes of Health and National Cancer Institute Grant 1SC1CA138658-05 and U54 CA118638, and DOD Idea Award BC 123427 to RGP; and facilities, and support services at Morehouse School of Medicine (NIH RR03034 and 1C06 RR18386) and NIH/NCRR grant 1G12RR026250-03.
Growth hormone receptor silencing sensitizes triple negative breast cancer cells to chemotherapy

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Body: Triple negative breast cancer (TNBC) is an aggressive form of breast cancer, which, accounts for approximately, 15% of breast cancers diagnosed in women. TNBCs lack expression of estrogen receptor, progesterone receptor and human epidermal growth factor receptor type 2 (HER2). So, there are no targeted therapies available for TNBCs and chemotherapy is the main choice for the treatment. Although the tumors respond initially, large numbers of patients develop recurrence due to chemoresistance. Growth hormone receptor (GHR) is a class I cytokine receptor, which plays vital role in the development of chemoresistance. Studies have shown that silencing of GHR sensitizes breast cancer cells to chemotherapeutic drugs. In this study we investigated the impact of GHR silencing in TNBC cells and further analyzed the possible mechanisms associated with GHR silencing induced sensitization. GHR was silenced using small interfering RNAs in metastatic breast cancer cells MDA MB 231 and MDA MB 468. Molecular analyses were performed to determine apoptosis, cytotoxicity, colony formation, invasion and migration in GHR knockdown cells. Silencing of GHR induced cytotoxicity and apoptosis in TNBC cells. Migratory and invasive potentials were drastically reduced in GHR silenced cells. Moreover, epithelial to mesenchymal transition markers were significantly down regulated by GHR siRNA treatment. GHR targeting significantly increased the efficiency of docetaxel against TNBC cells. Inhibition of GHR also inhibited the expression of BCRP, which is frequently associated with the development of chemoresistance in breast cancer. Further, treatment with GH induced the overexpression of drug transporter proteins involved in chemoresistance. Inhibition of GHR in breast cancer cells reverted the expression of these proteins and sensitized the cells to docetaxel. These findings support the hypothesis that targeting GHR could have a potential new therapeutic approach to overcome chemoresistance in TNBCs.
**Title:** Exosomes mediate the transfer of drug resistance in MCF-7 breast cancer cells by P-glycoprotein

Jinhai Tang\(^1\), Mengmeng Lv\(^2\), Shanliang Zhong\(^3\), Weixian Chen\(^4\) and Jianhua Zhao\(^5\). \(^1\)JiangSu Cancer Hospital, Nanjing, Jiangsu, China; \(^2\)JiangSu Cancer Hospital, Nanjing, Jiangsu, China; \(^3\)JiangSu Cancer Hospital, Nanjing, Jiangsu, China; \(^4\)JiangSu Cancer Hospital, Nanjing, Jiangsu, China and \(^5\)JiangSu Cancer Hospital, Nanjing, Jiangsu, China.

**Body:** Background: Drug resistance plays an important role in success or failure of anticancer therapies. Recently, exosomes are reported to maybe play an important role in acquired drug resistance. Our previous studies demonstrated that human breast cancer cell line MCF-7 could acquire drug resistance and the increased survival potential when it is co-incubated with the cell culture supernatant from its resistant variant MCF-7/Adr or MCF-7/Doc. In addition, the overexpression of P-glycoprotein (P-gp, a plasma membrane multidrug efflux transporter) is one of the most widely observed mechanisms contributing to multiple drug resistance. However, little research focuses on the role of P-gp in drug resistance transfer through exosomes in cancer. This study aimed to investigate the role of exosomes in drug-resistance transfer between breast cancer cells and explore the potential mechanism.

**Material and methods:** An isogenic docetaxel-resistant MCF-7 subline (MCF-7/Doc) was established by exposing MCF-7/S (parent drug-sensitive MCF-7) to gradually increasing concentrations of Doc in vitro. Exosomes from MCF-7/S (exo/S) and from MCF-7/Doc (exo/Doc) were respectively extracted from their cell culture supernatants and were identified by transmission electron microscope and Western blot. Exosomes were dyed by PKH26 with red fluorescence, and the absorption of dyed exosomes by cells was observed under a confocal microscopy. Drug resistance was assessed by apoptosis analysis and MTT assays of P-gp expression was analyzed by Western blot and Flow cytometry.

**Results:** Similar to MCF-7/S, MCF-7/Doc secreted exosomes, which exhibited round or elliptic shape ranging from 30 to 100nm in diameter with intact membrane, and only expressed Tsg101 protein marker of exosomes. After co-incubation of stained exosomes with MCF-7/S or MCF-7/Doc cells, the exosomes were observed to be absorbed by the receipt cells. The apoptotic rate of the MCF-7/S incubated with exo/Doc was reduced by 35% against Doc compared to MCF-7/S (\(P=0.01\)), but the apoptotic rates were not different between MCF-7/S alone and MCF-7/S incubated with exo/S (\(P=0.145\)). MTT showed the result similar to that of apoptosis assay. IC50 of MCF-7/S incubated with exo/Doc was higher (about 50%) than MCF-7/S incubated exo/S (\(P=0.027\)), but the IC50s between MCF-7/S with and without treatment with exo/S were not different (\(P=0.44\)). MCF-7/Doc expressed significantly higher P-gp in comparison with MCF-7/S, and exo/Doc also expressed higher level of P-gp than exo/S. In the analysis of P-gp expression by flow cytometry, there was a shift of the peak corresponding to MCF-7/S toward the regions of high P-gp levels after incubation with exo/Doc. The amount of exo/Doc might affect the P-gp expression, as the MCF-7/S co-incubated with 2-fold exo/Doc (80\(\mu\)g) expressed higher P-gp than the MCF-7 treated with 1-fold exo/Doc (40\(\mu\)g).

**Conclusions:** Both MCF-7/Doc and its exosomes overexpress P-gp. After co-incubation with exo/Doc, MCF-7/S acquire drug resistance in some degree and its P-gp expression also increase. Exosomes are effective in transferring drug resistance as well as P-gp from drug-resistant breast cancer cells to sensitive ones. The delivery of P-gp via exosomes may be a mechanism of exosome-mediated drug resistance transfer.
Body: Introduction: Obesity is associated with a more aggressive breast cancer and a worse outcome following chemotherapy treatment. Triple negative breast cancer is a highly aggressive subtype of breast cancer characterized by the absence of estrogen, progesterone, and human epidermal growth factor-2 (HER2) receptors. This subtype has also been correlated with a worse prognosis. This study aims to investigate the impact of obesity on triple negative breast cancer cells' response to chemotherapy treatment and elucidate potential therapeutic options to improve response.

Methods: Sera was collected from breast cancer patients at the Cancer Therapy and Research Center at The University of Texas Health Science Center at San Antonio (UTHSCSA) and pooled according to body mass index (BMI) category (Control:18.5 to 24.9 kg/m^2; Obese:≥30 kg/m^2). MDA-MB-231 cells, a triple negative breast cancer cell line, were grown in serum-free media supplemented with 2% obese or control patient sera to create an in vitro model of obesity. The effects of docetaxel, a chemotherapeutic agent, on cell viability in the presence of obese versus control sera were examined by MTT. Modulation of B-cell lymphoma 2 (Bcl-2), cyclooxygenase-2 (COX-2), and mammalian target of rapamycin (mTOR) were evaluated as possible mechanisms for obesity-induced chemotherapy resistance.

Results: The obese sera significantly reduced sensitivity of the MDA-MB-231 cells to docetaxel. In fact, there was no variance in the viability level of cells grown in obese patient sera for 96 hours with and without docetaxel treatment. The mechanism for resistance does not appear to involve Bcl-2 or COX-2, previously implicated in docetaxel resistance. Intriguingly, suppression of mTOR did provide significant benefit, in agreement with our previous in vivo studies investigating obesity and aromatase inhibitor (AI) response. The role of the mTOR pathway in modulating therapeutic response is still being investigated.

Conclusion: Exposure to obesity-associated circulating factors confers chemotherapy resistance in triple negative breast cancer cells. Further research will be conducted to determine the mechanism by which obesity promotes chemotherapy resistance and whether modulation of the mTOR pathway will provide significant benefit.
Body: Background: Defects in the homologous recombination (HR) DNA repair pathway sensitize tumors to therapeutics that target this pathway. A significant proportion of triple negative breast cancers (TNBC) carry HR defects. Recently three DNA-based measures (LOH, Abkevich et al.; TAI, Birkbak et al; LST, Popova et al.) have been developed and shown to be highly associated with BRCA1/2 mutation status and sensitivity to platinum-based chemotherapy in TNBC. Standard chemotherapy consists of some combination of an anthracycline and cyclophosphamide, with or without a taxane. This study assesses the association of LOH, TAI, LST and the sum of these measures, the HRD Score, with response to standard neoadjuvant chemotherapy in patients with TNBC.

Methods: Tumor samples were retrospectively obtained from 45 TNBC patients and 2 BRCA1/2 mutation positive hormone receptor-positive/HER2-negative breast cancer patients who received anthracycline-based neoadjuvant chemotherapy at Stanford University Medical Center or Cedars-Sinai Medical Center under IRB approved protocols. Measures of LOH, TAI, LST were obtained; tumor BRCA1/2 mutation analysis was conducted; and BRCA1 promoter methylation status was determined. Response was categorized by the residual cancer burden (RCB) score with responders defined as RCB 0 or 1, and non-responders as RCB 2 or 3. Data were also analyzed using the outcome of pathologic complete response (RCB 0). BRCA1/2 deficiency was defined as either BRCA1/2 germline or somatic mutant, or BRCA1 methylated with loss of the second allele in the tumor confirmed by LOH at the affected gene. Associations of LOH, TAI, LST, HRD Score, BRCA1/2 deficiency and mutation status with response to neoadjuvant chemotherapy were evaluated with univariate logistic regression models.

Results: All three metrics showed significant association with response (LOH: p=0.0018; TAI: p=0.0057; LST: p=0.0043) and pCR (LOH: p=0.0061; TAI: p=0.0044; LST: p=0.047). The sum of the scores, HRD Score, was also significantly associated with response (p=0.0021) and pCR (0.011). Neither BRCA1/2 mutation status (p=0.78 and p=0.31 respectively) nor BRCA1/2 deficiency (p=0.12 and p=0.34 respectively) were significantly associated with response or pCR in this cohort. When the analysis was restricted to BRCA1/2 intact samples (n=29) all scores were still significantly associated with pCR (LOH: p=0.019; TAI: p=0.0046; LST: p=0.013; HRD: p=0.0067), while only the sum was significant for response (p=0.042).

Conclusions: All three measures of HR deficiency, LOH, TAI and LST and the sum of these three metrics, the HRD Score, are significantly associated with response to standard neoadjuvant chemotherapy in TNBC. This observation is consistent with the mechanisms of action of doxorubicin and cyclophosphamide as DNA damaging agents, though we have yet to examine cohorts of patients treated with non-anthracycline-containing standard chemotherapy regimens, such as docetaxel and cyclophosphamide. The HRD Score could be used clinically to identify patients with increased sensitivity to DNA damaging therapeutics.
Title: Combination of the PARP inhibitor E7449 with eribulin +/- carboplatin in preclinical models of triple negative breast cancer

Sharon McGonigle1, Jiayi Wu1, Donna Kolber-Simonds1, Natalie C Twine1, Jue-Ion Shie1, Noel Taylor1, Sergei Agoulnik1, Zoltan Dezso1, Shannon McGrath1, Mark Matijevic1, Shanqin Xu1, Galina Kuznetsov1, Mary Woodall-Jappe1 and Kenichi Nomoto1. 1Eisai Inc, Andover, MA.

Body: Introduction: In a small neoadjuvant study in patients with triple negative breast cancer (TNBC) the combination of eribulin plus carboplatin was effective, with a pathologic complete response rate of 43% following 4 cycles of treatment. Significant numbers of sporadic TNBC tumors are deficient in DNA repair capacity and share clinical and pathological features with hereditary BRCA1 mutant disease. PARP inhibitors have demonstrated synthetic lethality in cancer cells with defective DNA repair and have therapeutic potential for TNBC. In this study we describe the combination of PARP inhibitor E7449 with eribulin +/- carboplatin in preclinical models of TNBC.

Methods: E7449, an orally available PARP inhibitor, was administered in combination with eribulin +/- carboplatin to 4 s.c. xenograft models of TNBC: MDA-MB-436 (BRCA1 mutant, PTEN deficient), MDA-MB-468 (BRCA wild type, PTEN deficient), HCC1806 and MDA-MB-231 (BRCA and PTEN wild type).

Results and Discussion: Addition of E7449 to eribulin significantly delayed tumor progression in PTEN deficient MDA-MB-468 xenografts. In the BRCA1 mutant and PTEN deficient MDA-MB-436 xenograft model, combination of E7449 with eribulin enhanced antitumor activity versus eribulin alone. Similar potentiation was observed for carboplatin upon combination with E7449. Treatment of MDA-MB-436 xenografts with the triple combination of E7449 + eribulin + carboplatin was more efficacious than any double combination and was well tolerated at the doses examined. In contrast, no significant combination activity was observed for E7449 plus eribulin in the BRCA and PTEN wild type xenografts HCC1806 and MDA-MB-231, and similarly no potentiation of carboplatin was observed in an MDA-MB-231 xenograft. Notably, combination activity was observed in the BRCA1 mutant (MDA-MB-436) and PTEN deficient (MDA-MB-436 and MDA-MB-468) xenografts and not in the BRCA and PTEN wild-type models (HCC1806 and MDA-MB-231). Data from ongoing studies to evaluate the combination activity of E7449 + eribulin in patient-derived xenograft (PDx) models of TNBC will be presented at the meeting.

Potential biomarkers of sensitivity to the combination are under investigation in both cell line xenograft and PDx models and will be described.

Conclusion: The addition of E7449 to eribulin +/- carboplatin increased antitumor activity in a subset of TNBC models. Biomarker studies aimed at a better understanding of the underlying cause of sensitivity are underway. The preclinical data support assessment of E7449 + eribulin + carboplatin combination therapy in the current phase I/II clinical trial.
The PARP inhibitor niraparib demonstrated activity in patient-derived triple-negative breast cancer xenograft models with high homologous recombination deficiency (HRD) score

Yan Wang1, Stefano Cairo2, Olivier Deas2, Anne-Renee Hartman3, Joshua Jones3, Alexander Gutin3, Jerry Lanchbury3, Zaina Sangale3, Cara Solimeno3, Jean-Gabriel Judde2, Kirsten Timms3 and Keith Wilcoxen1. 1Tesaro, Waltham, MA; 2Xentech, Evry, France and 3Myriad Genetic Laboratories, Inc, Salt Lake City, UT.

Body: Triple negative breast cancer (TNBC), which comprises 15% of all breast cancers, has a poor prognosis and currently lacks effective treatment. TNBCs are highly proliferative, genomically unstable and share molecular characteristics with that of BRCA1/2 mutation driven breast cancer. Poly(ADP-ribose) polymerase-1 (PARP) is a key DNA repair enzyme that mediates single strand break (SSB) repair through the base excision repair (BER) pathway. PARP inhibitors have been demonstrated to selectively kill tumor cells that harbor BRCA1 and BRCA2 mutations. In addition, pre-clinical and preliminary clinical data suggest that PARP inhibitors are selectively cytotoxic for tumors with homologous recombination repair deficiency caused by dysfunction of genes other than BRCA1 or BRCA2.

Niraparib is a potent, orally active PARP inhibitor that is being evaluated in Phase 3 clinical studies for ovarian cancer and BRCA related breast cancer. Previously, we demonstrated that a subset of basal breast cancer (BBC) patient-derived xenograft (PDX) models responded robustly to single agent niraparib treatment. To understand the selectivity observed, the samples from a collection of 37 BBC PDX models have been subjected to homologous recombination deficiency (HRD) analysis. HRD analysis is a DNA-based assay that is capable of detecting homologous recombination deficiency independent of its etiology. Genome-wide SNP data was generated from a custom Agilent SureSelect XT capture followed by sequencing on an Illumina HiSeq2500. SNP data was analyzed using three algorithms (LOH, TAI and LST scores), and the final HRD score is the sum of the LOH+TAI+LAST scores.

Niraparib’s antitumor activity was investigated in patient derived BBC models with various HRD scores. The correlation between niraparib efficacy, HRD score and BRCA deficiency will be discussed.
Title: miRNAs associated with DNA repair capacity in Puerto Rican women with breast cancer

Jaime Matta¹, Clara Isaza¹, Carmen Ortiz¹, Erick Suarez² and Luisa Morales¹. ¹Ponce School of Mediicne and Health Sciences, Ponce, Puerto Rico and ²University of Puerto Rico, San Juan, Puerto Rico.

BACKGROUND: MicroRNAs (miRNA) are short non-protein-coding RNAs that regulate gene expression at the post-transcriptional level via binding to 3′-untranslated regions of protein-coding transcripts. Some miRNAs have been used as diagnostic, prognostic and therapeutic markers of breast cancer (BC). It is well established that dysregulation of DNA repair capacity (DRC) is an important risk factor of BC. However, there is little published information as to what specific miRNAs are associated with DRC in women with BC. OBJECTIVE: The main objective of this study was to identify candidate miRNAs associated with dysregulation of DRC in women with BC. METHODS: Plasma samples from 30 BC cases and 30 controls selected based on their DRC levels (low, high) using a proprietary algorithm. Samples were analyzed for miRNA expression utilizing protocols from Applied Biosystems (Life Technologies). The miRNA expression profiling was performed utilizing the RT-PCR TaqMan Array Human MicroRNA A Cards v 2.0 (Applied Biosystems) containing 383 miRNA probes. Single-stranded cDNA was synthesized from 200 ng of total RNA in 8 Multiplex RT primer pool reactions containing stem-looped RT primers that were specific to mature miRNAs. U6 snRNA-001973 was selected for normalization based on our own experimental validations. To quantify the association of the miRNA expression the fold change () was estimated for every detector with the p-values calculated using the t-test. RESULTS: Candidate miRNAs that showed a statistically significant expression were: miR-146, miR-34a, miR-221, Let-7b, miR-193b, miR-132, miR-192, miR-21, miR-197, miR-24, miR-26b, miR-29c were identified based on a false discovery rate of 4%. The results showed that twelve miRNAs differentially expressed in patients with BC. Candidate miRNAs have been reported associated with the expression of twenty seven DNA repair genes. Two of these genes are part of the NER pathway which has been identified by previous studies as important in BC. CONCLUSION: Our preliminary data suggests that differential expression of specific miRNAs might be associated with dysregulation of DRC in BC. The molecular mechanisms by which miRNAs regulate DNA repair genes remain to be elucidated. However, our results lend further promise to the concept of miRNAs as a tool to study the regulation of DRC. We see potential future applications in prognosis and therapy of women with BC. Supported by grants S06 GM008239-20 and 1SCA157250 from the NCI Center to Reduce Health Disparities and NIH-MBRS Program (NIGMS) and NIH-NIGMS #GM082406 (CO).
Expression of APOBEC3B in primary breast cancer of Japanese women

Eriko Tokunaga¹ ², Nami Yamashita¹, Kimihiro Tanaka¹, Yuka Inoue¹, Hiroshi Saeki¹, Eiji Oki¹, Hiroyuki Kitao³ and Yoshihiko Maehara¹. ¹Kyushu University, Fukuoka, Japan; ²Kyushu University, Fukuoka, Japan and ³Kyushu University, Fukuoka, Japan.

Body: Background: Human cancer genomes contain tens of thousands of mutations. APOBEC (apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like) family of cytidine deaminases normally function in innate immune responses that protects against retrovirus and retrotransposon propagation. However, these enzymes can also deaminate cytosines in the host genome and generate C to T mutations. APOBEC3B is overexpressed in several human cancer types, and this overexpression correlates with the presence of the APOBEC3B mutation signature. Recent studies have demonstrated APOBEC3B as a source of mutations in various malignancies including breast cancers. However, the relationships between the expression of APOBEC3B in breast cancer and the clinicopathological features have not been fully elucidated.

Aims: To investigate the expression of APOBEC3B mRNA in primary breast cancers and to evaluate the relationships between the APOBEC3B mRNA expression and the clinicopathological characteristics and prognosis in the primary breast cancer of Japanese women.

Methods: Specimens were obtained from 305 patients with primary breast cancers who underwent surgery without neoadjuvant systemic therapy. APOBEC3B mRNA expression was analysed using quantitative reverse transcription-PCR (qRT-PCR). The APOBEC3B expression level was normalized to that of the constitutive housekeeping gene TATA binding protein (TBP). Four breast cancer subtypes were determined by the immunohistochemical analysis of ER, PR and HER2; hormone receptor (HR; ER and/or PR)+/HER2-, HR+/HER2+, HR-/HER2+(HER2) and triple negative (TN).

Results: Expression of APOBEC3B mRNA was detected in 277 tumors, while it was not detected in 28 tumors. The APOBEC3B expression was significantly higher in ER-negative, PR-negative, high grade tumors. The APOBEC3B expression was positively correlated with Ki67 index and highest in TN and lowest in HR+/HER2- subtype. There were no correlations between the APOBEC3B expression and age, tumor size, lymph node metastasis and the stage. The APOBEC3B expression was not statistically different between invasive ductal carcinoma and DCIS, suggesting that the APOBEC3B expression is related to the carcinogenesis of breast cancer. High expression of APOBEC3B was significantly associated with the poor recurrence free survival in all cases and ER-positive cases; however, APOBEC3B expression was not related to the prognosis in ER-negative tumors.

Conclusions: The high APOBEC3B expression was related to the aggressive phenotype of breast cancer and the poor prognosis.
Title: A novel role for breast cancer associated protein 2 (BCA2) in regulation replication-stress mediated DNA damage responses

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Body: Breast cancer associated gene 2 (BCA2) has been originally identified from invasive breast cancer cells and shown to be overexpressed in over 50% of invasive breast cancers. Its expression is known to be highly associated with estrogen receptor-alpha (ER-α) status and promote cell proliferation and invasive properties. Importantly, expression of BCA2 is minimal or undetectable in most normal cells and tissues, which makes it as a valuable biomarker for ER-α positive breast cancers and a potential therapeutic target. BCA2 protein is a RING and ZINC-finger domain containing E3 ubiquitin ligase that has been shown to auto-ubiquitylate and interact with several proteins including Rab7, tetherin, ubiquitin, Ubc9 and p21, which are involved in different cellular processes. However, most of these studies have been focused on tumor progression, migration and invasive properties and almost no information on its role in carcinogenesis. Since many RING and ZINC-finger domain containing ubiquitin ligases are implicated in oncogenic signaling and DNA damage responses (DDR), in this study we examined the role of BCA2 in regulation of spontaneous and chemotherapeutics induced DDR in different breast cancer cell lines (ER-α positive versus triple negative). Interestingly, siRNA mediated down regulation of BCA2 induced spontaneous DDR, such as activation of replication checkpoint, slow cell cycle progression and double strand breaks (γH2AX foci). Exposure of BCA2 knockdown cells to DNA topoisomerase inhibitors (camptothecin and etoposide) potentiated DDR induced by these drugs. However, the molecular basis for this enhanced DDR is yet to be determined. Consistent with the previous studies, BCA2 knockdown attenuated cell proliferation, and compromised migration and invasion properties of these cells. Moreover, this novel role for BCA2 in DDR strongly suggests its status may also influence tumor response to chemo and radiation therapies and in carcinogenesis process. Further evaluation of breast cancer cell’s responses to different chemotherapeutic agents revealed distinct cellular responses based on the status of BCA2 and ER-α status. Taken together, our studies implicate a novel role for BCA2 in regulation of DDR and its influence on tumor response to chemotherapy. Additionally, we aim to present E3 ligase dependent and independent roles of BCA2 in these processes and tumor responses to different therapeutic agents.

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Title: Granulin, a novel STAT3-interacting protein, promotes breast cancer tumorigenicity

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Body: Since the neoplastic phenotype of a cell is largely driven by its gene expression patterns, increasing attention is focused on transcription factors that regulate critical mediators of tumor formation and metastatic progression like the oncogenic transcription factor, signal transducer and activator of transcription 3 (STAT3). Whereas normal cells have transient activation of STAT3 due to tight control by negative regulators, cancer cells frequently have inappropriate constitutive activation of STAT3 which drives increased expression of genes involved in tumorigenesis. However, little is known about proteins that interact with STAT3 to modulate its function. To identify novel STAT3-interacting proteins, we performed liquid chromatography tandem mass spectrometry-based profiling of STAT3-containing complexes immunoprecipitated from the triple-negative breast cancer cell lines MDA-MB-468 and SUM159PT, which have constitutively active STAT3. We identified granulin (GRN) as a novel STAT3-interacting protein and validated the STAT3-GRN interaction in breast cancer cells by co-immunoprecipitation. To investigate the functional effect of GRN on STAT3 activity, we silenced GRN using small interfering RNA. We found that GRN was necessary for constitutive and maximal cytokine-induced STAT3 transcriptional activity in breast cancer cells. GRN modulated cytokine-induced STAT3 function by enhancing STAT3 DNA binding and increasing the time-integrated amount of STAT3 activation and nuclear translocation. Silencing GRN mirrored the effect of silencing STAT3 on reducing the viability, clonogenesis, and migratory capacity of triple-negative breast cancer cells. Furthermore, GRN mRNA levels were significantly and positively correlated with STAT3 gene expression signatures indicative of STAT3 activation as well as with reduced overall survival in breast cancer patients. These studies used a proteomics approach to identify GRN as a novel STAT3 interacting protein that may serve as an important prognostic biomarker and potential therapeutic target in breast cancer.
Title: Cross-species data fusion reveals NRF2, unfolded protein and MAPK pathways in the control of cytotoxic and inflammatory responses to alkylating agents

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Body: Despite toxicity, alkylating agents are used to treat a variety of cancers. We set out to determine the mechanisms of alkylation cytotoxicity, aiming to identify strategies to augment therapy efficacy, especially cyclophosphamide, a therapy used for breast cancer. By comparing gene expression alterations of evolutionary distant cell types (fly, mouse and human) in response to the alkylating agent MMS (methylmethane sulfonate) we found conservation across species at a pathway but not a gene level. We demonstrate that alkylation responses in fly (Kc167 cells), mouse (mouse embryonic fibroblasts, MEFs) and human breast cancer cells are dependent on a conserved NRF2-mediated glutathione (GSH) production, which was required for appropriate drug detoxification and control of unfolded proteins responses (UPR). UPR was activated in response to alkylation in the different cell systems tested, and the balance of activity between PERK, Ire1 and ATF6 signaling dictated cell fate. Restoring GSH levels with NAC (N-acetyl-cysteine) or GSH-ester completely blocked chemotherapy toxicity in both normal and breast cancer cells, whereas depletion of GSH with the GSH synthesis inhibitor BSO (buthionine suphoximine), or knockdown of NRF2, potentiated toxicity and UPR activation. Irrespective of the role for GSH in controlling cytotoxicity, alkylating agents also enhanced ROS, which did not impact cell survival, rather being a secondary effect of GSH depletion as confirmed by using GSH unrelated antioxidants. These phenomena were observed across the different alkylating agents (4-hydroperoxy-cyclophosphamide, temozolomide and the alkylating-like cisplatin), suggesting conservation. The role of NRF2-GSH and UPR was conserved across species and normal versus tumor cells, however, we noted that alkylation stimulated breast cancer cells, not fly, to produce a pro-inflammatory secretome. This response was characterized by accumulation of extracellular mediators such as IL8, CXCL2, prostaglandin E2/COX-2, MMP1, MMP3, amongst others. IL8 and MMP-1 are highly associated with poor prognosis as determined from Kaplan-Meier curves. This non-conserved inflammatory pathway was not involved in cell survival of alkylation, but accelerated extracellular matrix invasion and angiogenesis as assessed in vitro by using conditioned medium from alkylating agents-treated breast cancers. We observed that basal-subtype breast cancers were prone to secrete several inflammatory mediators compared to luminal A/B and Her2+ cells, in untreated and even more in alkylation-treated conditions. We observed that much of this effect was controlled by MAPKs and downstream activation of NF-kappaB and AP-1/ATF2 transcription factors. From RNA sequencing and western analysis, we validated that selective blocking of MAPK/NF-kappaB/AP-1 axis using the multi-kinase inhibitor sorafenib blocked the inflammatory secretome as well as inhibited invasion and secretome-driven angiogenesis; this allows a more controlled tumor killing by alkylation in xenografts. These findings indicate that blocking the inflammatory response to alkylation provides a potential strategy to improve the efficacy of these chemotherapeutics in treating otherwise aggressive breast tumors.
Characterization of a new canine inflammatory mammary cancer (IMC) cell line (IPC-366)

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Spontaneous canine inflammatory mammary cancer (IMC) shares epidemiologic, histopathological and clinical characteristics with the disease in humans and has been proposed as a natural model for human inflammatory breast cancer (IBC). Few cell lines are available to study IBC, such us SUM 149, SUM 190 and MDA-IBC3. The aim of this study was to characterize a new cell line from IMC (IPC-366) for the comparative study of both IMC and IBC. Tumors cells from a female dog with clinical IMC were collected. The pathological diagnosis of IMC was confirmed at the Veterinary Pathology Service of UCM-Veterinary Clinical Hospital. The cells were grown under adherent conditions in DMEM/F12 with 5% fetal bovine serum and 1% antibiotics (streptomycin and penicillin). The growth and mammospheres production capability, and cytological, ultrastructural and immunohistochemical (IHC) characteristics of IPC-366 were evaluated. Tumorigenicity and metastatic potential of IPC-366 were also assessed by inoculating the cells on the mammary fat pad of 18 female Balb/SCID mice and the development of tumor was monitored by imaging and luciferase assay. Microscopic examination of tumor revealed an epithelial morphology with marked anisocytosis. Doubling time of the tumor cells was approximately 24 h. Under non-adherent conditions, IPC-366 cells formed mammospheres in approximately 3 days. Cytological and histological examination of smears and ultrathin sections by electron microscopy revealed that IPC-366 is formed by highly malignant large round or polygonal cells characterized by marked atypia and prominent nucleoli and frequent multinucleated cells. Some cells had cytoplasmic empty spaces covered by cytoplasmic membrane resembling capillary endothelial cells, a phenomenon described as vasculogenic mimicry. IHC characterization of IPC-366 was basal-like: epithelial cells (AE1/AE3+, CK14+, vimentin+, actin-, p63-, ER-, PR-, HER-2 – (DAKO, HER2 P4 ), overexpressed COX-2 and high Ki-67 proliferation index (87.15 %). Imaging and luciferase assay revealed that at 3 weeks after inoculating the IPC-366 cells, a tumor mass was found in 50 % of mice. At 8 weeks after inoculation metastases in lung, liver and lymph nodes were found. Xenograph tumors maintained the original IHC characteristics of the female dog tumor. In summary, the cell line IPC-366 is a fast growing malignant triple negative cell line model of inflammatory mammary carcinoma that can be used for the comparative study of both IMC and IBC.
Title: Regulation of system Xc- by signal transducer and activator of transcription 3 and 5 in human breast cancer cells

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Body: In order to survive and proliferate, cancer cells adapt to high levels of oxidative stress by countering the accumulation of reactive oxygen species (ROS) with an increased production of intracellular antioxidant molecules such as glutathione. The cell surface transport system Xc- is a cystine/glutamate antiporter that exports glutamate while importing cystine, thereby mediating levels of cysteine required for glutathione synthesis and the maintenance of cellular redox balance. Transcription factors that regulate key antioxidant defense mechanisms, including system Xc-, may therefore be of therapeutic interest. Recently, signal transducer and activator of transcription (STAT) proteins have emerged as potential targets for the development of novel anti-cancer therapies. In particular, inhibitors of STAT3 and STAT5 may become clinically relevant as anti-cancer agents for breast and brain cancer, as well as leukemia. Interestingly, suppression of STAT3 has been linked with increases in ROS and the induction of apoptosis. Upon activation by phosphorylation, dimerization, and nuclear translocation, STAT proteins transcriptionally regulate diverse target genes by binding to promoter regions containing gamma-activated site (GAS) motifs. The human xCT (SLC7A11) gene encodes the functional subunit of system Xc-. We provide evidence that expression of xCT is regulated by STAT3, and potentially also STAT5, affecting antiporter function in both MCF-7 and MDA-MB-231 human breast cancer cells. Computational analysis of the xCT promoter region revealed the presence of a distal GAS site. Its truncation significantly increased luciferase activity in a reporter assay, with similar increases obtained after treating cells transfected with the full-length xCT promoter construct with various STAT3/5 pharmacologic inhibitors. Knock-down of STAT3 or STAT5A using specific siRNAs produced similar results, suggesting that these STAT proteins act in a transcriptionally repressive manner. We also demonstrated binding of STAT3 and STAT5A to the xCT promoter in MDA-MB-231 cells, which was disrupted by preincubating the cells with specific inhibitors. xCT mRNA and protein levels increased significantly following treatment with STAT3/5 inhibitors. Pharmacologically suppressing STAT3/5 activation also significantly increased glutamate release and total levels of intracellular glutathione. We hypothesize that blocking STAT3/5-mediated signaling induces an adaptive, compensatory mechanism that protects breast cancer cells from ROS by up-regulating Xc- antiporter expression and function. Our findings suggest that targeting system Xc- may synergize with STAT3/5 inhibitors, heightening their therapeutic anti-cancer effects, particularly in conjunction with traditional chemotherapy treatments.

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Exploring the involvement of TTK kinase in centrosome amplification and Her2+ breast cancer

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Body: The centrosome is the cellular organelle responsible for accurate chromosome segregation. In normal cellular function, regulation of the centrosome duplication cycle in concert with the cell cycle is necessary for accurate passage of genetic information. However, when modulators of the cell and/or centrosome duplication cycles are deregulated, this process can result in centrosome amplification (CA, the acquisition of more than two centrosomes), leading to improper segregation of chromosomes and genomic instability. CA generates low-level aneuploidy (which is tolerated) and polyploidy (selected against when checkpoints are present). An integral goal in the field of centrosome biology is to characterize how alterations in modulators of CA influence disease development. In cancer, CA is associated with aggressive tumor types and metastasis and is likely to affect response to treatment. However, the full role of CA in tumorigenesis is poorly understood. Specifically in breast cancer, CA is observed in pre-cancerous lesions, which suggests CA in conjunction with genomic instability is an early contributor to tumorigenesis. In an effort to study mechanisms associated with CA, we are investigating the function of CA in in vitro and in vivo breast cancer models. Our lab and others have detected that TTK (MPS1) kinase, a proposed modulator of centrosome duplication, is overexpressed in Her2+ breast cancer cell lines at the mRNA and protein level compared to non-transformed mammary epithelial cells. We hypothesize that overexpression of TTK leads to CA and that inhibiting TTK expression in cancer cells will prevent active generation of CA and CIN (chromosome instability), leading to suppressed tumorigenic properties and further cancer evolution. Preliminary data shows that transient knockdown of TTK via siRNA attenuates the degree of CA in Her2+ breast cancer cells. Additional preliminary data shows that stable abrogation of TTK via shRNA can inhibit the proliferation of Her2+ breast cancer cells but does not alter expression of some members in the intrinsic apoptotic pathway. Ongoing studies will address the impact of altering TTK expression on CA, CIN, downstream centrosomal duplication signaling and tumorigenic properties of breast cancer cells. The results of these studies will address the mechanistic complexities, such as what centrosomal signaling pathways associated with TTK are underlying CA in breast cancer. We believe this work will help discern how deregulated centrosome regulatory molecules influence mammary tumorigenesis and/or responses to treatment.
Combination of IGF1R/FAK inhibition and PI3K/mTOR inhibition in triple-negative breast cancers

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Introduction: Triple-negative breast cancers (TNBCs) account for 15%-20% of all breast cancers with limited treatment options and poor prognosis. The poor outcomes seen with TNBCs are in part due to a lack of viable therapeutic targets. Overexpression of insulin-like growth factor 1 receptors (IGF1R) and focal adhesion kinase (FAK) are closely associated with invasive breast carcinomas. However, in our previous study, we found that the combined use of IGF1R inhibitor and FAK inhibitor had produced limited effects on TNBC cells inhibition. NVP-BEZ235 is a potent PI3K/mTOR dual inhibitor and has been shown to be effective in TNBC cell lines, especially for the mesenchymal-like and luminal-androgen receptor subtypes. Unlike rapamycin, which produces a feedback activation of Akt, NVP-BEZ235 alone successfully blocks the Akt activation and effectively inhibits the cells proliferation. Our hypothesis is that the combined inhibition of IGF1R/FAK and PI3K/mTOR produces greater suppression on TNBC cell growth. Methods: We examined the effects of NVP-TAE226, the dual inhibitor of IGF1R and FAK, in combination with NVP-BEZ235 on human MDA-MB-231 and BT549 TNBC cell lines. SRB cell survival assays were performed following NVP-TAE226 or NVP-BEZ235 treatments alone and NVP-TAE226 in combination with NVP-BEZ235. Western blotting was used to detect expression and phosphorylation of down-stream signaling proteins and epithelial to mesenchymal transition (EMT)-related markers. Matrigel invasion chamber assay was performed to evaluate the TNBC cells invasion patterns under treatments of either NVP-TAE226 or NVP-BEZ235 alone or the two drugs in combination. Spheroid migration assay will also be used to assess combination effect on the metastatic nature of TNBC cells. Results: The combined IGF1R/FAK inhibition with NVP-TAE226 and PI3K/mTOR inhibition with NVP-BEZ235 resulted in significantly greater cytotoxicity than either single agent alone in MDA-MB231 and BT549 cell lines (P<0.05). The combination of IGF1R/FAK and PI3K/mTOR inhibition suppressed the PI3K/Akt and MEK/ERK signaling cascades, reduced FAK and ZEB1 activity and significantly decreased the cell invasion for TNBC cell lines (p<0.05). Our data indicated that the combination treatment targeting both PI3K/Akt pathway and EMT related protein molecules (IGF1R/FAK) lead to greater cytotoxic effect and suppression of EMT and invasion. Conclusion: These results suggest that the combined inhibition of IGF1R/FAK and PI3K/mTOR may be an effective strategy for TNBC and warrant further investigation in in vivo animal studies.
Title: Identification and characterization of a new TIMP-1 binding protein

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Body: Tissue inhibitor of metalloproteinases 1 (Timp-1), is one of the four known endogenous inhibitors of matrix metalloproteinases (MMPs); in recent years, Timp-1 has become increasingly recognized as a multifunctional protein that, independently of its MMP inhibitory activity, is able to regulate core cellular processes such as cell proliferation and apoptosis. Consistent with this pro-survival function, Timp-1 expression was shown to be able to protect cancer cells from epirubicin or paclitaxel-mediated cytotoxicity. Consistent with this effect, clinical studies have shown high Timp-1 tumor levels to be predictive of resistance to adjuvant anthracycline-based chemotherapy in metastatic breast cancer patients. In spite of abundant evidence directly involving Timp-1 in regulation of cell growth and apoptosis, the downstream mechanisms of Timp-1–mediated cell signaling underlying these effects, and its biological consequences, have remained unclear. In order to address this issue, we aimed to identify cellular binding partners for Timp-1, which may be able to induce signaling. Therefore, we performed yeast two hybrid screening using a mammary gland cDNA library. We report here the identification of a novel Timp-1 interactor, CD74. CD74, also known as MHC class II invariant chain (li), was mainly thought to function as an MCH class II chaperone promoting the exit of MHC class II molecules from the endoplasmic reticulum (ER). However, a fraction of cellular CD74 has been found to traffic to the plasma membrane where it functions as an accessory-signaling molecule, being quickly recycled back into the endosomal pathway. The interaction between TIMP-1 and CD74 was confirmed by co-immunoprecipitation studies in the triple negative breast cancer cell line MDA-MB-231, and we showed that CD74 is necessary for Timp-1 cellular internalization and Timp-1-mediated activation of Akt signaling. To determine the applicability of our findings to a broader context, we analyzed a breast cancer patient cohort for expression of CD74, CD63 and Timp-1 by immunohistochemistry (IHC) and in situ hybridization (ISH). We found that cancer cells which were negative for TIMP-1 mRNA but positive for Timp-1 protein, indicating an active transport of Timp-1 into the cells. These results raise the possibility that CD74 may be a useful target for effecting Timp-1 mediated chemoresistance.
Title: Tyrosine phosphorylation of p27kip1 regulates the activity of cyclin D-cdk4 complexes in breast cancer

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Body: The oncogenes Cyclin D and cdk4 are overexpressed in breast cancer, but the levels of these proteins are not always accurate indicators of oncogenic activity because p27Kip1 is required to assemble this otherwise unstable dimer. However, p27’s association activates or alternatively inhibits cyclin D-cdk4, serving as a bona fide ON/OFF “switch.” Tyrosine (Y) phosphorylation of residues Y88/89 in p27 displaces its C-terminus from the cdk4 active site, permitting both ATP binding and CAK phosphorylation of cdk4’s T loop. This model leads to the following hypothesis: modulation of p27 pY controls cdk4 activity, which in turn regulates efficient cell cycle passage, and in breast cancer where cdk4 activity is deregulated, p27 may be constitutively switched ON. Deregulated Src Family Kinase (SFK) signaling in cancer may increase p27 pY, constitutively activating oncogenic cdk4, causing continuous cell cycling. Using our p27 pY phosphospecific antibody, we have shown in primary tumors, that p27 pY is not detected in benign tissue regions, but is detected in grade 1 and progressively higher grade tumors, suggesting that p27 pY may be a marker for increased oncogenic cdk4 activity and cdk4 inhibitor sensitivity. We identified an SH3 recruitment domain within p27 that controls p27 pY, and in turn controls cdk4 activity. Blocking the SH3:p27 interaction with small peptides prevents p27 pY and cdk4 activity in vitro and in vivo. Using a phage-ELISA assay, we identified PTK6/Brk (Protein Tyrosine Kinase 6/Breast Tumor Kinase) that functions as a high-affinity kinase, able to phosphorylate p27 in vitro and associate with phosphorylated p27 in vivo. Overexpression of PTK6 in vivo increases p27 pY and increases resistance to specific cdk4 inhibition by the chemical inhibitor, PD0332991. An ALTeratively spliced form of PTK6 (ALT), which contains the SH3 domain, specifically associates with p27 in cells arrested by contact or serum-starvation, blocking pY and acting as an endogenous inhibitor of cdk4. As PTK6/Brk is overexpressed in more than 60% of human breast carcinomas, our data suggest that PTK6/Brk overexpression facilitates cell cycle progression by increasing cdk4 activity through direct p27 Y phosphorylation. As PD0332991 moves into the clinic, p27 pY could serve as a marker to identify tumors sensitive to cdk4 inhibition, while blocking the PTK6:p27 interaction with small molecules represents a novel therapeutic option to inhibit cdk4 activation.
Title: Inhibition of CDK4/6 induces senescence and autophagy in ER positive breast cancers

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Body: Deregulation of the cell cycle machinery is a hallmark of breast cancer, facilitating aberrant proliferation that fuels tumorigenesis and disease progression. This makes the cell cycle, particularly the cyclin dependent kinases (CDKs) an ideal choice for drug targeting in these tumors. Palbociclib or PD0332991, a potent CDK4/6 inhibitor is a known anti-proliferative agent that induces G1 arrest and prevents tumor growth in several cancers including ER+ breast cancer. This drug has shown tremendous success in Phase II clinical trials in ER+ breast cancers and is presently undergoing phase III trials in combination with letrazole. However, little is know about its precise mechanism of action and modes of resistance in ER+ breast cancers. This is critical in understanding the biology of treatment response, drug resistance and combination strategies, and this project aims at addressing these gaps in knowledge.

We have used a series of ER+ breast cancer cell lines to examine the mechanism of action of Palbociclib and identify the nodes that could mediate resistance to this agent. Our results have revealed that continuous treatment of ER+ breast cancer cells with Palbociclib results in a G1 arrest with concomitant downregulation of pRb. Treated cells undergo senescence and autophagy, but not apoptosis. Further, we have shown that the induction of quiescence or senescence and autophagy occurs in a time and dose dependent manner.

Since this agent is known to be a specific CDK4/6 inhibitor, we next downregulated these kinases in our model system and subjected them to Palbociclib treatment. Interestingly, downregulation of CDK4 or CDK6 did not significantly alter the sensitivity of these cells to the anti-tumor effects of Palbociclib. Next we asked if depletion of Rb can render the cells resistant to Palbociclib. Results revealed that the complete depletion of Rb was sufficient to reduce the sensitivity of ER+ cells to Palbociclib by 4-6 fold. However, the very steep pattern of the growth response curves suggest that in the absence of Rb, Palbociclib can effectively inhibit another target, albeit, at a higher drug concentration.

Lastly, to interrogate if deregulation of the G1/S checkpoint can render cells more resistant to this inhibitor, we examined if overexpression of the low molecular weight isoforms of Cyclin-E (LMW-E), which by themselves are sufficient to induce an oncogenic phenotype through constitutive phosphorylation of Rb, can mediate resistance in these cells. These results, which were similar to those from the Rb knockout studies, revealed that LMW-E overexpression can reduce the sensitivity of ER+ breast cancer cells to Palbociclib by 5-7 fold.

Collectively, our studies suggest that inhibition of G1/S transition is not the sole mode of action of Palbociclib and that there is another unidentified target, which is specifically inhibited by this agent, when the G1/S check point is compromised. Since the deregulation of the G1/S checkpoint is likely to occur clinically in patients treated with Palbociclib, identification of this second target will be instrumental in efforts to overcome resistance and identify biomarkers of sensitivity to this agent in breast cancer patients.
Title: Nottingham prognostic index plus (NPI+): Validation of the modern clinical decision making tool in breast cancer

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Body: Introduction
Current management of breast cancer (BC) relies on risk stratification based on well-defined clinicopathologic factors. The Nottingham Prognostic Index Plus (NPI+) is based on the assessment of biological class combined with established clinicopathologic prognostic variables providing improved patient outcome stratification superior to the traditional NPI+.* This study aimed to validate the NPI+ in an independent series of BC.

Methods
A Validation series of 469 primary early-stage BC cases treated in Edinburgh, UK were matched for size, stage and grade to cases from Nottingham, UK used to develop the NPI+ (Training series). Adjuvant therapy was similar in both series except that 143 Edinburgh cases received endocrine therapy whilst the matched Nottingham cases had no adjuvant therapy. However, there was no significant difference in 10 year BC specific survival (BCSS) between the Training and Validation series.

Cases, prepared as TMAs, were immunohistochemically assessed for Cytokeratin (Ck)5/6, Ck18, EGFR, Estrogen Receptor (ER), Progesterone Receptor (PgR), HER2, HER3, HER4, Mucin 1 and p53 expression. NPI+ biological class based on the expression of the 10 biomarkers was determined. Subsequent NPI+ prognostic scores were assigned using individual algorithms for each biological class developed using the Training series incorporating clinicopathologic parameters: positive nodes (including nodal stage), tumour size, tumour grade (including mitotic index) and PgR. NPI+ biological classes, prognostic scores and prognostic groups were compared between the Validation and Training series and their role in prediction of patient outcome. A p-value of <0.01 was considered significant.

Results
As anticipated, there was a comparable distribution of NPI+ biological classes between Training and Validation series: Luminal A, n=143 (31%) vs n=115 (25%); Luminal N, n=99 (21%) vs n=89 (19%); Luminal B, n=75 (16%) vs n=85 (18%); Basal p53 altered, n=54 (12%) vs n=72 (15%); Basal p53 normal, n=37 (8%) vs n=53 (11%); HER2+/ER+, n=31 (7%) vs 18 (4%); HER2+/ER-, n=30 (6%) vs n=37 (8%; X²=13.792, p=0.032). BCSS was analogous between the Validation and Training series in each of the NPI+ biological classes except Luminal B (p=0.042). Similar BCSS was observed in the NPI+ Biological classes of the Training versus Validation series when taking into consideration adjuvant treatment modalities.

The mean NPI+ score was similar between the Validation and Training series (2.30 vs 1.89, Pearson’s Regression p=0.079). The NPI+ prognostic groups significantly predicted patient outcome in each molecular class (BCSS, p<0.0001) in the Validation series irrespective of adjuvant treatment. Comparing the BCSS in each of the NPI+ prognostic groups demonstrated there were no significant differences between patient outcome in each of the NPI+ prognostic groups between the Validation and Training series.

Conclusion
This study validates the NPI+ in an independent series of primary BC confirming its` reproducibility. The NPI+ provides improved individualised clinical decision making for breast cancer for both prediction of clinical outcome and relevant therapeutic options.

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Title: The CYP19 RS4646 polymorphism is related to the prognosis of stage I–II and operable stage III breast cancers

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Body: Background: Aromatase, encoded by the CYP19 gene, catalyzes the final step of the conversion of androgens to estrogens. In premenopausal women, estrogen is mainly produced by the ovary, while in postmenopausal women, aromatization of androgen in extragonadal tissue, for example, adipose tissue, is the main source. Given the critical role of CYP19 in estrogen synthesis and the association of clinical outcome with rs4646 polymorphism, we investigated the clinical relevance of CYP19 rs4646 genotypes in early breast cancer.

Methods: Genotype for CYP19 rs4646 variants was performed on 406 Chinese women with early breast cancer. Associations were examined between rs4646 genotypes with histopathological characteristics and disease-free survival.

Results: In patients younger than 50 years, women who are homozygous for the minor allele (AA) have a longer disease-free survival (DFS)compared with those carrying the major allele(CC or AC) (87 months versus 48.7 months; HR 0.560; 95CI 0.318-0.985; P =0.041). This differences was further demonstrated by a multivariate analysis (HR 0.456; 95CI 0.249-0.836; P=0.011). Conversely, the same variants(AA) were found to be associated with a poorer DFS in women with age above 50 years (AA versus AC or CC: 38.9months versus 79.7 months; HR 2.758; 95% CI: 1.432-5.313; P=0.002). Furthermore, the differences was proved by the COX proportional hazards model (HR 2.983; 95CI 1.494-5.955; P=0.002).

Conclusions: The present study indicates that CYP19 rs4646 polymorphism is associated with DFS in early breast cancer and that the prognosis index of the homozygous for the minor allele (AA) may depend on circulating estrogens levels due to the different age. The founding is novel, if confirmed in a larger prospective independent cohort, rs4646 genotypes may provide useful information for routine management in breast cancer.
Title: Expression of the C9Orf72 long-isoform in cancer tissues prognosticates disease-free and breast cancer-specific survival

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Body: Background: An expansion of the GGGGCC hexanucleotide repeat in C9Orf72 gene promoter results in reduced mRNA expression of its long-isoform, and has been associated with amyotrophic lateral sclerosis, and frontotemporal dementia. Over-expression of C9Orf72 in cultured neuronal cells resulted in increased cell death, but its role in oncogenesis and, specifically, in brain metastasis is unknown.

Materials and Methods: C9Orf72 mRNA levels were measured by qPCR in 200 normal breast, 217 (cohort 1) and 100 (cohort 2) breast tumor samples, and correlated with clinico-pathological variables, breast cancer-specific (BCSS) and disease-free survival (DFS). MCF-7 tumor xenografts models were used to observe the effect of C9Orf72 on tumor metastasis.

Results: C9Orf72 mRNA levels were statistically higher in tumor than in normal breast tissues (P<0.0001). In cohort 1, comprised of 117 inflammatory and 100 locally-advanced breast cancers, C9Orf72 levels were (1) low in IBC than in LABC (P=0.79); (2) statistically significant lower in cohort 1 than in cohort 2 (P<0.001); and (3) associated with local and distant recurrences (P<0.001) in both cohorts. Kaplan-Meier analysis showed that low C9Orf72 levels were associated with both worse DFS and BCSS in the two cohorts (P<0.0001 for both). Cox proportional hazards model determined that ER status (Hazard Ratio [HR] = 0.65; 95%CI=0.44-0.98; p=0.04), lymph node ratios (HR=1.84; 95%CI=1.19-2.84;p=0.006), and low C9Orf72 levels (HR=37.4; 95%CI=17.08-81.72; p<0.0001) were prognosticators of DFS; and low C9Orf72 levels (HR=7.02; 95%CI=4.1-12.1; p<0.0001) were associated with worse BCSS in the training-cohort. In the validation cohort, low C9Orf72 levels were associated with worse DFS (HR=291.1; 95%CI=16.6-5121.1; p<0.0001). Tumor grade (HR=2.5; 95%CI=1.59-3.8; p<0.0001), and low C9Orf72 levels (HR=6.9; 95%CI=3.6-13.2; p<0.0001) were independent prognostic factors of worse BCSS. Overexpression of C9Orf72 in a MCF-7 orthotopic mouse xenograft also resulted in significant tumor regression and reduced metastatic events to inguinal, axillary lymph nodes, pancreas, liver and kidney.

Discussion: C9Orf72 low levels were highly specific and sensitive prognostic factors of worse DFS and BCSS in two demographically distinct cohorts of breast cancer patients, and increased C9Orf72 expression resulted in reduced metastatic events in a mouse xenograft model. The molecular mechanisms of C9Orf72-induced cell death are unknown and warrant deep investigation because of its possible association with brain metastasis. C9Orf72 has the potential to emerge as a very attractive prognostic biomarker and potential therapeutic candidate in breast cancers.

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Title: Reduction in raf kinase inhibitor protein predicts poor outcomes and correlates with promoter hypermethylation as well as matrix metalloproteinases expression in patients with breast cancer

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Body: Background: Invasion and metastasis are the direct causes of mortality in breast cancer patients. Reduction in raf kinase inhibitor protein (RKIP) expression has been shown to be an indicator of metastatic spread in numerous cancers. However, the role of RKIP alteration in the successive steps of breast carcinogenesis and its association with outcome variables has not been well established in breast carcinoma.

Materials and Methods: To elucidate the role of RKIP alteration in the successive steps of breast carcinogenesis and its association with outcome variables, immunohistochemical staining with anti-RKIP antibody was performed in a total of 324 patients with 26 normal breasts, 25 usual ductal hyperplasia, 76 ductal carcinoma in situ (DCIS), and 198 invasive breast carcinoma (IBC) using tissue microarray. In addition, we studied the promoter hypermethylation of RKIP as a mechanism for loss of RKIP expression in IBC. To investigate the potential involvement of RKIP in the modulation of matrix metalloproteinases (MMPs) in breast cancer, we also performed immunohistochemical staining for MMP-1, -2, -9, and -13 in IBC.

Results: RKIP expression appeared to decrease progressively along the continuum of neoplastic changes from normal breast epithelium to IBC (P < 0.001). Reduced RKIP expression in IBC was significantly higher than in DCIS (P < 0.05). Reduced RKIP expression was significantly associated with the metastatic relapse (P < 0.001). The patients with reduced RKIP expression had a significantly poorer prognosis for disease-free and overall survival than those with normal expression (P < 0.001 and P < 0.001, respectively). Reduced RKIP expression was one of the statistically significant independent risk factors for disease-free survival (P = 0.003). Reduced RKIP expression in IBC was significantly correlated with promoter hypermethylation (P < 0.05). MMP-2 and -9 expressions in IBC with reduced RKIP expression were significantly higher than that in IBC with normal RKIP expression (P < 0.05 and P < 0.01, respectively).

Conclusion: Our results suggest that tumor progression in breast epithelium is accompanied by reduced RKIP expression. Reduced RKIP expression may serve as a new parameter for the prognostic prediction in patients with IBC. In IBC, RKIP promoter hypermethylation may be involved in RKIP gene inactivation and RKIP can control tumor cell invasion and metastasis through regulation of MMPs, particularly MMP-2 and -9.
Title: Prediction of bone metastases of breast cancer using combined markers of bone metabolism and inflammation

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Body: Introduction
Bone metastases in breast cancer impair a patient’s QOL because of skeletal-related events such as bone pain, fractures, spinal cord compression, and hypercalcemia. It might be important to predict bone metastases and initiate adequate treatment early in the disease process. Screening and diagnosis of bone metastases are performed using serum markers and imaging systems such as CT, MRI, PET, and SPECT in postoperative testing. However, a method for predicting bone metastases for stratifying patients who require treatment has not been established. Although various markers of bone metabolism have been approved for monitoring of postoperative bone metastases, these are not considered clinically practical because of their low specificity. We selected TRACP-5b as a marker of bone metabolism; likelihood of bone metastases, and CRP as a marker of inflammation; likelihood of distant recurrence. We hypothesized that the combination of these two markers of different aspects would provide an accurate prediction of bone recurrence.

Patients and methods
Three hundred forty-nine breast cancer patients who underwent surgery in our hospital between August 5, 2010, and October 31, 2013, were enrolled in this study. Their serum levels of TRACP-5b and CRP were measured in a blinded manner at the R & D laboratory of Nittobo Medical Co., Ltd. Eighty-one patients were excluded (78 cases; neoadjubant chemotherapy administration, 3 cases; T4), and the data from the remaining 268 patients were included in the statistical analysis. The cutoff values were 380mU/dL for TRACP-5b and 0.016 mg/dL for CRP. Patients with both values above the cutoff value were classified as +/+ , and they were compared with the other patients. The odds ratio between +/+ and the others were calculated using MedCalc statistical software.

Results
Patients stratified into four classes according to the value of TRACP-5b and CRP: +/+ (n=60), +/- (n=49), -/+ (n=76) and -/- (n=83), (+ means above the cutoff value). Eight of the 268 patients had relapsed metastases: three in the bone only, one in the bone and lung, three in lymph nodes only, and one in the lung only). The Incidence of bone metastases was 5 % (3/60) in the +/+ patients and 0.5 % (1/208) in the others. The incidence was significantly higher in the +/+ patients than in the others (odds ratio: 10.9, 95% CI 1.11 to 106.74, p= 0.040). When the other relapses not including bone metastases were included in the analysis, no significant difference was observed between the two groups (odds ratio: 0.4, 95% CI 0.02 to 7.07, P=0.513). TRACP-5b concentration alone could not classify the patients into two groups according to significantly different incidences of bone metastases(odds ratio: 4.5, 95% CI 0.46 to 43.57, P=0.197).

Conclusion
The results presented here show that the prediction of bone metastases by the combination of TRACP-5b and CRP concentrations is clinically relevant. We plan to increase the number of patients to provide sufficient statistical power to confirm this diagnostic potential.
Title: Decrease of tumor F3 expression after neoadjuvant chemotherapy associates to lower survival in breast cancer

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Body: Background:
Pathologic complete response (pCR) is the main prognostic factor after neoadjuvant chemotherapy (nCT) for breast cancer (BC). However, in cases without pCR, additional prognostic biomarkers are needed for subsequent prognostic and therapeutic stratification of patients. Tissue factor (F3) is the protease initiator of blood coagulation cascade and is expressed in solid tumors including BC. F3 oncogenic functions derive both from coagulation activation and from its cytoplasmic domain, although they are not well known yet. Prognostic impact of F3 circulating levels and tumor expression seems to be variable, and no studies evaluating F3 expression changes in the residual tumor after nCT are available. Our aim was to evaluate chemotherapy-mediated changes in F3 mRNA levels and their prognostic value in BC treated with nCT.

Methods:
RNA was isolated from FFPE samples of pre- and post-CT tumors. Post-CT F3 levels were analyzed only in patients without primary tumor pCR. Quantification of F3 was performed by RT-qPCR. F3 expression was categorized as no expression or above/below median expression. Change in expression levels (∆F3), defined as pre-nCT minus post-nCT, was categorized by value of change (positive vs. negative). Association of F3 levels with clinical and pathological characteristics and analysis of paired samples was evaluated with non-parametric tests. Kaplan-Meier curves, log-rank test and Cox proportional hazard regression multivariate models were used for survival analysis. To externally validate our results, we also studied the correlation between F3 and the rest of the set of genes in the TCGA database. The best 150 directly and 150 inversely correlated genes (r >0.3 and <−0.3 respectively) were selected and functional prediction was performed using Genemania software for both groups.

Results:
We included 108 consecutive women with invasive BC, mostly with stages IIB or IIIA-C; Her2+: 25.0%, triple negative: 22.2%. After nCT including anthracyclines and taxanes, pCR rate was 19.4%. nCT significantly increased F3 expression (p<0.000001). Pre-CT F3 levels were not associated with prognostic or predictive variables in our series. Loss or low post-CT F3 levels were associated with poor prognosis only in the univariate analysis. However, a nCT-induced decrease in F3 expression had a negative impact on overall survival in both univariate and multivariate analysis including cN (p=0.001 and 0.013 respectively). Both in our series and in external databases, F3 mRNA levels have an inverse correlation with proliferative genes, and nCT enhances these correlations (pre-CT: MYBL2: r=-0.358, p=0.001; MKI67: r=-0.267, p=0.019; post-CT: MYBL2: r=-0.495, p<0.001; MKI67: r=-0.498, p<0.001). A functional strong inverse correlation between F3 and mitotic functions was also confirmed in TCGA database.

Conclusion:
Our data demonstrate that nCT consistently increases tumor F3 expression. However, those cases with decreased expression of F3 after chemotherapy show poor overall survival rates. Functional analysis in our series and in public databases demonstrate that decreased F3 mRNA expression correlates with an increased mitotic activity, suggesting that low F3 mRNA levels could be a marker of active and resistant to treatment tumors.
Title: Clinical relevance of TP53 mutations and genomic instability in node positive breast cancer

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Body: Background - Aim: Malignant tumors are currently understood as genetic mosaics. For breast cancer (BC) patient assessment, the challenge remains to use the increasingly available tumor genomic data along with standard clinicopathological parameters.

Methods: Histologically reviewed, paraffin tumor tissue DNA samples (N=1771), from patients who had received anthracycline-based adjuvant chemotherapy in the frame of 3 randomized trials by HeCOG (HE10/00, pre-trastuzumab [T]; HE10/05 & HE10/08, post-T era), were investigated with targeted massively parallel sequencing (tMPS) for variants in 58 genes previously implicated in BC (custom panel, Ion Torrent systems). Upon multiple stringent quality filters, pathogenic coding mutations (MUT) and allelic (im)balance (AI, for 5 or more imbalanced SNPs; AI-high: at least 3 AI loci per sample) were evaluable in 1388 cases (78.4%). IHC4 (HER2 ASCO 2013 guidelines, Ki67 20% cutoff) was used for BC subtyping (luminal A, luminal B, luminal HER2, HER2-enriched and TNBC).

Results: In total, 1264 tumors carried 1 or more MUT and 788 were positive for AI. No two tumors had the same genetic profile. Top 5 MUT genes (>5% of all cases) were TP53, PIK3CA, CDH1, GATA3 and MLL3; top 5 genes with >50% frequency within the tumor (possible drivers) were TP53, PIK3CA, GATA3, MAP3K1 and PTEN. Top imbalanced (unstable) loci included 5p15.33 (TERT), 7p12 (EGFR), 17p13.1 (TP53), 17q21 (BRCA1), 10p15 (GATA3) and 10q23.3 (PTEN). TP53 MUT were more prominent in HER2-enriched and TNBC (p<0.0001) and were strongly associated with AI (p<0.0001). Tumor genomic characteristics were modelled with 3-year disease-free survival (DFS) as end-point for the evaluation of tumor aggressiveness; patients without infiltrated lymph nodes (LN) were not evaluated in this analysis. In all 3 cohorts, 1-3 positive LN were favourable and >3 LN were unfavourable prognosticators. Among patients with HER2-negative tumors (evaluable N=851), those with >3 positive LN and AI-low tumors fared similarly to patients with 1-3 LN; those with AI-high tumors fared significantly worse (log-rank, p<0.0001). DFS for AI-low TNBC was similar to luminal A and luminal B tumors, while AI-high TNBC fared significantly worse (p=0.0007). Again in HER2-negative patients, TP53 MUT were associated with worse DFS irrespectively of subtype (p<0.0001). Among patients with HER2-positive tumors (evaluable N=246), DFS for T-treated patients (N=100) without TP53 MUT was similar to those who did not receive T, while T-treated patients with TP53 MUT fared significantly better (p=0.011); AI-high was an adverse prognosticator only for patients with >3 positive LN who did not receive T, while this feature did not interfere with outcome in T-treated patients or in non-T-treated patients with 1-3 LN (p<0.0001).

Conclusions: This prospective-retrospective translational research study, one of the largest presenting tMPS data from paraffin tissues, confirms the extensive genetic diversity of BC at the individual tumor level. Genomic (in)stability, as assessed with AI and TP53 MUT status, may help in further defining prognosis in BC patients with early aggressive disease. TP53 MUT are indirectly shown to predict for T-specific benefit, which merits validation in larger cohorts.
A copy number aberration driven endocrine response gene signature stratifies risk in estrogen receptor positive breast cancer

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Background: Many prognostic gene signatures have been developed for estrogen receptor positive (ER+) breast cancer (BC); however, most have been solely based on mRNA expression data without integrated information on underlying primary drivers such as genomic aberrations. We therefore coupled gene expression and copy number aberration (CNA) in an attempt to improve upon prognostic signatures for ER+ BC.

Methods: mRNA expression based discovery was conducted between 172/59 ER+ BC with low/high Ki67 levels after neoadjuvant aromatase inhibition and significant genes (significance analysis of microarray, q-value less than 0.05) were screened by correlation (Mann-Whitney-Wilcoxon test P less than 0.05) with CNA using Agilent comparative genomic hybridization array. Further interrogation on prognosis of relapse-free survival (RFS) by univariate survival analysis (P less than 0.05) in patients treated with adjuvant endocrine monotherapy from a public data set produced a Copy Number Aberration Driven Endocrine Response (CADER) signature consisting of treatment sensitive/resistant genes. MetaCore (GeneGo Inc) pathway analysis was conducted for enriched pathways. We subsequently applied Nanostring nCounter technology to formalin fixed archival tumor RNA from 620 ER+ adjuvant tamoxifen treated BC (UBC TAM-series) for CADER gene profiling. Patients in multiple independent public data sets and the TAM-series were classified into treatment sensitive defined by up-regulated sensitive gene centroid and down-regulated resistant gene centroid by the median cutoffs, treatment resistant defined with the reverse pattern and indeterminate otherwise. CADER risk stratifications were associated with patient survival outcomes in public cohorts and the TAM-series. The Kaplan-Meier (KM) analysis and Cox models were used for survival analysis. Published PAM50 intrinsic subtypes and subtype based risk of relapse (ROR-S) assignments were used (Nielsen CCR 16:5222, 2010).

Results: A 54-gene CADER signature, 27 resistant/27 sensitive genes, was derived. Pathway analysis indicated that CADER was enriched with sensitive genes of cell survival functions while resistant genes were largely drivers of cell cycle progression. CADER stratifications were significantly prognostic of relapse free survival (RFS) in all public cohorts (log rank test P=0.05 for all) and in the UBC TMA-series (P=0.0001, BC specific survival and RFS). CADER showed an additional value (likelihood ratio test P=0.05) in all cohorts when both standard clinical variables and ROR-S were incorporated in multivariate Cox models. CADER were highly concordant with intrinsic subtypes and ROR-S (p=0.0001) in all data sets. However, CADER may stratify risk within ROR-S medium risk patients (P=0.002 METABRIC; P=0.003 and 0.036 in TAM-series for BC specific survival and RFS).

Conclusions: We have developed a signature that is prognostic of long-term survival in postmenopausal BC, further splits risk within ROR-S medium risk group and identifies some highly resistant BC in presence of ROR-S and clinical variables (see Ellis et. al. abstract for evaluation of a CADER single sample predictor in the MA12 Phase 3 clinical trial).
Audit of the accuracy of immunohistochemical (IHC) testing of HER2 negative status of breast cancer in the United Kingdom

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Body: Background: The analysis of the level and distribution of HER2 protein expressed by cancer cells (HER2 status) is of great clinical value in the management of breast cancer patients both for the determination of the prognosis of disease and for identification of those patients who are eligible for anti-HER2 therapy. Accurate assessment of the HER2 status is essential for identifying patients which will benefit from HER2 targeted therapy. HER2 status in the UK is established using a two tier strategy with IHC as the initial test and subsequent reflex of equivocal results to in situ hybridization (ISH). IHC staining of the HER2 protein is graded as 0, 1+, 2+ or 3+ dependent upon the intensity of staining, cellular localisation and the percentage of cells positive in accordance with CAP/ASCO and UK guidelines. HER2 3+ cases are considered as positive, with HER2 2+ cases (equivocal) retested by ISH to ascertain the gene amplification status. Cases that are scored as 0 and 1+ by IHC have no additional testing and are classed as negative. The literature indicates that a subset of these IHC negative cases show HER2 gene amplification by FISH (range 1.1-11.5%). The aim of this audit is to evaluate the discordance rate of HER2 IHC negative, FISH positive breast cancer in the UK, with a secondary objective to resolve if this is related to the choice of antibody and assay platform used.

Materials and methods: This audit selected a total of 600 sequential cases reported as HER2 negative on IHC, from three UK reference centres receiving cases from 29 different hospitals. The cases were given a unique identifying number and anonymised. Each of the three centres used a different IHC method for frontline HER2 testing with centre one using HercepTestTM (DAKO), centre two Pathway 4B5 (Roche), and centre three, Oracle (Leica Microsystems). HER2 gene amplification status was determined using dual colour FISH analysis, PathVysion (ABBOTT) fluorescence ISH (FISH) in a single centre to provide standardised methodology and assessment. HER2 was classed as amplified when the HER2/CEP 17 ratio was two or greater in accordance with UK guidelines. All cases which showed discordance between IHC and FISH were re-tested with each of the HER2 IHC platforms to discover whether these are truly discordant results or if the discrepancy is a consequence of the choice of antibody.

Results: 16/600 (2.8%) unequivocal HER2 gene amplification (mean ratio >2.0) whilst 8/600 (1.2%) had borderline amplification status(mean ratio = or <2.0). The overall assay specific discordance rates were 3.0% (HercepTest), 2.5% (4B5) and 3.0% (Oracle), respectively.

Conclusion: The observed level of discordance is well within the range of discordance rates reported by previous studies. The discrepancies could be due to inadequate quality fixation and/or inadequate sensitivity of the assay platforms used, or under scoring. A detailed analysis of possible assay related source of discrepancy is currently underway by repeating the analyses of the 24 discordant cases using like for like three assay platforms at an independent expert centre.
Title: TRAIL receptor agonists target basal B triple negative breast cancer (TNBC) that expresses vimentin and Axl

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Body: Background:
Tumor Necrosis Factor Related Ligand (TRAIL) triggers apoptosis by binding to cell surface receptors. We previously showed that TRAIL receptor agonists preferentially kill TNBC cells with mesenchymal features (Basal B cell lines) through activation of TRAIL Receptor 2 (TRAIL-R2). We used preclinical models to identify predictive biomarkers of TRAIL sensitivity and tested expression of these markers in TNBC patient (pt) samples.

Methods:
We tested sensitivity to the TRAIL-R2 specific agonist drozitumab in vitro using cell viability and caspase assays. We examined expression of the mesenchymal proteins Axl and vimentin (vim) in breast cancer cell lines including TNBC by immunoblotting and using commercially available cDNA microarray data sets. Next, we performed an exploratory analysis on IHC tumor expression of vim and Axl in 53 African American pts with TNBC diagnosed between February 2003 and February 2009. In a retrospective cohort study, overall survival (OS) was calculated from date of surgery until date of death or last follow up. Disease-free survival (DFS) was calculated from the date that the pt was identified as being disease free until date of recurrence or date of last followed without recurrence. The significance of the difference among Kaplan-Meier curves was determined by a log-rank test. Axl, vim, and age at diagnosis values were divided approximately into quartiles based on data from all available pts before being used in actuarial analyses. A Cox proportional hazards analysis was also performed to determine if Axl or vim retained prognostic value after adjusting for other factors jointly associated with outcome. All p-values were two-tailed.

Results:
As previously demonstrated with TRAIL, drozitumab selectively killed Basal B TNBC cell lines. Gene analysis and protein expression demonstrated that vim and Axl were selectively expressed in drozitumab sensitive Basal B cells. Analysis of cDNA microarray data sets showed that approximately 40% of TNBC express high levels of both Axl and vim. IHC confirmed that expression of Axl and vim seen on cDNA microarray was in TNBC tumor cells. In an exploratory analysis of the relationship of vim and Axl expression to OS and DFS, Axl, vim, stage, and response to neoadjuvant chemotherapy were factors found to be potentially associated with OS in univariate analyses while Axl, vim, age and stage were associated with at least trends towards significance with respect to DFS in univariate analyses. Cox models showed that higher vim levels (p=0.08) and stage I and II disease (p=0.024) were potentially associated jointly with OS, while higher Axl levels (p=0.05), age (p=0.016) and stage I and II disease (p=0.0007) were jointly associated with DFS.

Conclusions:
Preclinical data suggest that expression of vim and Axl can identify TRAIL Receptor agonist sensitive TNBC cells. Based on microarray and IHC, a subset of TNBC tumors express these markers in tumor cells. In our exploratory analysis with limited pts, greater vim and Axl expression were associated with a trend towards better DFS and OS. Vim and Axl may be useful predictive biomarkers for identifying TNBC pts in whom to test TRAIL receptor agonists.

Research funding: Safeway Foundation and National Cancer Institute.
Title: Risk of recurrence estimates with IHC4 are tolerant of variations in staining and scoring

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Body: Aims

1. To determine the degree to which alterations in immunohistochemical (IHC) staining for ER, PgR, and Ki67 affect IHC4 scores and distant recurrence estimates.
2. To examine the level of scoring precision needed for accurate use of the IHC4 score.

Background

The IHC4+clinical (C) score combines assessment of protein expression levels of ER, PgR, HER2 and Ki67 with clinicopathological parameters to identify breast cancer patients at low, intermediate or high risk of distant disease recurrence so aiding treatment decision-making (Cuzick J, et al. JCO 2011;29:4273). The score is used in clinical decision making in our hospital (RMH). Our published studies have shown it provides information that improves decision-making on adjuvant chemotherapy in >65% of patients (Barton S, et al. BrJCancer 2012;106:1760; Yeo BJ, et al. AnnalsOncol 2014;25suppl_1:i2).

Despite its low cost and wide availability reported use of IHC4+C in other institutions remains limited (Lakhanpal R, et al. JCO 2014;32:abstr2549); one explanation is the perception that IHC-based methods and the assessment of them lack precision, reproducibility and portability. We have examined the effect of decentralized testing and easily reproduced estimate-based scoring methods on the IHC4 score to determine its suitability for wider adoption.

Methods

A TMA was constructed from 30 breast cancer cases representative of those for which IHC4+C is requested at RMH. Sections were distributed to three centers that undertake diagnostic breast cancer IHC and that use reagents and platforms from Dako, Leica or Ventana who provide most IHC for oncology globally. Centers carried-out staining using their standard procedures and returned slides for central assessment. Results were compared against those obtained at RMH using standardized methods previously described (Barton S, et al. BrJCancer 2012;106:1760), and were used to calculate IHC4+C and 10-year distant recurrence probability (%DRprob) scores. In parallel the TMA slide stained at RMH for ER was scored by a variety of simplified non-counting based methods. Results were compared with those produced by counted H-Score when used to calculate IHC4+C and %DRprob.

Results

There was a high-degree of correlation between individual IHC results produced by all three external centers and those of RMH, and of the IHC4 and %DRprob scores derived from them (Tables 1 & 2). Scoring method for ER could be adapted to require less precision without significantly affecting IHC4+C and %DRprob results (correlation coefficients range: 0.90 - 0.98).

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Table 1. Correlation coefficients for all pair-wise comparisons
### Conclusion
The IHC4+C algorithm is tolerant of variation in staining and ER-scoring method used. Although additional comparative studies are required to confirm them, these data support the use of IHC4+C in routine clinical practice outside its institute of origin.

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<th>Mean</th>
<th>RMH</th>
<th>Dako</th>
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<td>Ventana</td>
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Table 2. Showing for each center, the mean %DRprob score and median value obtained when the absolute difference was calculated between matched scores.
Title: The LiquidBiopsy in metastatic breast cancer (MBC): A novel diagnostic platform for next generation sequencing (NGS) of circulating tumor cells (CTCs)

William Strauss\textsuperscript{1}, Jessamine Winer-Jones\textsuperscript{1}, Laura Austin\textsuperscript{2}, Paul W Dempsey\textsuperscript{1} and Massimo Cristofanilli\textsuperscript{2}. \textsuperscript{1}Cynvenio Biosystems, Inc, Westlake Village, CA and \textsuperscript{2}Thomas Jefferson University, Kimmel Cancer Center, Philadelphia, PA.

Body: Background: Effective treatments for advanced breast cancer are limited leaving palliative care as the only option for patients. The development of effective treatments has been challenging in part due to the biologic heterogeneity of the disease. The challenges to improve patient outcomes are represented by the availability of diagnostic technologies allowing real-time access to the genetic drivers of disease in each individual patients and the ability to match such information with novel targeted therapies. Blood based tests have a long history of providing real-time evidence in medicine. Thus blood derived templates could fill this role in genetic diagnosis. To perform, any technology for molecular analysis of tumor cells from blood must overcome limiting amounts of template and high signal to noise. Current efforts are directed to develop blood based tests for circulating biomarkers that can describe the molecular changes in breast cancer. We investigated the ability to productively sequence tumor cells recovered from whole blood.

Methods: EpCAM expressing Circulating tumor cells (CTCs) were recovered from 49 patients with metastatic breast cancer on the LiquidBiopsy\textsuperscript{TM} platform (Cynvenio Biosystems, Inc). CTC events were characterized using a nuclear stain (DAPI), Cytokeratin expression and absence of CD45. DNA template recovered from this population was case control sequenced with the Life Technologies AmpliSeq targeted resequencing panel on an Ion Torrent Platform using a CLIA validated sequencing pipeline and a sensitivity of 1%. Somatic single nucleotide variants (SNV) present in CTC but not the germline were identified. Critically, the false positive score for 31 normal donors thus evaluated was zero.

Results: Circulating target cells were detected in all 49 patients with metastatic BC (CTC Median 28, range 2 - 4098). Upon resequencing, shared germline polymorphisms were observed in CTC and germline populations. In contrast, SNV were detected in EpCAM enriched populations but not germline in 32% of samples (allele frequency 1.0%-49.6% with 2417-22068 x coverage). Enumeration of cytokeratin expressing cells was predictive of neither the presence nor the allele frequency of detectable SNV. Interestingly mutations in the gene for PIK3ca accounted for 20% of the mutations observed including variants at K111E, E542K, E545K, and H1047L. Mutations were also observed in TP53 (8%), and MET, FGFR2, SMAD4. Twenty samples had tissue biopsy sequence data available and of those, 11 contained mutations that were evaluable in the CTC. All but one mutation were absent in the evaluable tissue biopsy

Conclusions: CTC recovered by EpCAM selection directly from blood can be sequenced by NGS. The predictive value of the CK+ phenotype as a surrogate marker for mutation bearing cells is not supported by these data. Even with a relatively narrow 50 gene target platform, cancer relevant SNVs were detected in >30% of the patients, making targeted NGS of CTCs a promising approach for biomarker discovery and validation in MBC.
Machine learning-based prognostication of breast cancer recurrence using tissue slide features from H&E and immunohistochemically stained slides

David Knowles¹, Chukka Srinivas², Christophe Chefd'hotel², Sherry Dean² and Michael Barnes². ¹Stanford University, Stanford, CA and ²Ventana Medical Systems, Inc, Mountain View, CA.

Introduction:
More accurate prognosis of hormone-positive early stage breast cancer patients offers the opportunity to make more informed follow-up choices, for example the addition of adjuvant chemotherapy. Traditionally, pathologists have prognosticated these cancers using conventional staging, tumor proliferation index, and a small set of morphological features (gland formation, nuclear grade, and mitosis) manually scored from H&E slides. This information along with the immunohistochemical (IHC) protein expression of the tumor (estrogen receptor (ER), progesterone receptor (PR), Her2 and Ki67), may be used to prognosticate a patient. While there is potentially a large amount of prognostic information for a given patient, this information is analyzed separately from a clinical standpoint without its holistic integration into a single comparative prognostic dataset. In this study, we investigated a machine learning approach to combine these different prognostic information sources. We constructed a prognostic model to predict recurrence risk from exhaustive set of image features computed from digitized immuno histopathology tissue slides.

Material and Methods:
A paraffin-embedded tissue microarray cohort consisting of 280 cases was used for analysis (3 cores per sample). Each case had matching H&E and ER, PR, HER2, and Ki67 immunohistochemical (IHC) stains. H&E and IHC slides were analyzed to extract image feature data and integrated with clinical/demographic information. Our analysis involves the following steps: 1. Tumor and stromal region segmentation in H&E image; 2. Nuclei detection and classification in each of the IHC slide images; 3. Computation of a large set of local and regional morphometric, image intensity, relational and co-expression features; 4. Learn a binary classifier model to predict low-risk and high-risk recurrence; 5. Performance evaluation using cross-validation.

Results:
On our microarray cohort, the image-feature based recurrence risk binary classifier showed an AUC (area under the receiver operator curve) of 0.78. Various features were used including nuclei size measured on the ER stained slides, a measure of nuclear atypia measured on the PR images, and co-expression of ER and Ki67.

Conclusions:
This machine learning approach might provide a way to discover and integrate information holistically from different clinical prognostic data sources including clinical/demographic, H&E slide-based features and, for the first time shown, IHC stained slides. We are currently investigating the above features on a 600 patient cohort.
Title: Correlation between germline and tumor CYP450 2D6 gene polymorphisms

Mehdi Dehghani¹, Anneliese O Gonzalez², Neda Hashemi-Sadraei², Songlin Zhang² and Kevin P Rosenblatt¹,². ¹CompanionDx Reference lab, Houston, TX and ²University of Texas Health Science Center, Houston, TX.

Body: Tamoxifen is used for the treatment and prevention of ER (estrogen receptor)-positive BrCa (Breast Cancer). Several studies have shown that genetic variations in the CYP2D6 gene and/or drug-dependent inhibition of CYP2D6 enzyme activity (drug-drug interactions or DDIs) are associated with significant reductions in the circulating levels of endoxifen the active tamoxifen metabolite. These studies demonstrated that changes in CYP2D6 metabolism, as predicted from CYP2D6 genotyping, affect efficacy. Recent negative results with regards to CYP2D6 genotyping from two large studies suggest that testing for CYP2D6 status has no practical clinical value. Tumor DNA was used for genotyping in a large fraction of the BrCa patients in these studies. Genetic bias may result when CYP2D6 genotyping is carried out on the tumor (somatic) genome rather than the host genome (germline DNA) since it is the host genome that determines CYP450 enzyme activity within the liver and GI tract. Also expanded CYP2D6 polymorphism coverage, using a more comprehensive genotyping panel, may improve risk stratification when using CYP2D6 genotyping as a prognosticator in BrCa patients treated with tamoxifen. We hypothesize that BrCa tissues will harbor numerous mutations, due to a mutator phenotype inherent in most cancers, including within the CYP450 family of genes and, specifically, within the CYP2D6 gene. We expect that comparisons between germline DNA isolated from peripheral blood, and mutated DNA isolated from BrCa (somatic DNA) specimens within the same patient will reveal extensive differences in CYP2D6 genotypes.

The aim of this study, is to extensively genotype 70 BrCa patients using a retrospective cohort with matched blood and tumor tissue from an existing biobank at UTHealth. We plan to perform genotype to phenotype conversions on each patient, for both germline and somatic DNA. We will look for discrepancies in genotypes and phenotypes and determine the magnitude of genetic bias possible in such cohorts.

Method: DNA samples were extracted from matched archived tumor cells, dissected by laser microdissection microscopy and blood. Genotyping was performed by clinically validated Taqman® discrimination assays on the most common alleles for CYP2D6. We also studied the genotype status of these paired samples for CYP2C9, VKORC1, Factor II, Factor V, MTHFR, CYP3A4 and CYP3A5 genes. CYP2D6 gene copy number and gene rearrangement with CYP2D7 pseudogene were also assessed by Taqman copy number assays at 3 three different sites within gene. Genotype-phenotype conversion was performed using an in-house developed, clinically validated genotype-phenotype translator package. Genotype agreement was assessed between the two DNA sources.

Results: Noticeable non-concordant results between DNA from breast tumors and blood were observed in the genotyping of polymorphisms in the CYP2D6 gene. However, strong agreement between DNA from breast tumors and blood was detected in the genotyping of polymorphisms in all other studied genes. These results suggest that previous publications refuting the association between CYP2D6 genetic polymorphisms and tamoxifen efficacy could have reached an inaccurate conclusion due to genetic bias and study design. Further research in this biomarker area is needed.
Title: Effect of prolonged cold ischemic time on immunohistochemical testing of estrogen receptor, progesterone receptor and HER2 expression in breast cancer

Tae-Kyung Yoo¹, Hyeong-Gon Moon¹, Jisun Kim², Jun Woo Lee³, Min Kyoon Kim¹, Eunshin Lee¹, Jongjin Kim¹, Wonshik Han¹, In-Ae Park⁴ and Dong-Young Noh¹. ¹Seoul National University College of Medicine, Seoul, Korea; ²University of Ulsan College of Medicine, Asan Medical Center, Seoul, Korea; ³Ewha Womans University School of Medicine, Seoul, Korea and ⁴Seoul National University College of Medicine, Seoul, Korea.

Body: Background: Estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) are the most important predictive and prognostic biomarkers in breast cancer. The American Society of Clinical Oncology/College of American Pathologists recommends that the time from tumor removal to fixation (cold ischemic time) should be kept within 1 hour. Through this study we mean to review the actual cold ischemic time in real practice and analyze whether delayed formalin fixation effects immunohistochemical (IHC) testing results.

Methods: Patients, who received surgery for invasive or in situ breast cancer in Seoul National University Hospital, Seoul, Korea between February and December 2013, were retrospectively reviewed. Cold ischemic time was calculated by extracting the time of formalin fixation from the time when surgery ended. All patients were equally divided into two groups (short ischemic group, long ischemic group) according to median cold ischemic time. Chi-square test was done for ER and PR positive/negative (0% negative, ≥1% positive) and student t-test was done for ER and PR percentage. Also χ² test was done for HER2 positive/negative and scoring system ranging from 0 to 3+.

Results: A total of 615 patients were included in this study. The median cold ischemic time was 2h 43min 4sec (range 6min 36sec – 84h 26min 20sec). Only 48 patients had a cold ischemic time shorter than 1 hour. No association between ER, PR expression and cold ischemic time was found in the χ² test (p=0.581, p=0.954) and student t-test (p=0.648, p=0.978). As for HER2 expression, in the long ischemic group, there were significantly more patients with positive immunohistochemical testing results (χ² test, p=0.016), and significantly higher grades in HER2 scoring system (χ² test, p=0.022). Compared to IHC results, FISH testing for HER2 amplification showed no significant difference (χ² test, n=145, p=0.500). This tendency was persisted when patients were divided into four groups by 25 quartile of cold ischemic time.

Conclusions: Our findings show that the actual cold ischemic time in practice is longer than recommended guidelines. Despite that, ER, PR expression was not associated with cold ischemic time. As for HER2 expression, longer cold ischemic time was associated with more HER2 positive and higher HER2 score. But this tendency was not showed in FISH testing for HER2 amplification.
Title: Comparison of the parallel quantitative expression profiling in paired FF and FFPE samples with IHC4 by using two prespecified prognostic models

Xiaoyan Huang¹, Genhong Di¹, Xiaojing Xu², Bingyu Sun², Rui Bi², Wentao Yang³, Rui Li², Guangyu Liu¹, Jiong Wu¹, Zhzhou Shen¹ and Zhimin Shao¹. ¹Fudan University Shanghai Cancer Center, and Shanghai Medical College, Fudan University, Shanghai, China; ²Shanghai Biotechnology Corporation, National Engineering Center for Biochip at Shanghai, Shanghai, China and ³Fudan University Shanghai Cancer Center, and Shanghai Medical College, Fudan University, Shanghai, China.

Body: Introduction: Breast cancer is one of the leading malignancies and causes of cancer death for women now in China especially in the developed cities. Early diagnosis and adjuvant therapy have helped to improve the survival of the patients. The technique limitation slowed the pace of development of gene expression profiling. QuantiGene Plex(QGP) assay of bDNA technique without requiring RNA extraction, cDNA synthesis or PCR amplification which is suitable for the FFPE samples analysis. QGP could quantify the expression of RNA in FFPE tissues. Our primary purpose was to verify the value of the QGP platform in conjunction with multi-analysis in paired FFPE and FF tissues. The second purpose is focusing the key genes (ESR1, PGR, ERBB2 and MKI67) expression signature in early breast cancer patients (T1-2N0-1M0), and compare the gene expression profiling with the immunohistochemistry(IHC) corresponding panels by using two prespecified models-power and linear, based on the different combinations of the variables (FF4_P, FF4_L, FFPE4_P, FFPE_L, IHC4_P, IHC4_L).

Methods: We retrospectively selected 103 paired archival tumor blocks and fresh frozen samples consecutively from the fresh tissue bank from the year 2006 to now. All the tissues are without neoadjuvant treatment. The tumor specimens with local recurrence or distance metastasis had the priority. The QGP data were harvested from a paralled bDNA assay with multiplex capability of the Luminex platform by coupling xMAP fluorescent beads. We compared the QGP result of FFPE samples with FF and IHC results head-to-head. We used automatic IHC staining instrument (BenchMark XT) to retest all the Ki67 staining. We divide the total sample into training and test groups. According to the data from the training group to create the two models, and through the cross-validation to build a model based on a subset of the provided data, then calculate their average value to create the final model. And then through the test group on the construction of group training model validation.

Results: The storage time of the FF and FFPE samples used in our study ranged from 20.5 to 96.6 months. There is no different expression in each storage times intergroup and intra-group. FFPE and FF of QGP assay (single gene markers ESR1, PGR, HER2, and MKI67) correlated with the IHC result. The two models created the different scores. Both the power and linear models scores of FF4, FFPE4 and IHC4 are the prognostic factors in univariate and multivariate analysis of disease free survival with significant statistic(p<0.01), but in the bootstrapping resampling the FFPE4_L was borderline (p=0.068). The AUC of ROC in the test group is from 0.760-0.820 of each scores, and there is no statistic difference between each AUC.

Conclusion: The multiplex bDNA assay is a reliable and accuracy technique, and could bridge the gap among the FF tissue to FFPE. The both models of QGP could be complement of the immunohistochemistry panels and provide more reproducible results. The parallel multiplex QGP could leading to a great potential for translational cancer research for future study and resolve the complex pathological interpreting.
Title: Low influence of tumor cell content on mRNA expression levels of ESR, PGR, HER2 and KI67 when performing the MammaTyper® RT-PCR kit

Ralph M Wirtz¹, Tilman Rau², Mark Laible³, Kornelia Schlombs³, Sotirios Lakis¹, David Wachter², Ugur Sahin³ and Arndt Hartmann². ¹STRATIFYER Molecular Pathology GmbH, Cologne, Germany; ²Institute for Pathology, University Clinic Erlangen, Erlangen, Germany and ³BioNTech Diagnostics GmbH, Mainz, Germany.

Body: Background
Breast cancer patient management relies on approximations of molecular subtypes by immunohistochemical staining (IHC) of ER, PgR, Her2/neu and Ki-67. However, routine application of IHC is subject to important pre-analytical, analytical and interpretational variations which result in significant inaccuracy (Hammond et al. 2010, Polley et al, 2013). In contrast, quantification of biomarker RNA expression by RT-qPCR using the MammaTyper® RTqPCR kit displayed a high concordance of single marker results of 100 % HER2, 96.8 % ESR1, 97.2 % PGR and 97.6 % KI67 based on predefined cutoffs (see Laible et al, abstract submitted). However, varying tumour cell content (TCC) could possibly affect the validity of quantitative assessment of ESR1, PGR, HER2 and KI67. Herein we aimed to investigate the performance of MammaTyper® RT-qPCR kit under extreme scenarios of TCC enrichment.

Materials and Methods
Ten extreme cases with low TCC (10 - 30%) and enriched in DCIS (15 – 70%) were selected. Two RNA samples were prepared for each case using the RNXtract® IVD kit. One sample contained at most 20% TCC whereas its pair contained > 80% after macrodissection by an experienced technician. ESR1, PGR, HER2 and Ki67 RNA were determined using the MammaTyper® RT-qPCR kit on a Roche Light Cycler. Differences in DDCT values and coefficient of variation were used to analyze differences between non-macrodissected and macrodissected paired samples. IHC served as a reference for biomarker status evaluation.

Results
Despite the varying TCC content of invasive carcinomas the median 40-DDCT Differences of the mRNA Expression of HER2, ESR1, PGR and HER2 between non-macrodissected and macrodissected samples were 0.34, 0.46, 0.27 and 0.41, respectively. When previously established clinical cut-offs for biomarker positivity were considered, only a single case for KI67 appeared to be affected by low TCC (negation). The concordance with IHC data was 88.9% for ESR1, 100% for PR, 88.9 for HER2 and 44.5% for KI67 in this series.

Relative mRNA expression differences between non-macrodissected and macrodissected tumor specimen

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Conclusion
The performance of the MammaTyper® diagnostic assay does not appear to be affected by fluctuations in the TCC of the original FFPE specimens under the presence of increased amounts of DCIS. Similar findings have been previously reported in a research setting (Kotoula, Virchows Arch 2013). Effective RNA extraction and optimal PCR output normalization provide sufficient robustness for tolerating up to 8-fold TCC specimen changes, independent of the presence of DCIS. Our analysis suggests that extra time spent on macro-dissection of specimens for routine RT-qPCR assays could be avoided with safety when using the MammaTyper® RT-qPCR kit.
Title: Centrosome amplification score: A quantifiable cancer cell trait with putative risk-predictive value in breast cancer

Vaishali Pannu¹, Padmashree CG Rida¹, Sergey Klimov¹, Karuna Mittal¹, Guilherme Cantuaria², Michelle Reid³ and Ritu Aneja¹. ¹Georgia State University, Atlanta, Gabon; ²Northside Hospital Cancer Institute, Atlanta, GA and ³Emory University Hospital, Atlanta, GA.

Body: Breast tumors harbor extensive intratumoral heterogeneity (ITH), both within primary and metastatic lesions. The generation of this genetic diversity relies on chromosomal instability (CIN), a dynamic and complex multilayered phenotype. CIN comprises of an increased propensity to missegregate chromosomes during mitosis and ostensibly can be regarded as a survival state adapted to aneuploidy, frequent aberrant mitosis and a sustained reshuffling of the genome. Centrosome amplification (CA), a well-established cancer cell-specific trait is known to compromise mitotic fidelity resulting in CIN. Essentially, CA assists cancer cells in concocting an array of diverse clones that drives tumor evolution by providing basic infrastructure for ITH. On this note, logical reasoning and rational thinking led us to hypothesize that CA, a cell-biological cancer cell selective feature, may be profoundly crucial and serve a causal role in driving ITH associated with tumor progression. While some studies suggest that CA is an indicator of aggressiveness, others report that extent of CA increases with grade. No study yet has ever quantified CA or emphatically demonstrated the extent of CA during the course of malignant evolution from a well differentiated to a poorly-differentiated tumor. Here we have developed first-of-a-kind novel method to quantitate the degree, extent and type of CA (both numeral and structural) within tumor samples to evaluate the trend in incidence and severity in breast tumors across grades. Tissue specimens from core biopsies of 200 breast tumors were immunostained for centrosomes and nuclei. Employing immunofluorescence confocal imaging, a stack of optical sections was acquired to capture all centrosomes and nuclei within 10 regions of interest (ROIs) per sample. Centrosomes were categorized as (i) individually-distinguishable centrosomes (iCTRs) or (ii) as megacentrosomes (mCTRs) comprised of several tightly clustered centrosomes whose precise number cannot be determined. For each ROI, number of nuclei as well as numbers and volumes of all iCTRs and mCTRs were determined and a cumulative Centrosome Amplification Score (CAS) was obtained for each ROI as CAStotal = CASi+CASm. Low grade (Grade I, n=75) tumors exhibited significantly higher CASi(3.9 vs 2.3), CASm (9.5 vs 5.4) and CAStotal (12.8 vs 8.05) values than high grade (Grade II and III, n=125) ones, which does not support the previously held notion that CA increases during disease progression. This postulation is additionally supported by the observation that low grade tumors that exhibit lymph node infiltration and metastasis (n=30) had higher CASm (7.1 vs 9.8) and CAStotal (9.5 vs 11.5) (reverse these numbers) values as compared to the non-invasive ones (n=50). In conclusion, our innovative method to quantitate CA in tumor samples establishes CA as a "quantifiable cell biological property", that can potentially predict the risk of a low grade tumor being or becoming an aggressive and invasive one.
Title: Empowering the Nottingham Grading System: An integrated Ki67-mitosis classifier yields a better patient stratification tool

Vaishali Pannu1, Padmashree CG Rida1, Sergey Klimov1, Nikita Wright1, Guilherme Cantuaria2, Michelle Reid3 and Ritu Aneja1.
1Georgia State University, Atlanta, Gabon; 2Northside Hospital Cancer Institute, Atlanta, GA and 3Emory University Hospital, Atlanta, GA.

Body: Therapeutic decision-making for personalized management of breast cancer relies on patient stratification based on the risk conferred by clinicopathologic factors, such as stage, Ki67 Index (KI) and tumor histological grade. The most widely used histologic grading system for breast cancer, the Nottingham Grading System (NGS) provides prognostic information about breast tumor samples by combining analysis of the extent of glandular differentiation, nuclear pleomorphism and mitotic activity present in the tumor sample. In the NGS, the mitotic Index (MI) is defined as the number of mitotic cells per ten high-power fields. KI is a universal prognostic indicator but is not part of the NGS. Although the NGS has been widely used owing to its reproducibility and significant prognostic value, its accuracy in predicting disease prognosis and aggressiveness is limited. Our earlier work has demonstrated that by rationally integrating KI and MI into a new metric called the Ki67-Adjusted Mitotic Score (KAMS), which is a measure of the proportion of mitotic cells amongst Ki67-positive cells, we are able to glean a new layer of prognostic information about metastatic risk. We found that for Nottingham Grade II and III patients, high KAMS values predicted relatively better overall survival (OS) than low KAMS values.

We therefore asked if the incorporation of a KAMS-based classifier subsequent to conventional Nottingham classification, would improve patient stratification to enable their funneling towards more optimal treatment choices. Ideal KAMS thresholds were established by analyzing the KAMS-stratified survival groups and selecting the thresholds which created the widest survival stratification that was additionally confirmed to be statistically significant via a Log-Rank test. For Nottingham Grade II and III patients, an above-threshold KAMS value was deemed as low-risk and a below-threshold KAMS value was deemed as high-risk for the purpose of grade adjustment. Grade adjustments were based solely on the patients’ KAMS values. Thus the adjusted Nottingham Grade I consisted of the original Nottingham Grade I patients along with KAMS determined low-risk original Nottingham Grade II patients. Adjusted Nottingham Grade II patients were composed of KAMS determined high-risk patients originally in Nottingham Grade II along with KAMS determined low-risk original Nottingham Grade III patients. Finally, the adjusted Nottingham Grade III was made up exclusively of high-risk patients from the original Nottingham Grade III.

We found that the adjusted system represents a wider separation between OS curves with adjusted Grade I (n = 774) OS being 95.48%, adjusted grade II (n = 727) OS at 87.62%, and adjusted Grade III (n = 110) having an OS of 78.18%. Overall survival groups between adjusted grade I and II and I and III (p < 0.001) showed statistical significance. We also found that the hazard ratios for all Nottingham grades are significantly better after adjustment (2.875 versus 2.034 for Grade II and 5.115 versus 3.456 for Grade III) indicating that the KAMS can function as an effective risk-stratification biomarker and that incorporation of a KAMS-based classifier enhances patient stratification.
Title: Evaluation of RT-qPCR and luminex-based methodologies in HER2 breast cancer testing

Elizabeth N Kornaga\textsuperscript{1}, John B McIntyre\textsuperscript{1,2}, Alexander C Klimowicz\textsuperscript{3}, Natalia Guggisberg\textsuperscript{1}, Don G Morris\textsuperscript{1,4} and Anthony M Magliocco\textsuperscript{5}. \textsuperscript{1}Translational Research Laboratories, Alberta Health Services, Calgary, AB, Canada; \textsuperscript{2}University of Calgary, Calgary, AB, Canada; \textsuperscript{3}Boehringer Ingelheim Pharmaceuticals Inc, Ridgefield, CT; \textsuperscript{4}University of Calgary, Calgary, AB, Canada and \textsuperscript{5}Anatomic Pathology, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL.

Body: *Co-First Authors

Background: Currently, patients diagnosed with breast carcinoma undergo HER2 testing to direct clinical treatment decisions. At present, immunohistochemical (IHC) and in-situ hybridization methodologies are employed in the clinical setting to ascertain HER2 status. While these tests represent the current standard, their interpretation and variability of results with respect to HER2 prognostic and predictive value remains an outstanding issue.

Aim: In this comparative study we assessed the utility of testing HER2 gene expression by RT-qPCR and the Luminex Quantigene® Plex 2.0 (Affymetrix) methodology against the clinically accepted IHC assay.

Methods: Local cases from 2008-2010 that were clinically evaluated for HER2 were identified and underwent further pathologist review. In cases where there was sufficient tumor, formalin fixed paraffin embedded samples were retrieved. A total of 207 cases were identified which met selection criteria. Tumour sections were stained for HER2 and scored 0-3, following ASCO/CAP guidelines. For molecular assessment total RNA was extracted from tumours and those samples with sufficient RNA yield and quality were assessed for Her2 transcript level expression by RT-qPCR (n=129) and Luminex Quantigene® Plex 2.0 assays (n=166).

Results: Results for RT-qPCR are relative to two normal breast calibrator samples and reported as the mean relative quantification (RQ) value. For HER2 IHC negative cases (0/1+), the mean score was 0.13 (0.004-1.84, SD ±0.22); equivocal cases (2+), mean score was 0.19 (0.007-0.72, SD ±0.18); and positive cases (3+), mean score was 1.51 (0.03-6.78, SD ±1.61). Student’s t-test was performed to compare the means between groups and results are as follows: negative vs. equivocal p=0.17; equivocal vs. positive p=0.0002; and negative vs. positive p<0.0001. Luminex methodology is reported as the normalized mean fluorescence intensity (nMFI) for each group. For HER2 IHC negative cases (0/1+), the mean score was 0.28 (0.01-1.19, SD ±0.23); equivocal cases (2+), mean score was 0.42 (0.10-1.23, SD ±0.27); and positive cases (3+), mean score was 4.74 (0.1-9.09, SD ±2.5). Student’s t-test to compare the means between groups was again utilized, and results are as follows: negative vs. equivocal p=0.0036; equivocal vs. positive p<0.0001; and negative vs. positive p<0.0001.

Conclusions: Results demonstrate that both RT-qPCR and Luminex Quantigene® Plex 2.0 methods are able to discern strong positive HER2 cases (IHC 3+) from negative HER2 (IHC 0/1+). For cases with moderate IHC staining (2+), the Luminex-based assay was found to perform better than RT-qPCR. For each reporting group, the range of HER2 gene expression values were observed to overlap; thus no distinct cut point could be assigned. While the results from this pilot study are promising, the adoption of molecular methods for HER2 diagnostic testing will require further rigorous investigation before any clinical considerations can be made.
Body: Background: Loss of progesterone receptor expression is known to be a predictor of Luminal-B subtype, and is of independent prognostic significance in luminal type breast cancer. However, the threshold of PgR status in IHC has been subject to debate, and the PgR status as reported by IHC not infrequently is at variance with the PgR result as reported by the Oncotype DX (ODX) assay.

Methods: We retrospectively reviewed tumor IHC and ODX data from resection specimens with ER+/HER2- breast cancer, and low to intermediate Ki-67 proliferation rates (Ki-67 < 30%). A total of 74 specimens were re-analyzed, all from resection specimens that had been selected for ODX assay for the purpose of planning of adjuvant chemotherapy, from two institutions (Univ. Heidelberg, Germany, and Univ. Zurich, Switzerland). PgR expression of < 10% using IHC (clone PgR636, Ventana and Dako systems) or PgR values of < 5.5 in ODX assay were considered negative.

Results: All cases were positive for ER by IHC and ODX, and 20% were negative for PgR by ODX assay. This compared to only 8% of negativity by IHC, but all except one case negative for PgR by IHC were also correctly identified as PgR negative by ODX. Median ODX recurrence score (RS) was 35 for PgR negativity by ODX assay, as compared to 30 for negative PgR status by IHC. For positive PgR status, median RS was calculated as 15 vs. 17 by ODX vs. IHC. Risk categories by ODX were high (RS >= 31) in 9 cases with negative PgR status by ODX, but only 3 of these high risk cases were negative for PgR by IHC. Median Ki-67 values were 22.5 for PgR negative cases by ODX as compared to 15 for PgR negative cases by IHC. Similar results were obtained when a higher PgR threshold of 20% was applied to IHC results.

Conclusions: In luminal type breast cancers, the evaluation of PgR status by IHC may underestimate the proportion of PgR negative cases and did not identify most cases of tumors with a high risk of recurrence. This may be caused by an overly high sensitivity of PgR status in routine automated immunohistochemistry.
The chromosomal genomic landscape and targetable co-amplified genes in HER2 positive breast cancer patients who relapsed on an adjuvant trastuzumab chemotherapy trial

Shelly Gunn¹, Chris McCaskill¹, Linda Daley¹, Agnes Puskas¹, Lina Asmar², Yunfei Wang² and Stephen Jones¹. ¹MolecularHealth, Woodlands, TX and ²McKesson Specialty Health, Woodlands, TX.

Body: Background
Early stage HER2 amplified breast cancer has a generally favorable prognosis with over 95% of patients showing 2-year disease free survival (DFS) when treated with adjuvant trastuzumab. However, a subset of these tumors are refractory to treatment and present a challenge for the oncologist, particularly when clinical and histologic parameters, including the patient’s nodal and hormonal status, are indicative of lower-risk HER2 positive disease. In this study we describe the genomic landscape of three clinically lower-risk patients with HER2 amplified tumors who relapsed on adjuvant docetaxel and cyclophosphamide plus 1 year of trastuzumab in a phase 2 study (Jones et al, Lancet Oncology 14: 1121, 2013). All patients’ tumors showed high-level HER2 amplification ratios by FISH (8.51-14.46) and were analyzed in parallel with a fourth clinically matched 2-year DFS patient’s tumor from the same trial with high HER2 gene amplification (FISH ratio 11.38).

Methods
Primary tumor genomic DNA analysis was performed from archival tissue by next generation sequencing (NGS) on the Illumina HiSeq 2500 platform in a CLIA certified laboratory. Tumors were screened for point mutations and copy number alterations (CNAs) by NGS using a targeted-whole exome 613 gene panel. CNAs detected by NGS were confirmed on a DNA microarray featuring high-density probe coverage of the same 613 genes on the targeted panel. CGH chromosome ratio plots were overlaid with algorithmically derived NGS copy number data to generate a map of the chromosomal genomic landscape for each patient’s tumor.

Results
High-level HER2 gene amplification status was confirmed, and co-amplified chromosome 17 genomic regions were detected in all three relapsed patients’ tumors. High-level HER2 amplification was also confirmed in the non-relapsed patient’s tumor but co-amplified regions were not detected on chromosome 17 or elsewhere in this patient’s tumor genome. Two of the relapsed ER negative tumors shared focal high-level CCNE1 gene amplifications on chromosome 19. High-level MAP3K3 gene amplification on chromosome 17 was detected in the one ER positive tumor from a relapsed patient. Focal PIK3CA gene amplifications were not identified in any of these tumors, but two tumors (one from the relapse group and one non-relapse) were positive for activating H1047R mutations.

Conclusions
Combined NGS and CGH analysis of HER2 positive early stage breast cancer can be performed in the clinical laboratory to reveal the tumor’s chromosomal genomic landscape. Combined with other test results, this tumor map can help identify patients at high-risk for relapse and reveal alternative predictive biomarkers of therapeutic response.
Correlation of the effects of potential and known chemopreventive agents on proliferation rates in normal mammary glands and mammary cancers with their chemopreventive efficacy

Brandy M Heckman-Stoddard¹, Clinton J Grubbs², Fariba Moeinpour², Vernon E Steele¹ and Ronald A Lubet¹. ¹National Cancer Institute, Rockville, MD and ²University of Alabama, Birmingham, AL.

Body: Core needle biopsy, fine needle aspiration, or imaging (e.g., mammography) are currently used to examine chemopreventive agent efficacy in Phase II breast cancer trials. However, biomarker endpoints, such as proliferation rates using Ki67 in normal or "at risk" breast tissue, have not been formally validated relative to cancer outcome. The aim of this study was to validate Ki-67 in "normal" mammary tissue from the methylnitrosourea (MNU) rat mammary tumor model with mammary cancer multiplicity within the same animal as surrogate biomarker for agent efficacy. Multiple studies were performed in female Sprague-Dawley rats to correlate this proliferation biomarker in mammary tissue after two weeks of chemoprevention agent treatment with mammary cancer incidence and multiplicity at the end of the study. In brief, MNU was given at 50 days of age, one week later administration of the agent was started, and after two weeks a biopsy of the mammary gland was taken. Treatment with the agents continued for approximately 150 days, at which time the study was terminated and mammary tumor multiplicity was measured. Changes in proliferation and mammary tumor multiplicity were compared to a control group of untreated group of animals within the same experiment using a two-sided Student t-test.

<table>
<thead>
<tr>
<th>Agent</th>
<th>Normal Mammary Gland Proliferation Index</th>
<th>Final Mammary Cancer Multiplicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorozole (1.25 mg/kg BW/day)</td>
<td>88%↓</td>
<td>90%↓</td>
</tr>
<tr>
<td>Lipitor (125 mg/kg diet)</td>
<td>38%↓</td>
<td>16%↓</td>
</tr>
<tr>
<td>Targretin (150 mg/kg diet)</td>
<td>90%↓</td>
<td>92%↓</td>
</tr>
<tr>
<td>Naproxen (200 mg/kg diet)</td>
<td>6%↑</td>
<td>45%↑</td>
</tr>
<tr>
<td>Iressa (10 mg/kg BW/day)</td>
<td>52%↓</td>
<td>93%↑</td>
</tr>
<tr>
<td>Tamoxifen (3.3 mg/kg diet)</td>
<td>77%↓</td>
<td>100%↓</td>
</tr>
<tr>
<td>Metformin (150 mg/kg BW/day)</td>
<td>25%↓</td>
<td>71%↑</td>
</tr>
</tbody>
</table>

In general, highly effective agents to preventing cancers (e.g., tamoxifen) also prevented normal mammary gland proliferation after only two weeks of treatment, while inactive agents (e.g., naproxen) had minimal effects on normal gland proliferation. The effects of the agents on established mammary cancers (in which the agents were given for 7 days to rats bearing small MNU-induced mammary cancers) showed similar correlations. Additional biomarkers, as well as the proliferation change and efficacy of these agents in rats fed a high-fat (Western) diet, will also be presented. In conclusion, determining the effect of a potential chemopreventive agent on cell proliferation following short-term treatment appears to be an effective method for predicting its efficacy in preventing mammary cancers. These data further confirm that Ki67 measurements are useful in Phase II prevention trials as a biomarker of agent efficacy.
Title: Inhibiting CDK1 and CDK2 activity prevents triple negative breast cancer

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1University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Background: Molecular analysis of human tumors has revealed that most carry mutations that result in deregulation of the cell cycle. In particular, breast cancers that do not express the estrogen receptor, progesterone receptor, or HER2, termed triple negative breast cancers (TNBCs), are characterized by a high proliferation rate and currently no therapy exists to prevent the development of TNBC. Mammalian cells express multiple cyclins and CDKs, but only five, Cdk1, Cdk2, Cdk3, Cdk4 and Cdk6, are thought to directly participate in cell cycle regulation. Therefore, we hypothesized that inhibition of CDK activity would prevent TNBCs.

Methods: Gene expression data was statistically analyzed using GraphPad and R. The effect of CDK1/2 inhibition on premalignant cell growth was determined by siRNA transfection or by treatment with dinaciclib (1 – 10nM) for 3-4 days. Cell cycle analysis was performed on PI stained cells and apoptosis was determined in cells stained with the FITC Annexin V. In vivo studies were performed using C3(1)-SV40 Tag transgenic mice. 10 week old mice were treated i.p. 3x/week with 20 mg/kg dinaciclib or vehicle. Mice were euthanized 2 months after treatment, or if tumor volume reached 1500mm3. Mammary glands were analyzed for the incidence of early lesions using Image J. Survival analyses used log-rank tests, and Kaplan–Meier curves were plotted using GraphPad. H&E and KI67 staining were performed in paraffin-embedded tissue sections. Immunoblot analysis of protein lysates was performed to detect CDK levels, CDK activity and downstream signals.

Results: In silico analysis of gene expression data sets revealed that genes associated with CDK1 and CDK2 complexes (CDK1, Cdk2, CCNA2, CCNB1, CCNB2, CCNE1) are higher expressed (>2-fold, p <0.05) in DCIS compared to normal breast tissues. Similarly, CDK1 and Cdk2 genes are higher expressed in TNBC compared to normal breast. In accordance with the in silico data, the protein expression levels and activity of CDK1 and Cdk2 were higher in the premalignant cell lines than in normal mammary epithelial cells. siRNA-mediated CDK1 depletion was sufficient to inhibit premalignant breast cell growth, however, Cdk2 knockdown did not totally impair proliferation. Treatment of premalignant cells with the CDK1/2 inhibitor dinaciclib dose dependently inhibited growth with an accumulation of cells in G2/Gm phase and induction of apoptosis. Furthermore, the activity of downstream CDK effectors such as Rb, RNA Pol II and FOXM1 was blocked upon treatment of premalignant cells with dinaciclib. In vivo in SV40 Tag mice, CDK1/2 inhibition by dinaciclib significantly reduced the total number of early lesions compared to the control group (3.917 ± 1.495 vs 18.64 ± 1.503) which was accompanied by a significant reduction in proliferation indicated by KI67 (32.45% ± 10.70% vs 47.96% ± 4.52%). Furthermore, dinaciclib significantly delayed (P < 0.05) TNBC formation by 3.4 wks with a median time to tumor formation of 167 days compared to 143.5 days of age in the control group.

Conclusions: These studies demonstrate that blocking CDK1/2 activity causes inhibition of premalignant cell growth and delays TNBC mammary tumorigenesis. These results suggest that CDK inhibitors may be useful for the prevention of triple-negative breast cancer.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-11-03
Average Grade: 6.25

Title: Development and implementation of a breast cancer risk identification and reduction program in a large health care system

Maureen A MacSweeney¹, Helen Roorda¹, Richard Lippert², Ivy Guardolia¹, Nefertari Burrell¹, Luis DeJesus¹, Augustus J Scarletto¹ and David A Decker¹. ¹Florida Hospital Cancer Institute, Orlando, FL and ²i/o Trak, Orlando, FL.

Body: Background: Multiple national and international organizations (USPSTF, ASCO, NCCN, and NICE) recommend identifying patients at an increased risk of breast cancer and counseling these patients concerning risk reduction strategies. Primary care providers have not complied with these guidelines. To fill this void, we initiated a program which identifies risk and offers risk reduction strategies. This abstract describes the development and preliminary outcomes of this program.

Material and Methods: Florida Hospital has 10 separate mammography center sites. Screening mammograms were performed on 48,917 women the year prior to the start of the program. The program was created to target this population. Data was collected between 4/1/2013 and 3/31/2014. Each of the 10 sites were incrementally added throughout one year. Women at the time of screening mammogram were offered participation in the program by the mammography technician. Mammography technicians were educated and trained. A tablet was developed with our IT department. Women that consented answered 8 questions from the NCI modification of Gail risk, 1 question addressing chest wall radiation, and 5 questions modified from the NCCN Guidelines BR/OV -1 to identify hereditary risk possibly missed by the modified Gail. The results, demographics and referring provider information was collected in a database. Patients and referring provider received letters tailored to their individual risks, by Mammologix, a mammography informatics company. High risk was defined as a modified Gail score of ≥1.7% for a 5 years, >17% lifetime risk, or a positive response to the 5 additional questions. Patients at risk were offered a risk and reduction consultation at our Breast Health Center. Consults were provided by a genetic counselor and/or ARNP. The risk and benefits of intervention were discussed. The referring provider was included in any discussion concerning risk reducing surgery, medication, and follow-up. Patients were referred back to their primary provider for continuing follow-up.

Results: 15,165 women were given a risk questionnaire at the time of screening mammography. 2752 patients opted out. 3243 were high risk by the modified Gail method. 1329 were high risk by both the modified Gail and our modified NCCN BC/OV-1 genetic guidelines questions. 14 were high risk due to chest wall radiation. Of the 4044 women at risk, 141 opted to be counseled (3.88%). 29 chose to begin prevention medication. 16 chose counseling regarding their hereditary risk.

FHCI Breast Health Center Data

<table>
<thead>
<tr>
<th></th>
<th>Q1</th>
<th>Q2</th>
<th>Q3</th>
<th>Q4</th>
</tr>
</thead>
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<td>Pts Screened</td>
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<td>5251</td>
<td>7344</td>
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<tr>
<td>Pts at risk by Modified Gail</td>
<td>196</td>
<td>290</td>
<td>1166</td>
<td>1569</td>
</tr>
<tr>
<td>Pts at risk by additional questions</td>
<td>51</td>
<td>75</td>
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<td>Pts seen in Center by ARNP</td>
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<tr>
<td>Pts seen in Center by Genetic Counselor</td>
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<td>2</td>
<td>9</td>
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<tr>
<td>Pts treated for Breast Cancer Prevention</td>
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<td>1</td>
<td>10</td>
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</tbody>
</table>

Conclusion: The program provides a comprehensive breast cancer identification and reduction option to a large number of women. It also provides the primary provider a service and resources regarding breast cancer risk identification and prevention.
Title: Inhibition of alternative NF-κB activity prevents the expansion of genetically unstable progenitor cells in BRCA1-deficient mammary glands

Andrea Sau¹, Rosanna Lau², Emma Nolan³, Peter A Crooks⁴, Jane E Visvader³ and Christine MA Pratt¹. ¹University of Ottawa, Ottawa, ON, Canada; ²UT MD Anderson Cancer Center, Houston, TX; ³Walter and Eliza Hall Institute of Medical Research, Parkville, Victoria, Australia and ⁴University of Arkansas for Medical Sciences, Little Rock, AR.

Body: Understanding the biological mechanisms underlying the initiation and progression of breast cancer is an important step for prevention and treatment. Individuals with mutations in the breast cancer-associated gene 1 (BRCA1) have a lifetime increased risk of up to 85% of developing breast cancer. BRCA1 participates in DNA damage repair and cell cycle checkpoint control, serving as a tumor suppressor gene to maintain global genomic stability. However, BRCA1 has also been shown to play a key role in the expansion of mammary stem/progenitor cells, which are the targets for carcinogenesis in individuals who have undergone loss of heterozygosity (LOH) for BRCA1.

We have used in vitro and in vivo models to demonstrate that BRCA1 loss or mutation induces alternative NF-κB pathway activation and leads to the expansion of a genetically unstable progenitor cell population in the mammary gland. Our data showed that BRCA1 loss or mutation is responsible for activation of the alternative NF-κB pathway evidenced by IκB kinase-α (IKKα) phosphorylation, processing of p100 to p52 and p52/RelB nuclear localization. Remarkably, we found that RelB and p100/p52 were highly expressed in 20-50% of the lobular structures in histologically normal breast tissue obtained from human BRCA1 mutation carriers.

After DNA damage, ATM directs the nuclear export of NEMO (NF-κB essential modifier) that in turn activates the alternative NF-κB pathway. We found high levels of phospho-ATM in breast tissue from human BRCA1-mutation carriers and in progenitor cells from BRCA1-knockout mouse mammary glands. Moreover, co-immunoprecipitation studies showed increased ATM/NEMO-containing complexes in BRCA1-deficient cells. Progesterone injections into MMTV-cre;BRCA1f/f mice induced an increase in Ki-67 and phospho-H2AX levels, demonstrating a role for progesterone in DNA damage amplification in mouse mammary glands.

Mammary epithelial progenitor cells obtained from BRCA1-mutated carriers as well as BRCA1-/- mouse glands can form acini in Matrigel in a progesterone-independent manner. We found that in vivo inhibition of IKKα/β using the IKK inhibitor dimethylaminoparthenolide (DMAPT) completely blocked mammary acini formation in Matrigel, requiring several estrus cycles post-injection to recover. Importantly, knockdown studies showed that p100/p52 was necessary for progenitor cell proliferation in Matrigel. Consistent with the continued dependence on alternative NF-κB activity in tumors derived from BRCA1-deficient progenitor cells, human breast cancer xenografts derived from BRCA1-mutation carriers infected with lenti-shp100/p52 showed a significant growth inhibition.

Overall, these results suggest an exciting new approach for the prevention of breast cancer in patients wherein intermittent or cyclic therapy using DMAPT (generic name LC-1) has the potential to mitigate breast cancer risk in BRCA1 mutation carriers through acute reduction of aberrant progenitor activity. As such, NF-κB-directed chemopreventive therapies may provide promising alternatives to prophylactic mastectomy in this high risk patient population.
Title: Pregnancy-induced epigenetic changes in the insulin-like growth factor signaling pathway

Tiffany A Katz¹, Serena G Liao¹, Thushangi Pathiraja³, Robert K Dearth², George C Tseng¹, Steffi Oesterreich¹ and Adrian V Lee¹.
¹University of Pittsburgh, Pittsburgh, PA; ²University of Texas, Pan American, Edinburg, TX and ³Genome Institute of Singapore, Singapore.

Body: Prevention will prove to be the single most effective way of eradicating breast cancer. Currently, the most effective natural breast cancer prevention is an early first full term pregnancy. While it is not feasible to use pregnancy to protect women from breast cancer, understanding how the protective effect is elicited will inform the development of new prevention strategies. Women who were pregnant in their twenties are protected thirty to forty years later creating a complicated mechanism to tease out experimentally. In order to understand the long-term protection we have investigated epigenetics, specifically DNA methylation, which is known to be stable over long periods of time. A cohort (Parous) of female FVB mice were bred, gave birth, and pups were weaned. A control group (Nulliparous) never saw male mice. Mammary glands were harvested immediately or 6 months after involution. These two time points allowed us to identify changes in DNA methylation that occurred in response to pregnancy, and additionally, changes that lasted long after parturition. DNA was isolated, and genome-wide DNA methylation was assessed using bisulfite-conversion and SureSelect Methyl-Seq. Bismark v0.7.12 was used for alignment of pair-end reads, followed by the R package "methylKit" for quality control and data analysis. A mapping efficiency of 50%–68.1% was achieved with 89,512,619 base pairs covered. CpG Pearson correlation plots and PCA analysis showed global similarity between samples. We then conducted a logistic regression to ascertain parity-induced differentially methylated regions and identified 153 and 236 persistently hypomethylated and hypermethylated genes, respectively. Among the differentially methylated genes were many signaling molecules involved in growth factor signal transduction, including insulin-like growth factor 1 and 2 receptors (IGF1R and IGF2R). It has previously been shown that circulating IGF1 levels are reduced in parous women, and similarly the growth hormone/IGF axis is altered in rodent models. Collectively, these findings suggest that the IGF pathway is regulated at multiple levels during pregnancy, and that its modification might be critical in the protective role of pregnancy. We are currently following up on these data, including protein analysis of the IGF pathway members and downstream signaling molecules in human specimens. Finally, we are expanding our analysis to additional genes and pathways epigenetically altered by pregnancy, with the ultimate goal to develop new prevention strategies.
Understanding predictive values of short-term morphologic assays of cancer chemoprevention for efficacy in animal mammary gland tumor assays

Barbara K Dunn¹, Vernon E Steele¹, Carol F Topp², Richard M Fagerstrom¹ and Barnett S Kramer¹. ¹National Cancer Institute, Rockville, Md and ²CCS Associates, McLean, VA.

Background: The predictive value of chemopreventive agent efficacy in morphologic (in vitro/in vivo) assays for efficacy in animal (mouse and rat) in vivo tumor assays is not well characterized. Over a 25-year period, the Chemopreventive Agent Development Research Group in the U.S. NCI's Division of Cancer Prevention has tested approximately 800 agents for potential chemopreventive activity. The current project focuses on a subset of 146 that were tested in both morphologic and mammary gland tumor assays in order to gain a deeper understanding of the relevant predictive value.

Materials and Methods: The early stages of the testing pathway involve two critical steps: (1) in vitro/in vivo morphologic assays and, for agents successful in these, (2) testing for tumor prevention (measured in terms of tumor incidence and multiplicity reduction) in animal tumor assays. The ultimate goal is to test agents that successfully decrease tumor incidence and multiplicity in animal tumor assays in humans. In the current project we evaluated the predictive values of the earlier-stage (morphologic) assays for efficacy in the later-stage (animal tumor, specifically mammary tumor) assays. Statistical modeling to determine how well the six most commonly used morphologic assays predicted efficacy of the 146 tested agents in mouse and rat mammary gland tumor assays was carried out by multimodel inference applied to ordinal logistic regression.

Results: The ability of these six morphologic assays to predict tumor outcomes was evaluated in the mouse and rat mammary gland cancer assays. Based on this statistical modeling, each morphologic assay was assigned a value describing how strongly it predicted outcomes in the mammary gland tumor assays. Selected morphologic assays (the mouse mammary organ culture (MMOC) and human foreskin epithelial cell (HFE) morphologic assays) in combination give a predictive value that meaningfully forecasts results for chemopreventive efficacy in the mouse and rat mammary gland tumor assays.

Conclusions: These predictive models can be used to guide our future decision-making with respect to agent selection as well as morphologic and animal tumor assay use. Our future work is focused on deepening our understanding of our Predictive Value approach by: (1) identifying the mammary gland tumor assays that best reflect anti-tumor efficacy in animals; (2) teasing apart those classes of agents that exhibit the highest predictive values overall; and (3) examining which agent classes show the highest efficacy in specific mammary gland tumor assays.
Title: Predicting the effect of tamoxifen on the breast: Change in measures of breast density, serum markers and SNPs

Anthony Howell¹, Sue Astley¹, Elaine Harkness¹, Julia Wiseman¹, Jill Fox¹, Paula Stavrinos¹, Mary Wilson¹, Yit Lim¹, Valerie Reece¹, Ursula Beetles¹, Anil Jain¹, Jamie Sergeani², Jack Cuzick¹, Ruth Warren² and Gareth Evans¹. ¹Nightingale Centre and Genesis Breast Cancer Prevention Centre, Manchester, Greater Manchester, United Kingdom and ²Centre for Cancer Prevention, London, Greater London, United Kingdom.

Body: Introduction In the IBIS I Prevention Trial we demonstrated that tamoxifen prevented breast cancer in women with greater than 9% absolute reduction in visually assessed mammographic density over a 12-18 month period as assessed by an expert radiologist, RW (1). In this study we investigated: whether RW obtained similar results in a different group of women treated with tamoxifen; how other expert mammographic film readers with experience of density assessment (radiologists, advanced practitioner radiographers and a breast physician) estimated mammographic change; how visual change relates to change in automated measures of volumetric density; and whether we can predict change by serum markers or SNPs. Methods 105 women aged 33 to 46 at greater than 1 in 6 lifetime breast cancer risk completed one year’s treatment with tamoxifen. RW assessed mammographic percentage density in increments of 5% for baseline and one year mammograms. Two or three of a pool of eight readers estimated % density using a visual analogue scale (VAS). Percent change was also estimated using a computer-assisted thresholding technique (Cumulus). Change in volume of dense tissue (Quantra™ & Volpara™) was measured. Changes in lipids, IGF1, insulin and relevant SNPs were measured to determine whether they predicted density change. Results Estimates of change obtained by RW were broadly consistent with those from our previous study, as were those from Cumulus, but could not be replicated using estimates from the pooled results of VAS readers. Tamoxifen was associated with marked reductions of dense volume of the breast (Table 1) However the different methods were at best only moderately correlated with RW (r less than 0.6). Change in serum triglycerides significantly predicted density change as measured by RW but none of the other serum measures or SNPs did.

Table 1 Numbers of women and percentage change in density measures after one year of tamoxifen

<table>
<thead>
<tr>
<th>% Density change</th>
<th>RW %change</th>
<th>Other radiologists %change</th>
<th>Cumulus %change</th>
<th>Quantra %change</th>
<th>Volpara %change</th>
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<tr>
<td>40+</td>
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</tr>
</tbody>
</table>

RW = Ruth Warren. CUMULUS was measured by JS, a trained operator of the software. * Percentage point change eg if density changed from 40% to 30% this was regarded as a 10% change. ^ Reduction in dense volume expressed as a percentage of baseline dense volume. We used Quantra version 2.0 and Volpara version 1.4.5

Conclusions Whilst RW remained consistent with previous density estimation it was not possible to use a pool of other expert readers to predict change. Cumulus gave similar results to RW but is difficult to use in practice. There was good agreement between the two objective volumetric measures used (r=0.5) and since these are automated they may be suitable for clinical practice. However their relationship to the long term breast cancer preventative effect of tamoxifen needs to be established. Reference 1 Tamoxifen-induced reduction in mammographic density and breast cancer risk reduction: a nested case-control study. Cuzick J, Warwick J, Pinney E, Duffy JW, Cawthorn S, Howell A, Forbes JF, Warren RM. J Natl Cancer Inst. 2011 May 4;
103 (9): 744-52.
Title: Metformin decreases Ki67 in HER2+ve ductal carcinoma in situ in a window of opportunity trial

Bernardo Bonanni², Andrea DeCensi¹, Aliana Guerrieri-Gonzaga², Giancarlo Pruneri², Matteo Puntoni¹, Massimiliano Cazzaniga², Andrea Vingiani², Davide Serrano², Oreste Gentilini², Harriet Johansson², Valentina Aristicò², Matteo Lazzeroni², Clara Varicchio², Marilena Petrera¹ and Giuseppe Viale². ¹E.O. Ospedali Galliera, Genoa, Italy and ²European Institute of Oncology, Milan, Italy.

Body: Background: In a presurgical trial in 200 non-diabetic women with breast cancer, we previously showed a heterogeneous effect of metformin on the Ki67, with a decreased proliferation in women with insulin resistance (HOMA>2.8) and an opposite effect in women with HOMA ≤ 2.8 (Bonanni et al. JCO 30:2593, 2012). Here we determined the effect of metformin on noninvasive proliferative disorders.

Methods: Patients with operable breast cancer were randomly assigned to metformin, 850 mg or placebo once daily on days 1-3 followed by two 850 mg tablets after dinner on days 4 to 28. A total of 3-5 specimens of adjacent (≤1 cm from tumor) and distant (>1 cm from tumor) tissue were obtained from the surgical specimens to assess systematically the prevalence of LCIS and DCIS adjacent to invasive cancer and of distant ductal hyperplasia (DH) in normal tissue. We also determined the effect of metformin on Ki67 in these lesions overall and by molecular subtype. All HER2 2+ DCIS by IHC were assessed by FISH.

Results: Overall, the prevalence of LCIS, DCIS and DH was 4.5% (9/200), 66% (132/200) and 35% (69/200), respectively. The Ki67 was positively associated with DCIS grade (p-trend<.001). The median levels of Ki67 by treatment arm in different premalignant groups is summarized below

<table>
<thead>
<tr>
<th>Premalignant group</th>
<th>Premalignant subgroup</th>
<th>Metformin arm</th>
<th>Placebo arm</th>
<th>p-value*</th>
<th>p-interaction‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>LCIS (n=9)</td>
<td></td>
<td>15 (5-15)</td>
<td>5 (4-6)</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>DH (n=69)</td>
<td></td>
<td>3 (1-4)</td>
<td>3 (1-4)</td>
<td>0.5</td>
<td></td>
</tr>
<tr>
<td>All DCIS (n=132)</td>
<td></td>
<td>12 (8-20)</td>
<td>10 (7-24)</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>DCIS grade 1/2 (n=108)</td>
<td></td>
<td>10 (7-16)</td>
<td>10 (6-17)</td>
<td>0.9</td>
<td>0.2</td>
</tr>
<tr>
<td>DCIS grade 3 (n=24)</td>
<td></td>
<td>33 (25-55)</td>
<td>40 (32-40)</td>
<td>0.2</td>
<td>0.04</td>
</tr>
<tr>
<td>HER2+ve (n=22)</td>
<td></td>
<td>22 (11-32)</td>
<td>35 (30-40)</td>
<td>0.06</td>
<td>0.04</td>
</tr>
<tr>
<td>HER2-ve (n=58)</td>
<td></td>
<td>16 (10-20)</td>
<td>17 (8-26)</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>ER+ve/HER2+ve (n=15)</td>
<td></td>
<td>12 (7-18)</td>
<td>32 (27-42)</td>
<td>0.004</td>
<td>0.001</td>
</tr>
<tr>
<td>ER+ve/HER2-ve (n=53)</td>
<td></td>
<td>16 (10-20)</td>
<td>15 (8-22)</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>PR+ve/HER2+ve (n=12)</td>
<td></td>
<td>18 (12-18)</td>
<td>32 (24-44)</td>
<td>0.02</td>
<td>0.05</td>
</tr>
<tr>
<td>PR+ve/HER2-ve (n=48)</td>
<td></td>
<td>16 (10-20)</td>
<td>12 (8-20)</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

*Wilcoxon rank-sum test; ‡ p-interaction between treatment and DCIS grade or HER2 status from a linear regression model, adjusted for age and BMI.

The effect of metformin on DCIS was different by HER2 status (p-interaction=.04) and, among this molecular subtype, by ER and PR status (p-interaction=.001 and .02, respectively). In HER2+ve DCIS, metformin decreased Ki67 by 40% overall (p=.06), by over 60% in ER+ve/HER2+ve DCIS (p=.004), and by 45% in PR+ve/HER2+ve DCIS (p=.02) There was no effect of metformin in HER2-ve DCIS, regardless of ER or PR status. Metformin did not affect Ki67 in DH overall, but showed a trend towards a decrease in women with abdominal adiposity (p-interaction=.05).

Conclusions: Window of opportunity pre-surgical models provide insight into a drug’s preventive potential by targeting intraepithelial proliferations adjacent to invasive cancer. The model shows a high prevalence of preinvasive lesions (field cancerization) in apparently normal breast tissue adjacent to breast cancer. Metformin selectively decreased Ki67 in HER2+ve
DCIS, particularly in the ER+ve subgroup, in line with a selective inhibitory effect on the HER2 pathway observed both in in vitro and in vivo preclinical models. Our results provide the background for a phase III trial of metformin in HER2+ve DCIS (ISRCTN16493703, Supported by AIRC, LILT and Health Ministry 2009-RF-153222).
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-12-03
Average Grade: 4.80

Title: Initial report of a randomized trial of letrozole in high risk women taking hormone replacement therapy

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Body: Background:
On the basis of positive results in a pilot study, we initiated a randomized, double-blind, placebo-controlled trial of the aromatase inhibitor letrozole in post-menopausal women at high risk for development of breast cancer who were taking hormone replacement therapy (HRT). The objective was to determine if risk biomarkers for breast cancer in benign breast tissue sampled by random peri-areolar aspiration (RPFNA) could be favorably modulated.

Methods
Women who exhibited cytologic hyperplasia +/- atypia and Ki-67 immunocytochemistry staining $\geq$1.5% on screening RPFNA were eligible to be randomized 1:1 between placebo and letrozole (2.5 mg daily) for six months, followed by repeat RPFNA. Women were then given the option to receive open-label letrozole for a second six months, and a third RPFNA. The primary analysis was a difference between the two groups for the change in Ki-67 between baseline and 6 months. The initial accrual goal was 108 subjects, with the expectation of 96 subjects evaluable for the baseline–6 months comparison.

Results
55 subjects were enrolled between March 2007 and March 2014, when accrual was closed. From the time of our successful pilot study to present, there had been a steady decline in the use of HRT by women in our high risk cohort, both in frequency of women using as well as the type and strength of HRT. The result was fewer potential subjects for screening and fewer still that satisfied the 1.5% Ki-67 criterion. Thus, the trial was closed early. Of 55 enrolled subjects, two dropped out prior to 6 months; 52 completed 6 months and provided evaluable RPFNA specimens for analysis, with one subject scheduled for repeat aspiration in September. Six subjects went off study between 6 and 12 months; 42 have completed the entire 12 month schedule, and 5 are still on trial. At baseline, 18 women displayed hyperplasia (Masood score 13-15) and 37 had hyperplasia with atypia (Masood score 14-17). Median Ki-67 was 3.0%, with a range from 1.6 – 15.4%. For 52 comparisons between baseline and 6 months, 8 women had no change by Masood score, 8 had an increase and 36 exhibited a decrease, i.e., less abnormality. Two women had no change in Ki-67 staining, 13 exhibited increased Ki-67 and 37 showed a decrease; median at 6 months 1.7%, median change -1.4%. For 42 comparisons between baseline and 12 months, 11 women had no change by Masood score, 9 increased, and 22 decreased. One woman had no change in Ki-67 staining, 10 exhibited increased Ki-67 and 31 showed a decrease. The decreases in Masood score and Ki-67 between baseline and 12 months (when all subjects had received letrozole, either for 6 or 12 months) were statistically significant (p<0.005, Wilcoxon signed rank test). When 6-month data are available for the final subject, the randomization will be unblinded by the statistician and the primary study question will be addressed.

Conclusion
While pending final analysis of the primary (blinded) endpoint, preliminary analysis indicates favorable modulation of cytomorphology and proliferation by the aromatase inhibitor letrozole in high risk post-menopausal women taking hormone replacement therapy.

Funding: NIH RO1 CA122577; Novartis Pharmaceuticals Corp.
Title: De-escalating doses of letrozole in post menopausal women at high risk for breast cancer

Ana Maria Lopez¹, Hsiao Hui Sherry Chow¹, Denise Frank¹, Sandhya Puthi², Judy Boughey², Paul Hsu³, Jose Guillen³, Marjorie Perloff⁴, Michelle Ley² and Julie E Lang⁵. ¹University of Arizona Cancer Center, Tucson, AZ; ²Mayo Clinic, Rochester, MN; ³University of Arizona Cancer Center, Tucson, AZ; ⁴National Cancer Institute, Bethesda, MD and ⁵Keck School of Medicine, University of Southern California, Los Angeles, CA.

Body: Background: Although breast cancer (BC) may occur at any age, its prevalence is greater postmenopause. Greater than 75% of postmenopausal BCs are hormone dependent. Aromatase inhibitors suppress postmenopausal estrogen biosynthesis. Letrozole has demonstrated efficacy against BC in the adjuvant and metastatic setting at the treatment dose of 2.5 mg daily. Its potential role in BC prevention has been inferred from reductions in contralateral BCs. Its side effect profile, similar to other AIs, includes exacerbation of menopausal symptoms that negatively impact quality of life (QOL) and may result in discontinuation of the drug. Both anastrazole and exemestane have been demonstrated to reduce BC in high-risk women.

Hypothesis: Lower and intermittent doses of letrozole effectively suppress estrogen in the high-risk postmenopausal woman and provide a better side effect profile.

Methods: A randomized, double-blind study comparing the impact of varying letrozole doses (2.5 mg daily, 2.5 mg MWF, 1.0 mg MWF, or 0.25 mg MWF) on estrogen suppression and side effects—lipids, bone resorption, menopause, and QOL—was conducted. Participants randomized to intermittent dosing received placebo on nontreatment days.

Results: 112 participants were enrolled at 2 clinical sites. Mean patient age was 62.8 years, and average BMI was 29.8. Analysis of available data after 24 weeks of therapy revealed statistically significant increase in triglycerides (N=94): 114.67±48.39 to 125.79±54.31 (p<0.01); vasomotor symptoms (N=95): 2.25±1.26 to 2.74±1.67 (p<0.01); and C-telopeptide (N=75): 0.39±0.23 to 0.55±0.28 (p<0.0001). Statistically significant decrease in estradiol (N=68): 5.57±5.19 to 1.26±1.41 (p<0.0001) and estrone (N=68): 22.39±13.02 to 1.64±2.66 (p<0.0001) were observed. No differences in QOL (SF 36) were noted after letrozole treatment. P-values were derived from paired t-test (signed rank test) for the difference between baseline and after 24 weeks of study drug.

Conclusions: De-escalating doses of letrozole suppress postmenopausal estrogen effectively and result in statistically significant increases in triglycerides, C-telopeptide and vasomotor symptoms without impact on QOL. Presentation will include unblinded intervention arm outcomes.
**Title:** Breast cancer (BC) following prophylactic mastectomy (PM), a clinical entity: Presentation, management, and outcomes

Robert W Mutter¹, Tanya L Hoskin², Marlene H Frost³, Joanne L Johnson⁴, Lynn C Hartmann⁵ and Judy C Boughey⁶. ¹Mayo Clinic, Rochester, MN.

**Body:**

**Objective:** Contralateral PM (CPM) and Bilateral PM (BPM) markedly decrease, but do not completely eliminate the possibility of development of a new BC on the side of the PM. Given the relative infrequency of its occurrence, little is known about the clinical characteristics, presentation, and management of patients who develop BC after PM. Our aim was to review our institutional experience of BC occurring after PM.

**Methods:** Between 1960 and 1993, 1,065 women underwent BPM and 1,643 women with unilateral BC treated with therapeutic mastectomy underwent a CPM. Medical records were reviewed and study-specific questionnaires were sent to all women at 10 years and 20 years after PM. BC after PM included locoregional invasive BC or DCIS on the side of the PM.

**Results:** Thirteen patients who underwent BPM developed BC after PM. Twelve patients who underwent CPM developed a subsequent BC on the side of the CPM. The median follow-up time from PM was 22 years (range 3-34). Detailed clinical characteristics of BC after PM are shown in Table 1. Presentations included: disease limited to the axilla without evidence of a local primary 4 (16%); synchronous local and axillary disease 1 (4%); synchronous local disease and distant metastases 4 (16%); clinically isolated local disease 17 (68%).

Of the 17 patients with isolated local disease, 11 (65%) underwent a completion/redo mastectomy, local excision of the tumor was performed in 5 (29%), and surgical management was unknown in 1 (6%). Ten of 17 (59%) underwent axillary lymph node dissection, 1 (6%) underwent sentinel lymph node biopsy, 1 did not undergo axillary staging, and axillary management was unknown in 5 (29%). Median tumor size was 0.9 cm (range 0.3-3.5) and only 1 of 17 (6%) patients was confirmed to have pathologic nodal involvement. Twelve of 17 (71%) received some type of adjuvant therapy: chemotherapy and/or endocrine therapy 3 (18%); radiotherapy 2 (12%); both 5 (29%); none 5 (29%). With a median follow-up of 7 years since diagnosis of local BC after PM, there has been one isolated local recurrence and 2 distant recurrences as first event.

**Conclusion:** BC can occur after PM. With rising rates of PM, understanding management of BC after PM is important. Most common presentation is local disease and can be managed with resection with consideration of adjuvant therapy. Multidisciplinary management of these cases is needed.

### Characteristics of BC Following PM

<table>
<thead>
<tr>
<th></th>
<th>All Patients (n=25)</th>
<th>BPM Cohort (n=13)</th>
<th>CPM Cohort (n=12)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age at diagnosis of BC after PM</td>
<td>56 (range 38-81)</td>
<td>58 (range 38-71)</td>
<td>54 (range 39-81)</td>
</tr>
<tr>
<td>Median time to development of BC after PM (years)</td>
<td>7 (range 1-25)</td>
<td>6 (range 2-25)</td>
<td>8 (range 1-21)</td>
</tr>
<tr>
<td><strong>Presentation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Self-detected abnormality</td>
<td>23 (92%)</td>
<td>12 (92%)</td>
<td>11 (92%)</td>
</tr>
<tr>
<td>Screening mammogram</td>
<td>1 (4%)</td>
<td>0</td>
<td>2 (16%)</td>
</tr>
<tr>
<td>Not known</td>
<td>1 (4%)</td>
<td>1 (8%)</td>
<td>0</td>
</tr>
<tr>
<td>Local disease only</td>
<td>17 (68%)</td>
<td>10 (77%)</td>
<td>7 (58%)</td>
</tr>
<tr>
<td>-Sub-areolar</td>
<td>7 (41%)</td>
<td>6 (40%)</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>-UOQ/axillary tail</td>
<td>2 (12%)</td>
<td>0</td>
<td>2 (16%)</td>
</tr>
<tr>
<td>-Lower/inframammary crease</td>
<td>2 (12%)</td>
<td>1 (10%)</td>
<td>1(8%)</td>
</tr>
<tr>
<td>Chest wall or unspecified</td>
<td>6 (35%)</td>
<td>3 (30%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Local &amp; regional (axillary) disease</td>
<td>1 (4%)</td>
<td>0</td>
<td>1 (8%)</td>
</tr>
<tr>
<td>Axillary BC without evidence of local primary</td>
<td>4 (16%)</td>
<td>1 (8%)</td>
<td>3 (25%)</td>
</tr>
<tr>
<td>Synchronous local and distant disease</td>
<td>3 (12%)</td>
<td>2 (15%)</td>
<td>1 (8%)</td>
</tr>
</tbody>
</table>
Body: Background: Breast cancer is the most commonly diagnosed cancer among adolescent and young (AYA) adult females, ages 15-39. Approximately 14% of all AYA cancers diagnosed in females are breast cancer. AYAs represent 7% of all female breast cancer diagnoses. AYAs diagnosed with breast cancer have larger proportions of cancers with lower estrogen receptor positivity and over expression of HER2, triple negative subtypes, and cancers associated with familial history. These factors suggest the need for increased surveillance of young women diagnosed with breast cancer and strong consideration for their enrollment into clinical trials.

Objective: To analyze geographic and socioeconomic factors that may increase risk and disparities in access to clinical trials for Texas AYA breast cancer survivors.

Research Questions: 1) What are the factors, including access to clinical trials, that affect Texas AYA breast cancer patients being diagnosed at later versus earlier stages? 2) What are the associations between various demographic and diagnostic risk factors and AYAs breast cancer survivors’ distance to clinical trials?

Data Sources, Population and Methods: Data sources include SEER 18 and Texas Limited Use data bases for incidence analyses. Data on breast cancer clinical trials was provided by www.BreastCancerTrials.org. Texas AYA breast cancer population data for 4153 women diagnosed from 2005 to 2009, was provided by the Texas Cancer Registry under IRB protocol 13-022. Methods for this study included use of SEER Stat for incidence analysis, ESRI ArcGIS 10.1 mapping and ESRI GIS Network Analysis tools to determine individual patient locations and distance to trials. Stata statistical software (12.1) was used to for bivariate and logistic regression modeling and analyses.

Selected Results:
1) In a comparison of U.S. SEER 18 and Texas Limited Use data, for the overall female AYA breast cancer populations and for nearly all of the racial and ethnic subgroups, Texas' age adjusted incidence rates for this age group (15-39) are higher than the U.S. incidence rates.
2) With insured patients as the reference, uninsured self pay patients were 70% (p-value< 0.000, CI 1.31, 2.20) more likely to be diagnosed at later stages;
3) Being of Hispanic/Latina ethnicity was associated with at 36% greater risk of late stage diagnosis (p-value <0.000, CI 1.16,1.59)
4) Black African American AYAs were 31% more likely to be diagnosed at a later stage (p-value 0.004, CI 1.08,1.58)
5) Using <45 miles distance to clinical trials as a reference, AYA breast cancer patients living 45-100 miles from an appropriate trial were 102% more likely to be diagnosed at later stages (p-value< 0.000, CI 1.65,2.48)
6) AYA patients living furthest from trials, over 200 miles, were 49% more likely to be diagnosed at later versus earlier stages of breast cancer (p-value 0.020, CI 1.06, 2.09).

Discussion: This is the first study to consider geographic, demographic and diagnostic factors, including distance to appropriate trials, in exploring early versus late staging of breast cancer among AYAs. The findings and discussion of results have both practice and policy implications related to access to care and clinical trials for AYA breast cancer survivors.
Title: Association between breastfeeding and breast cancer risk by receptor status: A meta-analysis

Marisa Weiss\textsuperscript{1,2}, Ying Liu\textsuperscript{3}, Paolo Boffetta\textsuperscript{4}, Graham Colditz\textsuperscript{5}, Ahmedin Jemel\textsuperscript{5} and Farhad Islami\textsuperscript{5}. \textsuperscript{1}Breastcancer.org, Ardmore, PA; \textsuperscript{2}Lankenau Medical Center, Wynnewood, PA; \textsuperscript{3}Washington University School of Medicine, St Louis, MO; \textsuperscript{4}Icahn School of Medicine at Mount Sinai, New York, NY and \textsuperscript{5}American Cancer Society, Atlanta, GA.

Body: Background:
The rising incidence of breast cancer is mainly due to changes in reproductive, lifestyle, and environmental factors, not inherited genetic mutations. Many risk factors can be modified, offering important opportunities for prevention.

In the era of personalized care, treatment is subtype dependent. While most prevention strategies are not subtype specific, we wanted to see if breastfeeding confers the same protection based on subtype, especially against poorer prognostic subtypes.

Methodology:
Relevant articles from case–control or prospective studies were identified by searching the PubMed and Scopus databases through 2014 and reference lists of relevant articles. Two researchers independently did the search and evaluated the articles. The summary risk estimates and 95% confidence intervals were calculated using random effects models (DerSimonian–Laird method) for the association between breastfeeding and breast cancer by receptor status. The reference category in most of the studies was never breastfeeding, but in a few studies this also included women who breastfed for a short time.

Results:
This meta–analysis of 27 articles (19 from case–control, 8 from prospective cohort studies) published between 1983 and 2014 included 36,881 women with invasive breast cancer from four continents. Ever breastfeeding was inversely associated with breast cancer risk in hormone receptor–negative subtypes, even in cohort studies and when the results were adjusted for age, body mass index (BMI), parity, and family history of breast cancer (RR= 0.88; 95% CI 0.74 â–“ 1.06 for non–luminal cancers). The inverse association between ever breastfeeding and triple–negative subtype was slightly stronger, but this was based on a limited number of cohort studies. In contrast, there was no association between breastfeeding and luminal subtypes in cohort studies.

Association between ever breastfeeding and breast cancer risk by receptor status

<table>
<thead>
<tr>
<th>Receptor status</th>
<th>No of studies</th>
<th>OR (95% CI)</th>
<th>I\textsuperscript{2} statistics (%)</th>
<th>P for heterogeneity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non–luminal (ER–, PR–)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort</td>
<td>7</td>
<td>0.84 (0.72 – 0.97)</td>
<td>50</td>
<td>0.06</td>
</tr>
<tr>
<td>Cohort, adjusted*</td>
<td>3</td>
<td>0.88 (0.74 – 1.06)</td>
<td>42</td>
<td>0.18</td>
</tr>
<tr>
<td>Case–control</td>
<td>13</td>
<td>0.76 (0.67 – 0.86)</td>
<td>59</td>
<td>0.004</td>
</tr>
<tr>
<td>Triple–negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cohort</td>
<td>3</td>
<td>0.74 (0.62 – 0.88)</td>
<td>0</td>
<td>0.46</td>
</tr>
<tr>
<td>Cohort, adjusted*</td>
<td>1</td>
<td>0.81 (0.62 – 1.04)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Case–control</td>
<td>8</td>
<td>0.73 (0.64 – 0.84)</td>
<td>12</td>
<td>0.34</td>
</tr>
<tr>
<td>Luminal (ER+ and/or PR+)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>Cohort</td>
<td>8</td>
<td>0.98 (0.89 – 1.07)</td>
<td>75</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cohort, adjusted*</td>
<td>3</td>
<td>1.04 (0.98 – 1.10)</td>
<td>0</td>
<td>0.75</td>
</tr>
<tr>
<td>Case–control</td>
<td>18</td>
<td>0.82 (0.76 – 0.89)</td>
<td>69</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

−, negative; +, positive; ER, estrogen receptor; PR, progesterone receptor

$I^2$ statistics show % of total variation across studies that is due to heterogeneity rather than random variation (chance). Arbitrarily, $I^2$ percentages may be interpreted as follows: –25%, low; –50%, moderate; and –75, high heterogeneity.

*Results adjusted at least for age, body mass index, parity, and family history of breast cancer.

**Conclusion:**
Breastfeeding is a powerful strategy to reduce the risk of several aggressive breast cancer subtypes, with a relative risk reduction of approximately 10% to 20%, depending on receptor status. To maximize breastfeeding use for the long–term health of mothers and babies, it is important to remove barriers in the home, community, and workplace as well as provide targeted education and support before and after delivery.
Goal: The goal of this study is to ascertain the beliefs, knowledge, understanding, attitudes and treatment access to breast cancer among rural women in Nigeria.

Background: Breast cancer has become a popular topic in recent years with several thousands of women diagnosed to be positive every year. The availability of care/treatment upon early detection is key to survival.

Methods: An interview guide was designed specifically for this study in which 200 rural women in Northern Nigeria, age 45 and over took part in. It contained questions about beliefs, knowledge, understanding and attitudes about Breast Self-Examination (BSE), Clinical Breast Examination (CBE) and mammogram. In addition, questions assessing the variables of the Health Belief Model and health motivations also were included. The data were obtained during face-to-face interviews in the primary language of the participating woman. The interviews were transcribed and translated into English.

Results: Out of the 200 women who participated, only 1% two (2) of the participants practiced BSE monthly, 8% had undergone at least one CBE during their lives, and 91% had never had a mammogram. There were little or no access to treatment even at early detection in these rural areas causing thereby vulnerability to loss of life. Majority of these rural women (95%) said they knew little or nothing about breast cancer. While 15% of the women said detecting cancer early was important, only 3% reported that cancer could be cured. Age, education, or mother tongue showed no statistically significant relationship with the breast health practice scores. However, proficiency with the English language (p = 0.009) and number of years exposed to awareness and education (p = 0.009) had a significant relationship with the breast health practice scores. The significant explanatory factor for the variable breast health practices was a cue to action (p = 0.009).

Conclusions: The level of awareness and treatment access to breast cancer amongst Northern Nigeria’s rural women is extremely low thereby making them not to engage in screening and/or detection practices. This alarming situation calls for urgent intervention of medical/health organizations to provide immediate breast cancer awareness, screening and care so as to reduce incidences or threat at early detection.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-14-01
Average Grade: 3.60

Title: Docosahexanoic acid’s modulation of survival and invasion is associated with altered CCL20/CCR6 chemokine levels and signaling in hyperplastic, DCIS and metastatic breast cancer cell lines

Ching Hui Chen¹, Laura Garcia¹, Carol Fabian², Stephen Hursting³ and Linda deGraffenried¹. ¹University of Texas, Austin, TX; ²University of Kansas Cancer Center, Kansas City, KS and ³University of North Carolina, Chapel Hill, NC.

Body: Introduction: High dietary intake of docosahexanoic acid is associated with a lower risk of breast cancer and reduced metastasis. Epidemiological and preclinical studies suggest that the regulation of monocyte recruitment may play an important role in lowering breast cancer risk and decreasing breast cancer metastasis. In addition to promoting the recruitment of pro-inflammatory leukocytes, the CCL20/CCR6 chemokine axis has been implicated in promoting breast cancer cell migration and invasion. We hypothesize that one mechanism by which DHA suppresses breast cancer progression and metastasis is through the suppression of CCL20/CCR6 signaling.

Methods: The 21PT, 21NT and 21MT-1 cell lines have been previously described as reflecting the characteristics of hyperplastic, in situ and metastatic breast cells, respectively. We measured the impact of physiological DHA concentrations on cell survival and cell proliferation using colony formation and MTT assays respectively. Invasion of 21PT, 21NT and 21MT-1 cells were evaluated using invasion chambers. Changes in CCL20 and CCR6 expression were measured using qPCR. The activity and expression levels of JNK, ERK1/2 and c-Jun, downstream modulators of the CCL20/CCR6 axis, were evaluated using Western blot analyses.

Results: Following a 24 hr exposure to 20 μM DHA and 5-7 days of recovery, colony counts of all three cell lines were significantly suppressed, with 21NT cells experiencing the largest percent reduction. Invasion capacity of 21PT, 21NT and 21MT-1 accurately mirrored the stages of breast cancer they represent. Treatment with DHA reduced the invasion capacity of 21MT-1 to the levels of its hyperplastic counterpart. CCL20 mRNA levels were reduced when cells were exposed to DHA for 48 hrs. Western blot analyses suggest that activation of ERK1/2 and JNK signaling may be critical in orchestrating DHA-associated reduction of invasion.

Conclusions: With the support from epidemiological and preclinical studies, the use of omega-3 based preventive regimen may prove useful for reducing breast cancer risk and breast cancer metastasis. In vitro studies using premalignant breast cancer can provide invaluable insights to the molecular mechanisms accountable for preventive properties of omega-3 fatty acids. DHA-associated regulation of CCL20/CCR6 signal transduction may be an important preventive mechanism and future studies will warrant deepened understanding of how nutraceutical compounds can prevent breast cancer.
Efficacy of tabebuia avellandae extract on a cell culture model for triple negative breast cancer

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Body: Background: The triple negative breast cancer (TNBC), a molecular subtype of clinical breast cancer consisting of epithelial cells that lack estrogen receptor-α (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (HER-2) expression, is non-responsive to either endocrine therapy or HER-2 targeted therapy. Chemotherapy is frequently associated with long-term systemic toxicity, acquired tumor resistance and resultant compromised treatment efficacy. These aspects emphasize a need to identify efficacious non-toxic agents for secondary prevention/therapy of TNBC. Non-fractionated aqueous extract from the inner bark of the Tabebuia avellandae (TA) tree found in the Amazon rainforest, available as a dietary supplement under the name of Taheebo or Pau d’arco, has documented efficacy in a cell culture model for the Luminal A breast cancer subtype, as well as for several other cancers. Present study examines the inhibitory effects of the TA extract, and identifies possible mechanistic targets for its efficacy in a cell culture model for TNBC.

Nutritional Supplement, Experimental Model and Biomarkers: Lyophilized powder of non-fractionated TA extract from Taheebo Japan, Osaka, Japan, provides the source material for the study. The ER-/PR-/HER-2- MDA-MB-231 cell line represents the cell culture model for TNBC. Anchorage dependent growth, cell cycle progression, status of cell cycle regulatory proteins and anchorage independent colony formation, represent the quantitative biomarkers for efficacy.

Results: Relative to the non-tumorigenic 184-B5 human mammary epithelial cells, the tumor derived MDA-MB-231 cells exhibited decreased population doubling time, increased saturation density, decreased G1: S+G2/M ratio and increased S+G2/M: Sub G0 ratio, indicating loss of homeostatic growth control. Additionally, unlike 184-B5 cells, MDA-MB-231 cells exhibited increased anchorage independent growth in vitro and tumor development in vivo, indicating enhanced cancer risk. Treatment of MDA-MB-231 cells with TA resulted in a substantial dose dependent cytostatic growth arrest (IC50:1.0%; IC90: 2.5%). Cell cycle analysis of TA treated cells revealed G1 arrest, leading to a progressive dose dependent increase in the G1: S+G2/M ratio. Mechanistically, TA decreased Cyclin D1 expression and attenuated RB phosphorylation, predicting Cyclin D-CDK4-pRB pathway as a molecular target for efficacy. Furthermore, TA effectively inhibited anchorage independent growth in a dose dependent manner.

Conclusions: Present data demonstrated pronounced efficacy of TA as a naturally occurring nutritional substance in a cell culture model for TNBC, and validated TA as a promising non-toxic natural agent for secondary prevention/therapy of clinical TNBC.
Title: Evaluation of sustained antiemetic efficacy over repeated cycles of anthracycline-cyclophosphamide (AC)-based chemotherapy: A phase 3 study of NEPA, a fixed-dose combination of netupitant and palonosetron for prevention of chemotherapy-induced nausea and vomiting (CINV)

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Body: Background: International antiemetic guidelines recommend co-administration of an NK₁ receptor antagonist (RA) and a 5-HT₃ RA in breast cancer (BC) patients receiving anthracycline cyclophosphamide (AC) chemotherapy as this population is at increased risk of developing CINV. NEPA, a novel, oral fixed-dose combination of a new NK₁ receptor antagonist (RA), netupitant (NETU 300 mg), and the 5-HT₃ RA, palonosetron (PALO 0.50 mg), was previously reported to be superior to oral PALO after a single cycle (Aapro et al, Annals of Oncology 2014) and multiple cycles (Aapro et al, ASCO 2014) of chemotherapy. This posthoc analysis evaluates sustained efficacy over multiple cycles when censoring patients who experienced CINV in the previous cycle.

Methods: This was a multinational, randomized, double-blind, parallel group study evaluating the efficacy/safety of single oral doses of NEPA versus oral PALO in chemotherapy-naïve patients receiving multiple cycles of anthracycline-based chemotherapy. All patients also received oral dexamethasone (DEX) 12 mg (NEPA) or 20 mg (PALO), only on Day 1. Overall (0-120 h) complete response (CR: no emesis, no rescue medication) was the efficacy endpoint evaluated. The analysis of sustained CR evaluates the probability that patients would remain complete responders over 4 cycles by censoring continuing patients who failed to have a CR in the prior cycle. A Kaplan Meier method and log-rank test comparing NEPA with oral PALO were utilized.

Results: 1455 patients were randomized; 1286 participated in the multiple cycle extension after cycle 1. Treatment groups were comparable with 98% females and 97% with BC; the mean age was 54. The percentage of patients who experienced a CR in cycle 1 and who sustained a CR over cycles 2-4 was greater for NEPA than for oral PALO (p <0.0001, log rank test). The table shows the percent of patients with continuing CR over time; N = the number of patients at risk.

<table>
<thead>
<tr>
<th>Time since first chemotherapy</th>
<th>NEPA + DEX</th>
<th>Oral PALO + DEX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cycle 1</td>
<td>74.3% (N = 724)</td>
<td>66.6% (N = 725)</td>
</tr>
<tr>
<td>Cycle 2</td>
<td>68.5% (N = 485)</td>
<td>57.1% (N = 434)</td>
</tr>
<tr>
<td>Cycle 3</td>
<td>65.7% (N = 423)</td>
<td>52.7% (N = 348)</td>
</tr>
<tr>
<td>Cycle 4</td>
<td>63.6% (N = 375)</td>
<td>50.6% (N = 300)</td>
</tr>
</tbody>
</table>

Conclusions: This multiple cycle analysis indicates that NEPA, a novel, fixed-dose antiemetic combination, more effectively demonstrates sustained control of CINV over multiple cycles than oral PALO. As females with breast cancer represent a particularly challenging population in terms of emesis control, it is especially crucial that antiemetic recommendations are followed to allow these patients to maintain their quality of life and continue their treatment plan over multiple cycles of chemotherapy. NEPA offers effective guideline-recommended prophylaxis in a convenient single dose.
**Title:** Safety of letrozole-gonadotropin controlled ovarian stimulation protocol in women with breast cancer undergoing fertility preservation before or after tumor resection via embryo or oocyte cryopreservation: A prospective cohort study

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**Body:** Purpose: We have previously described the concurrent use of aromatase inhibitors to reduce estrogen exposure in women with breast cancer undergoing controlled ovarian stimulation (COS) with gonadotropins for fertility preservation (FP) via oocyte or embryo cryopreservation. To purpose of this study was to investigate the impact of this letrozole-gonadotropin COS protocol on survival in women who underwent fertility preservation before or after breast surgery.

Patients and Methods: A total of 364 women with stage ≤3 breast cancer, who pursued FP consultation or FP treatments at our institution were prospectively evaluated. Of those, 146 elected to undergo COS with letrozole and gonadotropins for FP (120 prior to chemotherapy and 26 after chemotherapy). The remaining 218 patients elected to not to undergo a fertility-preserving procedure and served as controls.

Result(s): Demographic information and tumor characteristics at enrollment were similar between patients who pursued COS with letrozole and gonadotropins (COS group) and control groups.

Table 1. Demographics and tumor characteristics of patients who pursued fertility preservation vs. controls

<table>
<thead>
<tr>
<th>Treatment Group (n=120)</th>
<th>Control Group (n=218)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at FP consultation (years)</td>
<td>35.2±4.5</td>
<td>37.0±5.1</td>
</tr>
<tr>
<td>Age at cancer diagnosis (years)</td>
<td>34.8±4.5</td>
<td>34.9±4.7</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>22.8±3.7</td>
<td>23.1±3.9</td>
</tr>
<tr>
<td>Node involvement (%)</td>
<td>34</td>
<td>48</td>
</tr>
<tr>
<td>Tumor size (cm) Mean±SD</td>
<td>1.8±1.2</td>
<td>1.9±1.9</td>
</tr>
<tr>
<td>&lt;2</td>
<td>67</td>
<td>66</td>
</tr>
<tr>
<td>2-5</td>
<td>31</td>
<td>30</td>
</tr>
<tr>
<td>&gt;5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Lymphovascular space invasion (%)</td>
<td>31</td>
<td>37</td>
</tr>
<tr>
<td>Histologic grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>40</td>
<td>39</td>
</tr>
<tr>
<td>3</td>
<td>57</td>
<td>59</td>
</tr>
<tr>
<td>Estrogen receptor positive (%)</td>
<td>82</td>
<td>77</td>
</tr>
<tr>
<td>HER-2/neu positive (%)</td>
<td>35</td>
<td>33</td>
</tr>
<tr>
<td>Adjuvant tamoxifen use (%)</td>
<td>89</td>
<td>87</td>
</tr>
<tr>
<td>Length of follow-up (years, Mean±SD)</td>
<td>5.0±2.1</td>
<td>6.9±3.6</td>
</tr>
</tbody>
</table>

The median follow-up after diagnosis was 4.9 years in COS and 6.2 years in the control group. In the COS group, the hazard ratio (HR) for recurrence after IVF was 0.77 (95% CI: 0.28, 2.13) and the survival was not compromised compared with controls (P=0.61). In the COS group, survival was not different between patients with ER-positive and ER-negative breast cancer (P=0.75) and between patients who underwent COS before and after tumor resection (P=0.56). The survival was also not different between patients who pursued COS before and after chemotherapy (P=0.57).
Conclusion(s): Here we presented the largest prospective data with longest follow up on the safety of ovarian stimulation in women with breast cancer. COS with letrozole and gonadotropins for FP is unlikely to cause substantially increased recurrence risk in breast cancer, even in patients who have not yet undergone breast surgery. Larger studies are needed to confirm the findings from the subgroup analysis.

Support: Supported by NIH RO1 HD053112.
Title: Baseline joint pain predicts severity of subsequent joint symptoms in women initiating aromatase inhibitors for early stage breast cancer

Lea Baer¹, Katherine D Crew¹, Danielle Awad¹, Kevin Kalinsky¹, Matt Maurer¹ and Dawn L Hershman¹. ¹Columbia University Medical Center, New York, NY.

Body: Background: Aromatase inhibitors (AIs) are the standard adjuvant treatment of hormone-sensitive breast cancer (BC) in postmenopausal women. However, these therapies are associated with musculoskeletal complaints which may lead to non-adherence and early discontinuation. The aim of this study was to characterize the natural history of the AI-induced arthralgia.

Methods: Postmenopausal women with stage I-III BC were prospectively enrolled at the onset of adjuvant AI therapy. Subjects completed the Brief Pain Inventory (BPI) questionnaire at baseline, 3, 6, 9, and 12 months. BPI worst pain scores were categorized as 0-3, 4-6 and 7-8. Multiple logistic regression analysis was used to evaluate the association between baseline factors and having a 2-point worsening in BPI worst pain score. A Cox-proportional hazards model was used to evaluate factors that influenced the time to first 2-point worsening in pain score. Clinically significant change was defined as ≥2-point increase from baseline. Those with a baseline BPI worst pain score of ≥9 were removed from analysis.

Results: Among 180 consented subjects, 1 was found to be ineligible due to being perimenopausal, 42 subjects were lost to follow up, 17 subjects came off their AI and 8 subjects dropped out. Mean age was 61; 60% were white, 32% Black, 10%, Asian and 31% were Hispanic; 86% started on anastrozole; 24% had a change in AI. At baseline, 76 women had a BPI worst pain score between 0-3; 28 between 4-6; and 18 had 7+. Seventy subjects (64%) experienced at least a 2-point worsening of BPI from baseline and women who developed a 2-point worsening had a lower mean baseline BPI (2.57 vs. 3.75) compared to those who did not. Those experiencing an initial worsening in the first 6 months of therapy improved over time; while the patients experiencing BPI worsening at 9 months, continued to have progressive increase in BPI. In a multiple logistic regression model adjusting for AI switching, BMI, age, baseline score, prior chemotherapy, osteoarthritis, and prior hormone replacement therapy, those who had a baseline worst pain score between 4-6 (p=0.04) or 7+ (p=0.005) were less likely to develop a 2-point worsening in BPI at 12 months from baseline. Similarly, those with a baseline worst pain categorization of 7+ had a hazard ratio of 0.34 for time to 2-point worsening.

Conclusions: Women with low baseline BPI score are more likely to develop worsening BPI score over time. Women who develop symptoms later in the course of their therapy are at greatest risk for persistent symptoms. This has implications for studies evaluating interventions for prevention of AI arthralgias.

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Three</th>
<th>Six</th>
<th>Nine</th>
<th>Twelve</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPI mean score</td>
<td>Patients with &gt;2 point increase in BPI at any time</td>
<td>Patients with &gt;2 point increase in BPI at 3 months</td>
<td>Patients with &gt;2 point increase in BPI at 6 months</td>
<td>Patients with &gt;2 point increase in BPI at 9 months</td>
</tr>
<tr>
<td>3.75</td>
<td>2.57*</td>
<td>2.59</td>
<td>2.30</td>
<td>2.11*</td>
</tr>
<tr>
<td>4.25</td>
<td>4.52</td>
<td>6.08*</td>
<td>4.68</td>
<td>4.13</td>
</tr>
<tr>
<td>4.50</td>
<td>4.83*</td>
<td>5.32*</td>
<td>6.37*</td>
<td>5.07</td>
</tr>
<tr>
<td>4.00</td>
<td>5.10</td>
<td>5.11</td>
<td>5.13</td>
<td>5.75*</td>
</tr>
<tr>
<td>4.25</td>
<td>5.37*</td>
<td>5.48</td>
<td>4.86</td>
<td>5.66</td>
</tr>
</tbody>
</table>
*p value <0.05 compared to baseline
Title: Prognostic understanding and associations with mood and quality of life in patients with metastatic breast cancer

Amanda Parkes¹, Jennifer A Shin¹, Helen Knight¹, Stephen M Schleicher², Areej El-Jawahri¹, Lara Traeger¹ and Jennifer S Temel¹. ¹Massachusetts General Hospital, Boston, MA and ²Brigham and Women’s Hospital, Boston, MA.

Body: Background:
Data suggest that patients with metastatic cancer who understand their prognosis are more likely to prefer and receive care concordant with their wishes. Despite the importance of prognostic information for patients’ decision-making, there are no current data describing prognostic understanding in patients with metastatic breast cancer (MBC). The aims of this study were to describe prognostic understanding in patients with MBC and to explore its associations with mood, distress, and quality of life (QOL).

Methods:
We conducted a cross-sectional study of 50 patients who were receiving first- or second-line chemotherapy for MBC. Participants completed a series of questionnaires. We used a 13-item questionnaire to assess patients’ perceptions of their prognosis and goal of therapy. We evaluated mood, level of distress, and QOL using the Hospital Anxiety and Depression Scale (HADS), the Distress Thermometer (DT), and the Functional Assessment of Cancer Therapy-Breast (FACT-B), respectively.

Results:
The majority of patients (92%) reported it was at least somewhat important to know details about their prognosis. 19/47 (40%) patients reported that the primary goal of treatment was to cure their cancer, and 21/46 (46%) patients reported that the chance of cure was at least somewhat likely (≥25% chance of cure). 24/49 (49%) patients viewed themselves as terminally ill. There was a high prevalence of psychological morbidity in our patient cohort. 24/48 (50%) patients screened positive for distress (distress thermometer ≥4), 17/50 (34%) patients reported significant anxiety symptoms (HADS-Anxiety ≥8), and 11/50 (22%) patients reported significant depression symptoms (HADS-Depression ≥8). Distress, depression, and anxiety were each associated with lower QOL scores (93.0 vs. 114.0, p<0.001; 87.9 vs. 107.8, p=0.002; and 85.5 vs. 112.8, p<0.001, respectively). Patients who acknowledged their illness as terminal reported higher depression than those who did not perceive themselves as terminally ill (M=6.1 vs. 2.5, p=0.0006).

Conclusion:
Although the majority of patients with MBC receiving first- or second-line chemotherapy feel it is important to know detailed information about their prognosis, many incorrectly perceive that their cancer is curable. Accurate prognostic understanding was associated with increased depression symptoms. This study highlights the need to develop interventions to enhance patients’ prognostic understanding while providing adequate psychosocial support.
Evaluation of the effect of low level laser therapy on oral mucositis in breast cancer patients: A retrospective analysis

Jeroen Mebis¹,²,³, Sandrine Censabella¹, Annelies Maes¹,³, Leen Noé¹,³ and Paul Bulens¹,³. ¹Jessa Hospital, Hasselt, Belgium; ²Hasselt University, Diepenbeek, Belgium and ³Limburg Oncology Centre, Hasselt, Belgium.

Body: Background

The goal of this retrospective study was to investigate the effectiveness of low level laser therapy (LLLT) in managing chemotherapy-induced oral mucositis (OM) in breast cancer patients.

Methods

Breast cancer patients treated with chemotherapy at the Jessa Hospital (Hasselt, Belgium) and having received LLLT for OM were retrospectively selected from our database, provided sufficient data with regard to OM was available. LLLT treatment was provided using an AsGA diode laser (λ = 665 nm; output power: 100mW) combined with an infrared laser (continuous emission, output power: 500mW), delivered by an optical fiber with a diameter of 600µm. The energy delivered was 4 J per point of application. Treatment was applied to a maximum of seven sites (tongue, palate, tonsil, left and right inside of the cheek, floor of the mouth, and lips), depending on the location of OM. Patients received treatment two times a week until healing of each lesion.

Endpoints were the number of treated areas and the severity of OM at the start and the end of LLLT, graded by trained nurses according to the WHO scale (0 = no change; 1 = soreness, erythema; 2 = erythema, ulcers, can eat solids; 3 = ulcers, requires liquid diet only; 4 = oral alimentation not possible; if more than one location was treated, the highest grade was taken into account). An OM score was calculated for each patient by summing the WHO grades of all treated areas. Finally, where available, pain scores (obtained through a visual analogue scale ranging from 0, no pain, to 10, worst possible pain) were taken into account.

Results

Data from 93 patients with stage 0-IV breast cancer were included in these analyses. Mean age was 55.37 years (standard deviation [SD] = 9.72, median = 56). Most of the patients received anthracycline-based chemotherapy (65%). At the start of LLLT, mean time since start of chemotherapy was 48.92 days (SD = 39.43, median = 39). The median duration of LLLT was 2 weeks. OM outcomes at the start and the end of LLLT are presented in Table 1. At the end of LLLT, the number of areas that had to be treated significantly decreased. More importantly, there was a significant improvement in the severity of OM (highest WHO grade and OM score) and in pain. This improvement was also observed when patients were categorized according to their status at the end of LLLT (for each OM outcome: worsened, unchanged, or improved).

Table 1. Patients' status at the start and the end of Low Level Laser Therapy (LLLT)

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Start LLLT</th>
<th>End LLLT</th>
<th>N improved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean number of treated areas</td>
<td>3.89</td>
<td>2.16*</td>
<td>66 (71%)</td>
</tr>
<tr>
<td>Mean OM score</td>
<td>6.60</td>
<td>2.78*</td>
<td>75 (80.6%)</td>
</tr>
<tr>
<td>Mean pain scorea</td>
<td>5.14</td>
<td>1.64*</td>
<td>20 (90.9%)</td>
</tr>
<tr>
<td>N (%) WHO grade 1</td>
<td>11 (11.8%)</td>
<td>60 (64.5%)*</td>
<td>60 (64.5%)</td>
</tr>
</tbody>
</table>

a Pain scores were available for 22 (of the 93) patients. * p < 0.0001 (t-test or chi-square, as appropriate).

Conclusion

This retrospective analysis showed that LLLT, a standard management strategy for OM in head and neck cancer, significantly reduced the severity of chemotherapy-induced oral mucositis and relieved pain in patients with breast cancer. This is the first study in this population. Further research, preferably high-quality randomized controlled trials, is warranted to better investigate its usefulness in this population.
Open-label randomized parallel controlled study comparing bone mineral density between alendronate plus alfacalcidol combination and single administration of alfacalcidol in postmenopausal women receiving aromatase inhibitor as adjuvant therapy

Mitsue Saito¹ and Joe Matsuoka¹. Juntendo University, Bunkyo-ku, Tokyo, Japan.

Body: <Background>
One of the most worrisome side effects of endocrine therapy is loss of bone mineral density. Optimized bone therapy is thus warranted.

<Objectives>
The primary endpoint of this study was to assess the difference in bone mineral density between aromatase inhibitor-treated breast cancer patients receiving alfacalcidol alone versus also prescribed alendronate. Secondary endpoints were measured levels of surrogate markers for bone health and adverse events associated with bone-preserving therapies.

<Material and method>
Postmenopausal breast cancer patients (stage I-III) receiving any form of aromatase inhibitor (anastrozole (ANA), exemestane (EXE) or letrozole (LTZ)) whose bone mineral density as measured by DEXA (Dual-energy X-ray absorptiometry) was lower than the adult mean and from whom written informed consent had been obtained between March in 2008 and September in 2010 were studied. The study period was 2 years after enrollment. This study was approved by our institutional review board.

Patients were randomized into two arms stratified by age (<70 or not), use of an aromatase inhibitor (ANA, EXE or LTZ) and T score on DEXA (<-1.0 or not). Patients enrolled in arm A were treated with 35mg of oral alendronate weekly and 1µg of alfacalcidol daily. Patients in arm D were given 1µg of alfacalcidol daily.

Patients underwent DEXA (L2,3,4) every 6 months and blood tests for 1CTP (carboxyterminal telopeptide of type I collagen), BAP (bone alkaline phosphatase) and urine testing for NTX (type I collagen cross-linked N-telopeptide) every 3 months. Adverse events were monitored by physicians every 3 months.

<Results>
We enrolled 58 patients. Nine out of the 29 patients in arm D dropped out due to adverse events caused by alfacalcidol (2), caused by the aromatase inhibitor (2), metastasis (3) and severe bone loss (1). Six out of the 29 patients in arm A dropped out due to adverse events caused by alendronate plus alfacalcidol (1), caused by the aromatase inhibitor (2), metastasis (2) and financial difficulties (1). Improvement from the DEXA baseline in arm A was significantly better (p<0.0001) than that in arm D by analysis of covariance. NTX and BAP improved significantly in arm A (p<0.0001), but 1CTP did not (p=0.0382).

<Considerations>
Strict indications for and durations of bone therapy require further investigation. The dosage of alendronate was lower in this study than the international recommendation because the dosage approved by the Japanese government is half that in other countries and this applies to all the oral bisphosphonates because of the ethnic differences in absorption rate demonstrated in phase II studies.

<Conclusions>
Co-administration of alendronate and alfacalcidol contributed to preserving bone mineral density during adjuvant aromatase inhibitor treatment without producing severe adverse events. NTX and BAP are possible surrogate markers for bone therapy.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-15-07
Average Grade: 6.00

Title: Association of patient preference for adjuvant chemotherapy (chemo) at baseline (BL) with toxicity, mental health, function, quality of life (QoL) and survival in older women with early stage breast cancer (ESBC) [CALGB 49907 Alliance]

Ajeet Gajra⁵, Linda McCall⁶, Hyman B Muss³, Harvey J Cohen³, Aminah Jatoi⁴, Karla V Ballman⁴, Ann H Partridge⁵, Linda Sutton⁶, Barbara A Parker⁴, Gustav Magrinat⁷, Jaqueline M Lafky⁴ and Arti Hurria⁸. ¹Upstate Medical University, Syracuse, NY; ²Duke University Medical Center, Durham, NC; ³University of North Carolina Cancer Center, Chapel Hill, NC; ⁴Mayo Clinic, Rochester, MN; ⁵Dana-Farber Cancer Institute, Boston, MA; ⁶UC San Diego, Moores Cancer Center, LaJolla, CA; ⁷Cone Health Cancer Center, Greensboro, NC and ⁸City of Hope Comprehensive Cancer Center, Duarte, CA.

Body: Background: In CALGB-49907 (NEJM 2009;360:2055), older patients (pts) with ESBC were randomized to standard adjuvant chemo (AC or CMF versus capecitabine). The objective of this secondary QoL analysis is to assess if pts’ BL chemo preference (CP, defined as high or low), is associated with the following during and after completion of chemo: self and professional-reported toxicity, changes in mental health, function, QoL, recurrence-free (RFS) and overall (OS) survival.

Patients and Methods: Of 633 trial pts 350 participated in the QoL substudy; 145/350 pts completed the BL assessment regarding CP. CP was measured by asking the amount of benefit women would require to choose adjuvant chemo in a hypothetical situation, irrespective of chemo agents. Women who chose chemo for an increase in OS of \( \leq 12 \) months (mo) were designated as high chemo preference (HCP) and those who chose >12 mo were designated low chemo preference (LCP). CP associations were evaluated with: BL perception of self-health and perceived QoL on chemo; patient reported outcomes (PROs), changes in function and QoL (based on EORTC-QLQ-C30); anxiety and depression (Hospital Anxiety and Depression Scale); observed grade 3-5 adverse events (AEs) by NCI common toxicity criteria (CTC v2.0). Pts were assessed at midtreatment and at 1, 12, 18 and 24 mo post-treatment. Chi-square tests, t-tests, and Cox models were used for categorical, continuous, and time-to-event variables, respectively.

Results: The demographic and tumor characteristics of women (median age 71) who provided CP at BL were not different from women in the QoL subset or from non-QoL pts. 68/145 (47%) women had a HCP. CP groups did not differ based on age, surgery type, tumor and nodal stage, hormone receptor status, performance status, chemo assignment, education, marital or employment status except the LCP group had a higher proportion of white women (95% vs. 78%, \( p=0.004 \)). At BL, there were no differences in perception of self-health based on CP but women with LCP predicted QoL on chemo to be worse than women with HCP (\( p=0.006 \)) and reported greater nausea/vomiting. Mid-treatment, LCP pts reported worse nausea/vomiting, financial worries, and cancer symptoms. Post-treatment, LCP pts had worse constipation (at 1 mo) and financial worries (at 24 mo).

There were no differences based on CP for dyspnea, pain, fatigue, insomnia, anxiety or depression at any timepoint.

Mid-treatment, LCP women reported lower QoL and worse social, emotional and physical function compared to HCP women. These scores were not significantly different after treatment completion. LCP women had significantly higher rates of grade 3-5 AEs (53 vs. 34%, \( p=0.02 \)) during treatment but these did not persist post-therapy. CP was not significantly associated with OS (HR =0.75, \( p=0.36 \)) or RFS (HR=0.94, \( p=0.84 \)).

Conclusions: LCP at BL was associated with lower QoL, worse physical symptoms, AEs and function mid-therapy, but not mental health. Mid-therapy declines in women with LCP largely reversed post-therapy. This information may be useful for oncology professionals to counsel older ESBC pts with LCP receiving adjuvant chemo.
Title: Exercise intervention to run away from breast cancer treatment side effects: An integrative approach

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Body: Background. Some studies have shown that exercise increases breast cancer (BC) patients’ quality of life (QoL) during and after treatments. The aim of this study was to investigate the effect of an specific exercise intervention in QoL and in the exercise levels of these patients.

Methods. A randomized controlled trial evaluated an intervention (IG) vs a control group (CG) in early stage BC patients who recently finished chemo & radio therapies. Intervention consists on controlled group classes combining aerobic and resistance activities. CG maintained their previous lifestyle. QoL, leisure-time exercise levels (LTEL), chest (CMS) and legs maximal strength (LMS), physical capacity (PC) and some psychological variables, were assessed at baseline and after 3 months in both groups. Women who participated in the IG were followed up (FU) after 6 months.

Statistical analysis (SA) were performed using unpaired t-test on continuous variables comparing CG vs IG, considering possible confounders. Pearson correlation analyses were employed to examine possible correlations. 6 months FU data were analyzed comparing baseline and after intervention results using non-parametric test (n=13). SA was performed with SPSS v18 software. 95% CI was calculated and statistical significance level of p < 0.05 was used.

Results. 59 women (median 48.97±8.35 years old) completed this study. No differences between groups were observed at baseline. Average attendance rate to the program was 89%. There was a significant rise in LTEL (t123=16.33; p=0.0001) and in QoL (t123=2.88; p=0.005) comparing IG vs CG. Results also showed a correlation between QoL and LTEL in patients of IG only (r=0.22; p=0.013). Significant differences between groups were observed in both physical and psychological variables after training program.

Table 1. Results summary comparing intervention and control group.

<table>
<thead>
<tr>
<th>VARIABLES</th>
<th>Intervention Group (n=27) Mean±SD</th>
<th>Control Group (n=32) Mean±SD</th>
<th>Follow-Up (n=13) Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>QoL*+</td>
<td>112.88±17.74±</td>
<td>103.11±19.26</td>
<td>110.96±14.40</td>
</tr>
<tr>
<td>LTEL*+</td>
<td>45.11±14.61</td>
<td>14.87±4.78</td>
<td>34.56±19.51</td>
</tr>
<tr>
<td>Body Fat Mass</td>
<td>34.63±6.7</td>
<td>36.47±7.47</td>
<td>35.74±4.56</td>
</tr>
<tr>
<td>Muscle mass</td>
<td>41.3±3.16</td>
<td>38.78±8.51</td>
<td>41.48±2.01</td>
</tr>
<tr>
<td>Strength Index*+</td>
<td>2.6±0.83</td>
<td>2.12±0.69</td>
<td>2.32±0.58</td>
</tr>
<tr>
<td>CMS*+</td>
<td>45.89±11.07</td>
<td>31.47±9.87</td>
<td>53.76±12.42</td>
</tr>
<tr>
<td>LMS*+</td>
<td>93.07±27.3</td>
<td>69.00±24.94</td>
<td>114.65±24.29</td>
</tr>
<tr>
<td>PC*+</td>
<td>32.58±4.96</td>
<td>27.08±3.73</td>
<td>32.11±7.10</td>
</tr>
<tr>
<td>FACT-F *+</td>
<td>135.94±18.20</td>
<td>124.00±24.20</td>
<td>138.24±17.49</td>
</tr>
<tr>
<td>SF-36 Physical Dimension*+</td>
<td>48.49±4.83</td>
<td>45.61±5.82</td>
<td>49.75±2.75</td>
</tr>
<tr>
<td>SF-36 Psychological Dimension*+</td>
<td>43.00±8.11</td>
<td>37.03±12.58</td>
<td>44.33±7.33</td>
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<tr>
<td>Depression*+</td>
<td>6.83±7.83</td>
<td>12.55±10.77</td>
<td>5.88±5.19</td>
</tr>
</tbody>
</table>

*Significant differences were found between control and intervention group. + Significant differences were maintained between baseline and follow-up assessments.
No differences taking into account confounders were observed. Variable changes were maintained in FU participants assessed after 6 months. Correlation between QoL and LTEL ($r=0.52; \ p=0.008$) and significant weight loss ($\chi^2=6.08; \ p=0.048$) were observed.

Conclusion. These results suggest that a specifically designed BC exercise program increases LTEL, which correlates to a better QoL. This may reduce psychological and physical side effects of systemic treatment in patients with early BC that have recently finished treatments, even producing lifestyle changes in BC patients that could be long lasting.
**Title:** National survey of chemotherapy-induced appearance issues in breast cancer patients

**Body:** Background: Many breast cancer patients suffer hair loss due to chemotherapy, and not only scalp hair loss, but also eyebrow loss, eyelash loss and nail changes induced by chemotherapy are traumatic for patients. These side effects diminish self-esteem and greatly reduce quality of life. However, there has been little research in this field until now. To clarify the actual situation concerning appearance issues in breast cancer patients who received adjuvant chemotherapy, and to consider a support system for these patients, we conducted a questionnaire survey.

Methods: Disease-free breast cancer patients who have received adjuvant chemotherapy containing anthracycline and/or taxane within 5 years were recruited from 47 hospitals or clinics in Japan from April to October 2013. The patients participating in this survey completed a 65-question questionnaire concerning appearance issues (48) and their perception of physical and non-physical side effects (17). The drugs administered and treatment period were filled out by their doctors beforehand. The completed questionnaires were mailed directly to the data center by the patients.

Results: A total of 1511 patients returned the questionnaire to the data center with a response rate of 82% (1511/1853). Since 33 patients did not meet the entry criteria, the questionnaires returned by 1478 patients were analyzed in this survey. The mean age was 54.7 years (+-10.4, range 17-79). The distribution of the patients by time from the end of chemotherapy to this survey was as follows: < 1 year: 28%; 1 to 2 years: 24%; 2 to 3 years: 19%; 3 to 4 years: 15%; 4 to 5 years: 13%. In this survey, the side effect that most patients (92%) considered traumatic was hair loss. The second most traumatic side effect was fatigue (83%), while the 7th place was taken by nail changes (72%) and nausea/vomiting was in the 10th place (56%). During chemotherapy, scalp hair loss occurred in 98% of patients. Eyebrows fell out in 90% and complete eyebrow loss occurred in 36%. Eyelashes fell out in 88% and complete eyelash loss occurred in 37%. Fingernail changes occurred in 77% and toenail changes in 62%. In 60-70%, scalp hair, eyebrow and eyelashes recovered to the original appearance by 1 to 1.5 years after chemotherapy, but in 3-7%, scalp and face hair loss did not recover at all by 1 to 1.5 years. This proportion remained almost the same for 1.5 to 5 years too. During or after chemotherapy, 84% of patients used wigs. This decreased to 47% by 1 year after chemotherapy and 15.2% by 1.5 years. However 10% of patients were still using a wig 4 to 5 years after chemotherapy. Approximately 30% of the patients had trouble using and selecting a wig. In 51% of the patients, sufficient information on scalp hair loss was obtained. However, sufficient information on eyebrow loss, eyelash loss and nail changes was only obtained from 28%, 25% and 31%, respectively.

Conclusions: Our survey demonstrated the outline of hair loss and appearance issues in breast cancer patients who received chemotherapy. Hair loss is the most distressing and occasionally long-lasting side effect. Lack of information is a serious problem. These facts suggested a need for long-time and careful support of these patients.
Title: A combination natural product therapy attenuates common side effects associated with chemotherapy

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Body: Background: The taxanes and platinum agents provide substantial improvement in the treatment of breast and other cancers. Neuropathy is often the dose limiting toxicity of these agents and can devastatingly affect the patient due to diminished fine motor skills and pain in the hands and feet that diminishes the ability to exercise and interact in normal life. Clinically significant neuropathy occurs in 40% of patients and >10% will persist past a year causing permanent effects on quality of life. Perhaps more devastating, patients often have to discontinue effective treatment due to the development of these symptoms. Additionally, anemia contributes to dose reductions and delays that compromise therapy and cause significant fatigue that diminishes quality of life.

Interventions that could reduce these symptoms would provide a substantial improvement in the ability to care for these patients. Toward this end we have developed a non-toxic dietary approach that incorporates supplementation with several natural products. Together, this therapeuidic approach, called CS.001 is able to alleviate many of the negative side effects of breast cancer chemotherapy.

Methods: C57BL/6 mice were treated with Paclitaxel weekly or Oxaliplatin three times weekly. Animals were tested for neuropathic pain using a cold sensitivity test [acetone test] and sensitivity to mechanical stimulus [Von Frey Test]. A CBC and CMP were performed to assess systemic toxicity. Animals were provided with a nutritional complete diet that limited their carbohydrates to 10% of total caloric input and were additionally supplemented with the following natural products: [1] Medium chain triglycerides [30g/kg], [2] Curcumin [1200mg/kg], [3]EGCG [1200mg/kg] and [4]broccoli sprout powder [20g/kg]. Animals were placed on the CS.001 diet 1 week before beginning chemotherapy.

Results: Paclitaxel & Oxaliplatin treatment resulted in a statistically significantly increase in sensitivity to cold stimulus [Acetone test, p<0.001] that was reduced to control levels in the CS.001 treated animals. Mechanosensitivity was increased with Oxaliplatin & reduced with Paclitaxel treatment, and addition of CS.001 resulted in a statistically significant attenuation of the chemotherapy treatment [Paclitaxel vs Paclitaxel+CS.001, p<0.005 & Oxaliplatin vs Oxaliplatin+CS.001, p<0.05]. Additionally, reductions in RBC, Hemoglobin and Hematocrit with Oxaliplatin treatment [p<0.001] were significantly attenuated [p<0.05] with CS.001 treatment.

Conclusion: Dietary intervention combined with a supplementation of 4 natural products was able to attenuate chemotherapy induced anemia and neuropathy following chemotherapy treatment in mice. This combination is a promising quality of life intervention to evaluate in cancer patients receiving taxanes or platinums.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-15-11  
Average Grade: 4.00

Title: The distress screening tool: Initial experience with electronically curated patient reported measures  

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Body: Background: In June 2013, our health system transitioned to an electronic medical record (EMR) which included collecting patient quality of life data at each clinic visit. We used the NCCN distress thermometer (DT), a short, simple to use, self-report measure which uses a 10-point scale from 0 (no distress) to 10 (extreme distress) as well as an associated problem checklist which queries the source(s) of their distress. Among our breast cancer clinic population, we studied the severity and sources of distress as well as whether the DT score was associated with stage at diagnosis and time interval since diagnosis.  

Methods: Between October 1, 2013 and April 30, 2014, starting 3 months after implementation of a comprehensive EMR, all patients seen at our tertiary breast cancer clinic were asked to complete the DT survey at each clinic visit. DT data were collected and entered into the EMR at point of care. The DT tool was correlated with demographic and tumor information from our prospectively curated electronic datamart.  

Results: We collected 7276 DT surveys from 3267 unique patients over seven months. Median age of the cohort was 60 years; 73% were white and 21% were black. Among those with available staging data and a diagnosis of breast cancer, stage distribution was 10% stage 0, 34% stage I, 37% stage II, 15% stage III and 4% stage IV. The median reported distress score was 1.0 (range 0-10) with score distribution shown in Figure 1. The most commonly reported source of stress was fatigue (8.0%) followed by pain (6.8%). For new patient appointments the most commonly reported sources were worry (9.5%) followed by nervousness (8.0%). There was no significant correlation between overall distress score and stage at diagnosis. Among patients who were seen more than once during the study interval, the DT score changed for 33.7% of patients. The lowest distress scores were reported among women >3 years from initial diagnosis.  

Conclusions: The transition to an integrated EMR system has allowed collection of analyzable patient reported data to inform medical and psychosocial intervention. Structured data collection at point of care allows for efficient identification of and management for the major sources of distress among patients during breast cancer treatment and survivorship.
Title: Clinico-biological characteristics of patients surviving more than two years with metastatic breast cancer (MBC): Results of a transversal national multicentric survey

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Body: Introduction:
MBC cancer patients may have prolonged survival, and MBC cancers can be considered as a chronic disease. The main goal of the present study was to describe the clinico-biological features of patients surviving more than two years with MBC.

Method: During 4 months, we conducted a national multicentric survey about patients aged ≥ 18 in metastatic setting (all solid tumors) for more than 24 months. Clinico-biological data from 200 patients were collected in 39 French centers. Preliminary results of MBC patients (N=88, 87 women/one man) are presented.

Results: Most of them were ductal carcinoma (88%), expressing hormonal receptor HR (77%). 43% of tumors overexpressed HER2 (HER2+ tumors: 43%; Triple negative tumors: 6%). Median age at MBC diagnosis was 53 years [29-85]. 18% had metastatic disease at diagnosis and 82 % were localized with a disease-free survival of 65 months [4-312]. Median time of MBC disease was 4,5 years [2-20]. At the time of MBC diagnosis, 64% of patients were not single; 55% were working while 30% were retired. At data collection, 89% of non-single patients were not separated, and 43 % of working patients at diagnosis were still working.

Mean number of treatment lines in advanced disease was 4.7 [1-13]. 89% of MBC patients received at least one chemotherapy, 68% hormonotherapy, 70% targeted therapy and 38% had been included in at least one clinical trial. 97% of patients had a local treatment of their primary tumor. Concerning metastasis, 23% had a surgical treatment and 40% radiotherapy treatment. 80% of patients remain with PS of 0 or 1. Only 9% of patients were followed by a palliative care team, 24% by a psychologist and 23% by a nutritionist.

Conclusion: Our preliminary results of suggest that an important proportion of MBC cancer patients who live more than 2 years are young, have been treated with chemotherapy, hormonal and targeted therapy, have participated to clinical trials and still have good performance status. No change in marital status was observed. Half of working patients at MBC diagnosis continue to work. Few of them received palliative care. This study may help to better describe long-term survivors with MBC, and socio-medical burden as cancer became a chronic disease.
Title: Restoration by compression therapy of skin blood perfusion levels decreased during breast cancer chemotherapy, alleviating peripheral neuropathy

Tsuyoshi Ohno¹, Takashi Mine², Hiroki Yoshioka², Mikiko Kosaka², Kazuhiro Matsuda², Maiko de Kerckhove² and Charles de Kerckhove². ¹Nagasaki Prefecture Shimabara Hospital, Shimabara, Nagasaki, Japan and ²Nagasaki Harbor Medical Center City Hospital, Nagasaki, Japan.

Body: background Nanoparticle albumin-bound paclitaxel (nab-PTX) has become a key drug used in chemotherapy for breast cancer, but it often causes adverse effects such as peripheral neuropathy (PN). No effective prophylactic management has so far been established. We have applied a “3S” approach to prevent and treat PN based on two concepts: compression therapy using stockings and sleeves, and medication therapy using selected prophylactic medications. We previously reported better CTCAE v4.0 PN grades and notably superior nab-PTX dose maintenance in a 3S group compared to a control group. However, little is known about the effects of compression therapy on a patient’s level of skin blood perfusion; it is also unknown whether any such effects might vary by 1) PN grade or 2) the number of nab-PTX cycles. <Patients and Methods> To establish whether a compression therapy-skin perfusion relationship exists, the skin perfusion of the lower limbs was measured before and after stocking use in a 3S prophylactic treatment group for nab-PTX therapy (n=44), and in a control group of healthy volunteers (n=50). The skin perfusion was measured using a laser Doppler blood flow meter with an integrated probe (NL-101 Nahri Nexis Japan). To find how compression therapy affects skin perfusion by PN grade, the 3S group was subdivided into three PN grade subgroups (n = 12 for Grade 0, n = 20 for Grade 1, and n = 12 for Grades 2 and 3 combined). To find how compression therapy affects skin perfusion by number of nab-PTX treatment cycles undergone by patients, the 3S group was subdivided into the following three subgroups: 1 to 5 cycles of nab-PTX treatment (first period group; 1P, n=18), 6 to 10 cycles (second period group; 2P, n=16), and more than 10 cycles (third period group; 3P, n=12). <Results> In the control group of healthy volunteers, stocking use tended to increase the median skin perfusion level (mL/min/100g) from 10.9 ± 3.8 to 11.8 ± 4.3 (p=0.06). Interestingly, the median skin perfusion level for the 3S group as a whole significantly increased from 8.7 ± 3.3 before stocking use to 11.3 ± 3.8 after stocking use (p< 0.001). When examining the effects of stocking use on skin perfusion by PN grade, the increase in median skin perfusion level was significant in the Grade 0 subgroup (8.2 ± 2.7 to 12.4 ± 4.5, p=0.005), in the Grade 1 subgroup (8.3 ± 3.8 to 10.4 ± 4.0, p=0.0499), and in the Grades 2 and 3 subgroup (7.9 ± 2.3 to 10.6 ± 2.4, p=0.005). When examining the effects of stocking use on skin perfusion by number of treatment cycles, the increase in median skin perfusion level was significant in the 1P subgroup (7.9 ± 3.0 to 11.4 ± 4.8, p=0.007) and in the 2P (7.9 ± 2.9 to 10.8 ± 2.9, p=0.005), but not in the 3P (9.3 ± 3.5 to 10.8 ± 3.2, p=0.15). <Conclusion> This study demonstrated that the skin perfusion of the lower limbs is decreased following nab-PTX chemotherapy, and also that the skin perfusion is improved by compression therapy. Compression therapy appeared to successfully restore the skin perfusion levels across every grade of chemotherapy-induced PN. Therefore, our 3S approach is suitable for alleviating CIPN by proactively maintaining skin perfusion from the beginning of nab-PTX therapy.
Introduction

The introduction of an enhanced recovery programme for breast cancer surgery in our institution has provided an opportunity to redesign the service and the way in which patients receive pre-operative information. We have encouraged pre-operative consultations with physiotherapists to demonstrate post-operative arm exercises to see if this can improve compliance with exercises and reduce morbidity.

Aim

To audit patient reported upper limb symptoms after breast cancer surgery both before and after the introduction of an enhanced recovery programme with more detailed patient information and input from physiotherapy.

Methods

An enhanced recovery programme was first introduced in our institution in Sep 2012. Between December 2012 and December 2013, 80 patients having breast cancer surgery in Wishaw were sent a detailed questionnaire asking them about all aspects of their treatment. Two patients were excluded from the questionnaire one because of learning difficulties and the other because of acute psychiatric illness. There was an 89% response rate with 71 responses having been received to date. A previous similar questionnaire was sent to patients at 2 and 4 weeks post surgery between November 2011 and April 2012 before the introduction of the enhanced recovery programme. On this occasion there was a 73% response rate with 63 responses having been received from 86 sent. The two groups were compared.

94% of patients reported having received advice about physiotherapy and those who had a session with a physiotherapist increased from 16 to 39% with the introduction of the enhanced recovery programme. 80% of patients received a photographic exercise leaflet in addition to verbal and DVD information on arm exercises.

Postoperatively in the enhanced recovery group, 39% of patients reported some numbness in their ipsilateral upper arm (57% in the group who had undergone axillary node clearance compared with 28% in the sentinel node group). 11% of patients reported persistent ongoing pain after surgery and 9% felt that discomfort affected their daily activities. This contrasts with the previous cohort where 43% of patients reported reduced arm function and almost all of these patients (93%) felt that this impacted on their daily activities. As expected performance across a range of arm and shoulder functions was worse in for axillary node clearance than sentinel node biopsy for both groups.

Conclusion

Introducing an enhanced recovery programme changed the way in which information was provided to patients about post-operative arm exercises and reduced self reported arm morbidity.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-15-15
Average Grade: 6.00

Title: Survival duration and quality of life expectations in patients with metastatic breast cancer: The role of treating oncologists in influencing patients’ expectations of therapy – A questionnaire-based study

Anis Toumeh¹, Ria Kundu¹ and Iman Mohamed¹. ¹University of Toledo, Toledo, OH.

Body: Introduction
Breast cancer is the most common female cancer in the United States. Median survival for patients with metastatic disease is 18 to 24 months with some patients enjoying long term survival¹². Multiple studies have documented that cancer patients tend to overestimate survival duration and that their understanding of their prognosis is insufficient. This is due in part to vague doctor-patient communication, Lack of information concerning the effect of alternative therapies on overall outcomes and Lack of patient understanding of the likely outcomes of their disease³⁴.

Objectives:
To investigate the expectations of patients with metastatic breast cancer in regards to survival duration and quality of life from different treatments, and identify oncologists’ role in influencing these expectations.

Methods:
Electronic charts were used to select women diagnosed with metastatic breast cancer between the age of 35 and 80 treated at the cancer center at the University of Toledo medical center. A detailed questionnaire evaluated the level of comfort between patients and oncologist/s regarding survival duration and quality of life. The questionnaire also evaluated patients’ expectations from treatments they received, are receiving.

Results:
40 patients were identified. 26 out of which completed the survey. The majority of patients (65-77%) expected different treatments for their metastatic disease to prolong their survival for more than 5 years. 57 to 66% of our patients stated that their expectations regarding QOL outcomes from different treatments changed after discussion with their oncologist. 7% of our patients stated that their oncologist does not spend enough time explaining what to expect from the treatment in regards to quality of life outcomes.

Conclusion:
Our study highlights the importance of communication between oncologists and metastatic breast cancer patients and the influential role they play in patients’ expectations regarding survival and quality of life. It is also important to recognize patients’ concerns and spend enough time explaining expected outcomes from different treatments. Although this could be challenging in busy practices, short interval follow up visits and re-addressing those concerns along with involving the palliative care team early could be a potential steps for improvement.

References:
2014 San Antonio Breast Cancer Symposium

**Publication Number:** P5-15-16  
**Average Grade:** 5.40

**Title:** Breast cancer survivor advocacy at the University of Wisconsin Breast Center

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**Body:** Peer-to-peer support programs provide unique psychosocial and educational support for breast cancer patients. With the support of funding from the South Central Wisconsin Affiliate of Susan G. Komen for the Cure, we developed a Patient-Survivor Advocacy (PSA) program to complement University of Wisconsin Breast Center (UWBC) patient navigator to facilitate peer-to-peer support between those who have completed primary breast cancer treatment and those newly diagnosed patients.

We evaluated the feasibility and utility of this peer-to-peer support program for both patients and advocates.

**Methods:**
We recruited advocates from the pool of women previously treated for breast cancer at UWBC. PSAs completed 3 training sessions over a 5 month period, including a volunteer orientation and the After Breast Cancer Diagnosis (ABCD) program. Training topics included a patient survivor advocacy orientation, guidance for communication with patient matches, and documentation and reporting of patient contact to the UWBC patient navigator. Following training, PSAs were matched to patients based on age, type of breast cancer and other life factors identified as important by each referee. PSAs contacted matches via phone and documented communication frequency and content. PSAs and patients then completed surveys describing their experience and satisfaction with the PSA program. Survey questions were tailored to the program strengths, deficits, and areas for improvement. PSAs were also surveyed regarding curriculum and the training process.

**Results:**
Between 11/2012 and 4/2014, 14 PSAs were recruited and trained, and 40 patients were referred to the program. Half of patient referrals (20) were from UWBC physicians, while 18 were from UWBC nursing staff, in addition to 9 self-referrals (this includes overlapping referrals from multiple providers). Six decided not to participate, and one was transferred to hospice before being matched, while 8 patients were referred to the ABCD program due to lack of suitable UWBC PSA matches. Twenty six patients were successfully matched to active PSAs. The number of patients matched to each PSA ranged from 1 to 8; an average of 3 phone and 4 email contacts were made by each PSA-patient dyad. Communication topics included the effect of diagnosis and treatment on mood and mental health, physical health, daily life, and interpersonal relationships, as well as additional community resources for support. Patients and PSAs expressed positive impressions of the program: feeling supported by UWBC staff, experiencing a sense of accomplishment (PSAs) or direct benefit (patients). Responses suggested new topics for PSA training and cancer education, including surgical interventions, radiology, chemotherapy, integrative medicine, medical updates, and new topics in social support.

**Conclusions:**
The first year of the UWBC PSA program saw a successful peer-to-peer psychosocial support infrastructure for newly diagnosed breast cancer patients. PSA and patient survey responses provided useful feedback critical to development of the program. Future goals for this program include increased patient utilization, provision of additional education topics and materials during PSA training, and documentation of the impact of PSA program support on the UWBC nursing burden.
Background: Altered appearance due to chemotherapy is a very distressing adverse event and can remain unrecovered for a long time after chemotherapy. To clarify the current status of appearance change and its support systems, we conducted a national questionnaire survey of patients with breast cancer who had received chemotherapy in Japan. Here, we report on the long-term recovery of scalp hair loss during and after chemotherapy.

Patients and methods: A questionnaire was distributed to patients in hospitals throughout Japan between April and October 2013. The questionnaire was regarding the current status of the patients' appearance issues (scalp hair, eyebrows, eyelashes, nails, skin) related to chemotherapy and its support systems, including chemotherapy regimens received, endocrine therapy received, and duration after chemotherapy. Eligible patients were women with breast cancer without any recurrence who had received adjuvant or neoadjuvant chemotherapy containing anthracycline (A) and/or taxanes (paclitaxel, P; docetaxel, D) and who were within 5 years from the last chemotherapy treatment. The physicians of each hospital asked their patients to fill out the questionnaire and mail it directly to the data center. The scalp hair status was analyzed in a cross-sectional manner according to the duration from chemotherapy.

Results: The questionnaires were returned from 1511 patients in 47 hospitals (response rate, 82%; 1511/1853). Thirty-three patients were excluded, mainly because >5 years had passed since chemotherapy. In total, 1478 questionnaires were ultimately analyzed. The median age was 50 (range, 17–79) years. The distribution of patients according to time from the last chemotherapy treatment was as follows: <1 year, 28%; 1–2 years, 24%; 2–3 years, 19%; 3–4 years, 15%; and 4–5 years, 13%. During chemotherapy, scalp hair loss occurred in 98.4% of the patients, and 94% experienced >80% hair loss. Hair growth began during chemotherapy in 13.1% of patients and after chemotherapy in 80.3% (6.6% left the question unanswered). Within 6 months from the start of hair growth, 65% of patients felt a change in hair thickness, while 82% felt it was becoming thin. Of the patients, 70% felt a change in quality, while 48% felt that it had become unruly; 44% felt a color change, while 80% felt that they were growing more gray hair. Of the patients who answered the questions, >80% hair volume recovery was seen in 52.7% of patients within 1 year; in 63.5%, in 1–3 years; and in 61.7%, even after 3 years. After 3 years, volume recovery was seen in 67.8% of patients after an A+P–containing regimen; in 43.4%, after A+D; in 63.5%, after D; and in 88.9%, after A. Patients who had received A+P, D, and A+D had significantly less volume recovery than patients who had received A (P<0.001 for all).

Conclusions: Almost all patients with breast cancer experienced severe hair loss during standard chemotherapy, but a recovery trend was noted after chemotherapy. However, hair remained unrecovered to various degrees in a significant number of patients even 3–5 years after chemotherapy, especially in those who had received taxane-containing regimens. We should consider the support needs of patients who experience chemotherapy-induced hair loss.
Title: Hemoglobin levels and quality of life in patients with breast cancer and symptomatic chemotherapy-induced anemia enrolled in the eAQUA study

Mario Airoldi¹, Dominique Spaeth², Joan Van den Bosch³, Charalambos Christofyllakis⁴, Laura Belton⁵, Chet Bohac⁶, Jan-Henrik Terwey⁷ and Giuseppe Tonini⁸.
¹Azienda Ospedaliera Universitaria S. Giovanni Battista Le Molinette, Torino, Italy; ²Centre d'Oncologie de Gentilly, Nancy, France; ³Albert Schweitzer Hospital Location Dordwijk, Dordrecht, Netherlands; ⁴401 Military Hospital, Athens, Greece; ⁵LB Biostatistics, London, United Kingdom; ⁶Amgen Inc, Thousand Oaks, CA; ⁷Amgen (Europe) GmbH, Zug, Switzerland and ⁸Università Campus Bio-Medico, Roma, Italy.

Body: Background: Fatigue associated with chemotherapy-induced anemia (CIA) is common in patients with breast cancer, and can have adverse effects on quality of life (QoL). Erythropoiesis-stimulating agents (ESAs) reduce the need for transfusions and may improve QoL in patients with symptomatic CIA. Information on hemoglobin (Hb) levels and effects of fatigue on QoL in patients with breast cancer and CIA in real-world clinical practice is limited.

Methods: The Electronic Assessment of Quality of Life in Patients With Symptomatic CIA (eAQUA) study evaluated improvements in QoL for patients with CIA receiving ESAs who had an increase in Hb of ≥1 g/dL by week 9. This phase 4, international, longitudinal, prospective, observational study enrolled patients with solid tumors who received chemotherapy and had symptomatic anemia. Patients received ESA therapy for up to 13 weeks based on European indication. The primary outcome was the proportion of patients with increase in Hb ≥1 g/dL and improvement in fatigue-related QoL based on the Functional Assessment of Cancer Therapy-Fatigue (FACT-F; scale = 0 to 52 with lower scores indicating worse fatigue) subscale scores and fatigue Visual Analog Scale (VAS; scale = 0 to 100 with higher scores indicating worse fatigue) from baseline to week 9. FACT-F change scores were anchored to VAS change scores to determine the minimally important difference (MID) for improvements in QoL. Patients with a FACT-F change score that was ≥ the MID were considered to have an improvement in QoL. For Hb and QoL outcomes, week 9 data were those assessed closest to on-treatment day 57 (after initiation of ESA) and within on-treatment days 43 to 70 inclusive, to account for different ESA dosing schedules and the observational nature of the study. Secondary outcomes included rates of red blood cell (RBC) transfusions or iron supplementation during the study.

Results: Of 1262 patients enrolled in eAQUA, 289 had breast cancer and were included in the full analysis set (FAS; had at least one ESA dose); of these, 152 patients were eligible to be included in the primary analysis set (PAS; had QoL and Hb data available at baseline and week 9). At baseline, mean (standard deviation [SD]) Hb was 9.4 (0.6) g/dL; mean (SD) FACT-F and VAS scores were 27.1 (10.5) and 52.7 (22.8), respectively, in the FAS. Mean (SD) change from baseline at week 9 was 1.3 (1.3) g/dL for Hb; 4.1 (10.8) score change for FACT-F; and 4.7 (25.9) score change for VAS in the FAS. A total of 54 (18.7%) patients in the FAS required an RBC transfusion and 79 (27.3%) received iron supplementation. At week 9, 77 of 152 patients in the PAS had achieved improvement in fatigue-related QoL (50.7%; 95% confidence interval [CI] = 42.7%, 58.6%); 93 patients had increased Hb ≥1 g/dL (61.2%; 95% CI = 53.4%, 68.9%); and 59 patients (38.8%; 95% CI = 31.1%, 46.6%) had achieved both improvement in fatigue-related QoL and increased Hb ≥1 g/dL.

Conclusions: In this exploratory subgroup analysis, patients with breast cancer and symptomatic CIA treated with ESAs achieved clinically meaningful improvements in fatigue-related QoL and Hb levels.
**Title:** Prophylaxis of chemotherapy-induced febrile neutropenia with biosimilar filgrastim: Description of patients, treatment patterns and outcomes in the MONITOR-GCSF study in the breast cancer cohort


**Body:**


Objectives: To describe patients, treatment patterns of EP-2006, and outcomes in the breast cancer cohort of the MONITOR-GCSF study.

Methods: Prospective observational study following 466 evaluable patients from 23 centers in Europe for up to 6 cycles within a single chemotherapy line including a total of 2714 cycles.

Results: Median age was 56y (range 25-91); all but 3 patients were female. Table 1 presents chemotoxicity in terms of % FN risk and prophylaxis type. GCSF was correctly initiated as either primary or secondary prophylaxis per EORTC guideline recommendations (considering chemotherapy-related FN risk and patient-related factors) in 62% of patients. Eleven percent were undertreated, i.e., secondary prophylaxis when primary was indicated—either when CIN/FN risk >20% or when CIN/FN risk was 10-20% in combination with patient-related risk factors. Twenty-seven percent were overtreated, i.e., primary or secondary when not indicated—either primary prophylaxis in <10% risk of CIN/FN or in 10-20% risk of CIN/FN in the absence of risk factors, or secondary prophylaxis in <10% risk of CIN/FN in absence of prior CIN/FN. EP-2006 was started on average 2.8±2.6 days after chemotherapy was initiated and given for 4.9±2.1 days. Dosing was 45% at 30MIU/day, 55% at 48MIU/day. CIN (any grade) occurred in 12.0% of all cycles and 32.8% of patients had one or more episodes of CIN. 19.5% of patients had at least one episode of Grade 3 or 4 CIN of which 6.2% were febrile. CIN/FN-related hospitalizations were experienced by 5.2% of patients. CIN/FN-related chemotherapy disturbances (dose reduction, delay or cancellation) occurred in 9.4%.

Conclusions: Variation in treatment with biosimilar GCSF in breast cancer patients is evident in terms of decision to treat with primary prophylaxis relative to guideline recommendations as well as day of initiation, duration and dose, yet incidence of CIN/FN and related events is low. Forthcoming analyses will determine whether variability in treatment is associated with differential outcomes.

### Table 1

<table>
<thead>
<tr>
<th>Chemotherapy toxicity (risk of FN)</th>
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<tr>
<td>&gt;20%</td>
<td>250</td>
<td>53.8</td>
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<tr>
<td>10-20%</td>
<td>170</td>
<td>36.5</td>
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<td>&lt;10%</td>
<td>45</td>
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<table>
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<tr>
<th>Phrophylaxis</th>
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<tr>
<td>Primary</td>
<td>372</td>
<td>79.8</td>
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<tr>
<td>Secondary</td>
<td>94</td>
<td>20.2</td>
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<table>
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<tr>
<th>Prophylaxis decision relative to guidelines</th>
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<tr>
<td>Undertreated</td>
<td>50</td>
<td>10.8</td>
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<tr>
<td>Correct</td>
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<td>62.1</td>
</tr>
<tr>
<td>Overtreated</td>
<td>126</td>
<td>27.1</td>
</tr>
</tbody>
</table>
Title: What we know and what we must do: A metastatic breast cancer alliance quality of life landscape analysis

Musa Mayer¹, Katherine Crawford-Gray², Shirley Mertz³, Ginny Knackmuhs³ and Marc Hurlbert². ¹AdvancedBC.org, New York, NY; ²Avon Foundation for Women, New York, NY and ³Metastatic Breast Cancer Network, New York, NY.

Body: The Metastatic Breast Cancer Alliance (MBCA)[1] is comprised of non-profit advocacy, funding and industry organizations and individuals who seek to transform and improve the lives of women and men living with metastatic breast cancer. Stage IV or metastatic breast cancer is different from early breast cancer. The disease is not curable. People with MBC are always in treatment, switching treatment regimens as their disease progresses. Metastasis is the cause of virtually all breast cancer deaths, and nearly 40,000 die annually of MBC. Because their time is limited, patients daily experience a host of psychosocial and quality of life issues.

Objective: To review prior literature and patient survey reports related to quality of life needs for patients with MBC, interview key experts in the field, and to assess the extent to which non-profit organizations, clinical providers and others are meeting those needs.

Methods: We conducted (1) a literature review of >140 recent articles and studies in psychosocial research as well as survey findings from over 6,000 patients living with MBC; (2) a desk research analysis of MBCA members’ efforts in patient advocacy, research, policy, education and support, and public awareness; analysis of websites (n=24) and print materials (n=27); (3) interviews with MBC Alliance members about their information and services for MBC patients (n=16); and (4) an online survey of hospital-based patient navigation programs (n=31) and telephone helplines (n=8) provided by breast cancer and all-cancer organizations.

Results: We found inconsistent and incomplete development of patient education materials about metastatic disease and treatment options. Health care teams are often not taking time to educate patients on treatment options, or to routinely assess and treat their side effects and symptoms. Resources, staffing and time are not available to meet patients’ quality of life and psychosocial needs. Anxiety and depression in MBC patients remain untreated in many cases. Palliative care is misunderstood and still associated with end of life care by both doctors and patients. Yet, quality of life for MBC patients can be improved with increased access to palliative care earlier in the diagnosis of the disease.

Conclusion: Psychosocial research and patient surveys identify the information and service needs that would improve the quality of life for MBC patients; however, our study finds that those needs are rarely met. Advocacy groups and health care providers need to act strategically to put into place programs and support services that address the psychosocial and quality of life needs of patients living with MBC.

[1] https://www.mbcalliance.org/
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-15-21
Average Grade: 5.75

Title: SurvivorLink: Evolution of 1:1 peer support to connect young women with breast cancer

Jean Rowe¹, Michelle Esser¹, Megan McCann¹ and Stacy Lewis¹. ¹Young Survival Coalition, New York, NY.

Body: Background
There are 250,000 breast cancer survivors living in the United States today who were diagnosed under the age of 40. Compared to older women, young women (YW) generally face more aggressive cancers, lower survival rates, an increased risk of metastatic recurrence, and a higher rate of anxiety and depression.

YW also face unique issues as a result of their diagnosis. They are more likely to be single and dating, starting a career, raising young children, or possibly starting a family. Cancer treatments may impact fertility, cause premature menopause, and sexual dysfunction. Due to these and other concerns, YW diagnosed with breast cancer strongly desire to connect with other young survivors.

Young Survival Coalition (YSC) is the premier global organization dedicated to the critical issues unique to YW and breast cancer. It is YSC’s goal to ensure that no young woman goes through breast cancer alone.

Methods
In order to connect YW diagnosed with breast cancer with others similarly situated, YSC initiated its SurvivorLink program (SL) in 2006 then known as Point of Contact (POC). YW seeking to connect with another young breast cancer survivor called or emailed a YSC staff person, who connected her to a trained POC. POCs received training through 2-day in-person sessions held periodically throughout the country. In 8 years, there were 89 volunteers trained in 7 in-person training sessions. Between 2008 and 2010, 776 YW requested connections through SL. In 2011, usage of SL dropped significantly.

In 2013, YSC re-examined the SL program to determine whether it was a needed resource. If so, YSC sought to determine how to revitalize and expand it.

Results
During the analysis, a few key issues arose. Despite the stated desire of YW to connect with others, SL was not well-utilized from 2011 on. Further research determined that many young survivors were not aware of SL, even if they knew of YSC. The in-person trainings were expensive and not all interested volunteers were located in the same geographic area. This limited the ability to maintain a pool of fresh volunteers with diverse experiences. Data on the program had not been maintained in one central location. It was difficult to discern who was matched through SL, when, and the result of their interaction.

YSC believes that the program is important to continue. While other organizations offer call-in support or peer matching, none focus solely on YW with breast cancer nor have a diversity of YW with different diagnoses and experiences who could serve as potential matches.

Taking these issues into consideration, YSC took steps to bring renewed energy to this program. First, an online training portal for SL volunteers was developed. Through eight on-line modules, YSC can train more volunteers in a shorter period of time for less cost. Individuals can complete the training in their own time from the comfort of their own homes. In order to make YW aware of the program, SL is now regularly advertised on Facebook, the YSC homepage, and elsewhere. Finally, a central database was initiated to capture information on our trained volunteers, those who call-in, the matches made and the results of their interaction. This allows YSC to track the use and success of the program.
Title: End-of-life outcomes and hospice and palliative care utilization in hospitalized patients with metastatic breast cancer

Amanda Parkes¹, Jennifer A Shin¹, Helen Knight¹, Areej El-Jawahri¹, Lara Traeger¹ and Jennifer S Temel¹. ¹Massachusetts General Hospital, Boston, MA.

Body: Background:
Hospitalizations in patients with metastatic cancer occur commonly at the end of life but have not been well-described in those with metastatic breast cancer (MBC). The goal of this study was to describe the reasons for admission, end-of-life outcomes, and hospice and palliative care utilization in hospitalized patients with MBC.

Methods:
We identified all patients with MBC who had their first hospital admission (index admission) at Massachusetts General Hospital since their diagnosis of metastatic disease between 1/1/2009 and 12/31/2010 through a centralized clinical data registry. We collected demographic and clinical information and data on all hospital admissions and utilization of palliative care and hospice services during a three-year follow-up period.

Results:
We identified 123 patients hospitalized for the first time since their diagnosis of MBC. The median number of hospital admissions during the three-year follow-up period was 2 (range 1 to 17). Uncontrolled symptoms accounted for half (62/123, 50%) of the index admissions [20 (16%) with central nervous system systems, 17 (14%) with respiratory symptoms, 10 (8%) with pain, 9 (7%) with gastrointestinal symptoms, 4 (3%) with failure to thrive, and 2 (2%) with dehydration]. The majority of patients (93/123, 76%) died during the follow-up period. The median time from first hospitalization to death was 6 months (range 0 to 53). Among patients who died, 20/93 (22%) died in the hospital and 49/93 (53%) died within 14 days of hospital discharge. Among the deceased group, the inpatient palliative care team evaluated 53/93 (57%) of patients at least once during an admission, but only 18/93 (19%) patients attended an outpatient palliative care clinic appointment. A minority of patients who died (27/93, 29%) were referred to hospice upon discharge. An additional 34/93 (37%) patients who died were referred to hospice from the outpatient setting.

Conclusions:
Hospitalized patients with MBC are commonly admitted for uncontrolled symptoms. They have a poor prognosis, and approximately half die within two weeks of a hospital admission. However, only a minority receive outpatient palliative care or are referred to hospice services from the inpatient setting. These findings highlight the need to develop interventions to improve end-of-life care for patients with MBC who are hospitalized.
Title: Prognostic factors predicting overall survival after radiotherapy for metastatic spinal cord compression in breast cancer patients treated in the Brazilian public health system

Tomas Reinert1, Ricardo Zylberberg1, Frederico MT Lima1, Christiane S Pinto1 and Alexandre Boukai1. 1Instituto Nacional do Câncer (INCA), Rio de Janeiro, RJ, Brazil.

Body: Objective: To evaluate potential prognostic factors for survival after radiotherapy of metastatic spinal cord compression (MSCC) on breast cancer patients treated at the Brazilian public health system.

Methods and materials: We evaluated 51 consecutive patients treated in a single cancer center between May 2011 and May 2012. The following potential prognostic factors were investigated retrospectively: age, performance status, interval between tumor diagnosis and MSCC, presence of visceral metastases, interval between the development of symptoms and radiotherapy, site of compression and Tokushashi index (prognostic criteria index that divides patients into 3 groups with different life expectancy according to their total number of scoring points).

Tokuhashi Score Parameters

<table>
<thead>
<tr>
<th>General condition (ECOG PS)</th>
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</thead>
<tbody>
<tr>
<td>No. extraspinal bone metastasis foci</td>
<td></td>
</tr>
<tr>
<td>No. metastasis in the vertebral bodies</td>
<td></td>
</tr>
<tr>
<td>Metastases to the major internal organs</td>
<td></td>
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<tr>
<td>Primary site of cancer</td>
<td></td>
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<tr>
<td>Spinal cord palsy</td>
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Results: Patients characteristics are shown in Table 2.

Patients characteristics

| Age: median (range) | 54y (28-79) |
| Interval between BC diagnosis and MSCC: median (range) | 32mo (0-131). |
| Days of hospitalization: median (range) | 10 (2-67) |
| Visceral metastases (yes/no) | 49%/51% |
| Site of compression (cervical/thoracic/lumbar) | 24%/55%/49% |
| Spinal cord palsy (none/incomplete/complete) | 31%/47%/21% |
| Tokuhashi score risk (low/intermediate/high) | 30%/35%/33% |

Inpatient mortality was 18%. Among the 16 patients who had non-ambulatory status at admission only 2 (12%) were able to walk after treatment. Overall survival was 13.6 months. Overall survival according to the Tokushashi index was 1.5mo high risk, 13.6mo intermediate risk and 23.3mo low risk.

The following prognostic factors for inferior OS were identified on univariate analysis: high risk Tokuhashi score - HR 3.7 (p 0.0001); PS4 - HR 3.2 (p 0.0004); Presence of visceral metastasis - HR 4.0 (p 0.0001); interval between symptoms and radiotherapy >14d - HR 2.5 (p 0.01). On multivariate analysis, a high risk Tokuhashi score was statistically associated with inferior survival.
Conclusion: Metastatic spinal cord compression is an oncological emergency associated with significant morbidity and mortality. Life expectancy is a key factor for therapeutic planning. In the limited resource setting of the Brazilian public health system, the validation of prognostic factors is essential to guide the clinician on referring the patient to a tertiary cancer care center or to provide palliative care avoiding burden for debilitated patients resulting from multiple daily trips to the radiation oncology department and painful positioning on the treatment couch. This study validated the Tokuhashi index as a useful prognostic tool in this population.
Title: The impact of skeletal-related events on pain interference in patients with advanced breast cancer and bone metastases

Lesley Fallowfield¹, Donald L Patrick², Roger Von Moos³, Charles S Cleeland⁴, Ying Zhou⁵, Arun Balakumaran⁵ and Yi Qian⁵.
¹University of Sussex, Sussex Health Outcomes Research and Education in Cancer (SHORE-C), Brighton, United Kingdom; 
²University of Washington, Seattle, WA; ³Cantonal Hospital Graubünden, Chur, Switzerland; ⁴University of Texas MD Anderson Cancer Center, Houston, TX and ⁵Amgen Inc, Thousand Oaks, CA.

Body: Background: Patients with advanced breast cancer and bone metastases are at an increased risk for experiencing skeletal-related events (SREs), which include pathological fracture (PF), surgery to bone (SB) radiation to bone (RB), and spinal cord compression (SCC). The pain of SREs can be severe enough to interfere with daily functioning. Here we evaluated the impact of SREs on pain interference in patients with advanced breast cancer and bone metastases.

Methods: In a double-blind, double-dummy, placebo-controlled trial, patients were evenly randomized to receive monthly denosumab 120 mg SC or zoledronic acid 4 mg IV, (adjusted for renal function). Pain interference (overall, emotional well-being, and physical function) was assessed at baseline and each study visit using the Brief Pain Inventory-Short Form (BPI-SF) with scores that ranged from 0 (no interference) to 10 (complete interference). A change of ≥ 2 points from baseline was considered clinically meaningful. To evaluate the overall impact of SREs on pain interference, we conducted a post-hoc analysis using a Cox Proportional Hazards model adjusting for SREs as time-dependent covariates and stratified by treatment and randomized stratification factors. The impact of on-study SREs was evaluated using patients’ first on-study SRE, starting 28 days before the SRE occurrence.

Results: 687 first on-study SREs were reported (450 PF, 201 RB, 20 SB, 16 SCC). SCC, RB, and PF were associated with a greater risk of a clinically meaningful increase in overall pain interference (Table 1). For the subdomains, RB and SCC were associated with an increased risk of pain interference with emotional well-being, while PF, RB, and SB were associated with an increased risk of pain interference with physical function.

Impact of on-study SREs on time to ≥ 2-point increase from baseline in pain interference BPI score

<table>
<thead>
<tr>
<th></th>
<th>PF</th>
<th>RB</th>
<th>SB</th>
<th>SCC</th>
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<tr>
<td><strong>Pain interference - overall (n = 1829)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.31 (1.05, 1.63)</td>
<td>2.41 (1.80, 3.23)</td>
<td>1.85 (0.68, 5.05)</td>
<td>4.26 (1.38, 13.19)</td>
</tr>
<tr>
<td>P value</td>
<td>0.0159</td>
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<td>0.2322</td>
<td>0.0120</td>
</tr>
<tr>
<td><strong>Pain interference - emotional well being (n = 1806)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.20 (0.97, 1.48)</td>
<td>2.25 (1.72, 2.95)</td>
<td>1.11 (0.41, 3.00)</td>
<td>4.74 (2.15, 10.44)</td>
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<tr>
<td>P value</td>
<td>0.1003</td>
<td>&lt; 0.0001</td>
<td>0.8432</td>
<td>0.0001</td>
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<tr>
<td><strong>Pain interference - physical activity (n = 1690)</strong></td>
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<td></td>
</tr>
<tr>
<td>HR (95% CI)</td>
<td>1.35 (1.09, 1.69)</td>
<td>2.30 (1.70, 3.10)</td>
<td>2.86 (1.09, 7.47)</td>
<td>2.26 (0.45, 11.37)</td>
</tr>
<tr>
<td>P value</td>
<td>0.0070</td>
<td>&lt; 0.0001</td>
<td>0.0326</td>
<td>0.3232</td>
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</table>

Includes patients with baseline BPI score ≤8; HR = hazard ratio; CI = confidence interval.

Conclusions: In patients with advanced breast cancer, SREs are associated with an increase in pain interference. Effective treatments that prevent SREs may reduce the burden of pain on patients’ daily functioning.
Title: Effective alleviation of pegfilgrastim-induced pain with high-dose loratadine in breast cancer patients

Jiaxin Niu¹ and Ashish Sangal¹. ¹Western Regional Medical Center at CTCA, Goodyear, AZ.

Body: Background: Pegfilgrastim is widely used clinically in breast cancer patients receiving myelosuppressive chemotherapy. The incidence of pegfilgrastim-induced bone pain (PIP) was reported ranging from 26% to 59%. Severe PIP may impair patients' quality of life, leading to dose reduction, interruption or even discontinuation of chemotherapy. There have been some anecdotal reports regarding the use of loratadine to prevent PIP. Intriguingly, loratadine failed to decrease the incidence of PIP in a randomized phase II study. Nevertheless, loratadine has been commonly used in clinical practice despite lack of high-level evidence.

Methods: We first identified eligible breast cancer patients on planned multiple cycles of chemotherapy who developed clinically significant PIP requiring rescue use of analgesics after receiving an initial dose of pegfilgrastim (6 mg subQ on the day after chemotherapy) and standard prophylactic dose of loratadine (10 mg po daily beginning on the day of pegfilgrastim administration and continuing for 7 days). Then all the eligible patients were invited to receive higher-dose loratadine (10 mg po three times a day beginning on the day of pegfilgrastim administration and continuing for 7 days) in the following cycles with same chemotherapy and other supportive care as the previous cycle. Patients were advised to report any unexpected adverse events promptly. Each patient was assessed at the beginning of each following cycle by the treating physician; pain scale with duration and analgesic use was documented. Significant PIP, assessed by Worst Pain Scale (0-10) of the Brief Pain Inventory, was defined as worse pain score > 5 during the 7 days after pegfilgrastim administration. Significant improvement was defined as 3-point decrease in pain scale with or without decreased analgesic use.

Results: A total of 20 female breast cancer patients (15 patients receiving adjuvant chemotherapy, 5 patients receiving palliative chemotherapy) with significant PIP volunteered to receive off-label dose of loratadine in our observational study. In the following cycle, 2 patients (2/20, 10%) had complete resolution of pain, 11 patients (11/20, 55%) had significant improvement, and 7 patients (7/20, 35%) did not benefit from the higher dose of loratadine. No unexpected adverse events were reported. The results were reproducible in the remaining cycles of the same chemotherapy.

Conclusions: Administration of loratadine po three times a day for 7 days appears to be safe, and very effective to alleviate PIP in this observational study. Prospective studies are warranted to confirm the finding in order to address this crucially important clinic issue.
Title: Development and validation of the Penn arthralgia aging scale among breast cancer survivors on aromatase inhibitors

Moriah J Brier¹, Dianne L Chambless¹, Laura Lee², Angela DeMichele² and Jun J Mao². ¹University of Pennsylvania, Philadelphia, PA and ²Perelman School of Medicine at University of Pennsylvania, Philadelphia, PA.

Body: Background: Aromatase inhibitors (AIs) have been shown to decrease the recurrence rate and increase the overall survival rate of hormone receptor positive breast cancer among post-menopausal women. Unfortunately, arthralgia is a frequently experienced side effect of AIs, leading some women to discontinue AIs prematurely. Qualitative investigations suggest that joint pain may cause women on AIs to feel they are aging faster than they should be. Since general perceptions of aging have been shown to predict important health outcomes, such as adherence and mortality, aging perceptions related to joint pain are worth further exploration. Objective: The purpose of this study was to develop and validate a measure that captures perceptions of aging related to joint pain. A psychometrically validated tool will advance our ability to quantify and further understand the importance of this construct. Method: We developed the eight-item Penn Arthralgia-Aging Scale (PAAS) from interviews with 67 patients on AIs. The scale was pilot-tested, and changes to items were made based on patient feedback, as well as feedback from oncologists, nurses, and physical therapists. To validate the scale, participants suffering from joint pain were selected from a larger study examining the genetic determinants of symptom distress and disease outcomes among women on AIs. Five hundred and fifty-six breast cancer survivors completed the PAAS, as well as the Hospital Anxiety and Depression Scale, the pain interference and pain intensity subscales of the Brief Pain Inventory, and a demographic questionnaire. Exploratory factor analysis using oblique rotation was conducted to examine the factor structure of the scale. Convergent validity was assessed by correlating the PAAS with joint-pain severity. To determine whether the scale provides important information beyond existing measures, we used hierarchical regressions to calculate whether it predicted incremental variance in anxiety, depression, and pain interference outcomes. Results: The resulting scale had a one-factor structure (eigenvalue = 6.21), high internal consistency (Cronbach’s alpha = 0.94), and strong convergent validity (Spearman r = .55, p < 0.01 for joint pain). Additionally, the PAAS was found to explain additional variance in anxiety (7%, p < 0.001) and depression (28%, p < 0.001) after pain severity and age were controlled. The PAAS also explained additional variance (5%, p < 0.001) in joint pain interference above and beyond the variance accounted for by anxiety, depression, joint pain severity, and age. Conclusions: These findings suggest that the PAAS is a reliable and valid tool that captures a meaningful construct of perceptions of aging attributable to arthralgia. With further research, the PAAS may advance our understanding of breast cancer survivors’ emotional, behavioral, and clinical outcomes.
Title: Quality of life in patients receiving first-line eribulin mesylate for HER2- locally recurrent or MBC

Lee Schwartzberg1, Kristi McIntyre2, Joyce O’Shaughnessy3, Stefan Glück4, Erhan Berrak5, James Song5, David Cox5 and Linda Vahdat6. 1West Clinic, Memphis, TN; 2Texas Oncology, Dallas Presbyterian Hospital, US Oncology, Dallas, TX; 3Baylor Charles A. Sammons Cancer Center, Texas Oncology, US Oncology, Dallas, TX; 4Sylvester Comprehensive Cancer Center, University of Miami Health System, Miami, FL; 5Eisai Inc, Woodcliff Lake, NJ and 6Weill Cornell Medical College, New York, NY.

Body: Introduction: Eribulin mesylate is a nontaxane microtubule inhibitor approved to treat MBC in patients (pts) who previously received ≥2 chemotherapeutic regimens for MBC. A phase 2 study of first-line eribulin for HER2-negative (HER2-) MBC showed an overall response rate of 29%, median 6.8 m progression-free survival, and tolerability consistent with earlier studies. We present prespecified quality of life (QoL) results for this trial.

Methods: Pts (N=56) received eribulin mesylate 1.4 mg/m² IV on days 1 and 8 of each 3-wk cycle (median: 7 cycles). QoL was assessed using the EORTC QoL assessment (QLQ-C30) and a breast-cancer specific questionnaire (QLQ-BR23) pretreatment (baseline) and on day 1 of every other cycle during treatment. Percentage of pts with at least ±10-point change from baseline was summarized descriptively. Linear mixed-effects models were used to evaluate changes over time and compare responders vs nonresponders controlling for baseline score and time effect. Time-to-event analysis was performed on time to deterioration, defined as time from 1st dose to 1st occurrence of worsening in QoL score that reached minimally clinically important difference (MID; eg, 10 points in global health status in QLQ-C30) from baseline without further improvement of at least MID.

Results: For QLQ-C30 at cycle 6 (n=29), more pts had at least a 10-point improvement from baseline in role, emotional, and social functioning; fatigue, nausea/vomiting, pain, dyspnea, and insomnia item scores, than had worsening. More pts had worsening in global health status/QoL, cognitive functioning, and diarrhea (Table).

<table>
<thead>
<tr>
<th>Category</th>
<th>Improved</th>
<th>Stable</th>
<th>Worsened</th>
</tr>
</thead>
<tbody>
<tr>
<td>Global health status/QoL</td>
<td>2 (7)</td>
<td>16 (55)</td>
<td>11 (38)</td>
</tr>
<tr>
<td>Physical functioning</td>
<td>9 (31)</td>
<td>13 (45)</td>
<td>7 (24)</td>
</tr>
<tr>
<td>Role functioning</td>
<td>14 (48)</td>
<td>10 (35)</td>
<td>5 (17)</td>
</tr>
<tr>
<td>Emotional functioning</td>
<td>9 (31)</td>
<td>14 (48)</td>
<td>6 (21)</td>
</tr>
<tr>
<td>Cognitive functioning</td>
<td>2 (7)</td>
<td>13 (45)</td>
<td>14 (48)</td>
</tr>
<tr>
<td>Social functioning</td>
<td>10 (35)</td>
<td>13 (45)</td>
<td>6 (21)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>16 (55)</td>
<td>5 (17)</td>
<td>8 (28)</td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>8 (28)</td>
<td>16 (55)</td>
<td>5 (17)</td>
</tr>
<tr>
<td>Pain</td>
<td>15 (52)</td>
<td>8 (28)</td>
<td>6 (21)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>9 (31)</td>
<td>18 (62)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>12 (41)</td>
<td>15 (52)</td>
<td>2 (7)</td>
</tr>
<tr>
<td>Appetite loss</td>
<td>7 (24)</td>
<td>16 (55)</td>
<td>6 (21)</td>
</tr>
<tr>
<td>Constipation</td>
<td>5 (17)</td>
<td>18 (62)</td>
<td>6 (21)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2 (7)</td>
<td>21 (72)</td>
<td>6 (21)</td>
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</tbody>
</table>

Median time to deterioration in global health status/QoL was 5.06 m (responders, 8.54 m; nonresponders, 3.71 m; hazard ratio=0.60, P=0.22). In linear mixed models, responders (n=16) performed better than nonresponders (n=40) in role functioning (P=0.011), emotional functioning (P=0.031), fatigue (P=0.007), pain (P=0.047), insomnia (P=0.018), and appetite loss (P=0.032).
Mean symptom scores were significantly correlated with corresponding adverse event rates for nausea and vomiting, dyspnea, appetite loss, constipation, and diarrhea; Spearman rank correlation coefficients ranged from 0.31 to 0.54. For QLQ-BR23 at cycle 6, symptom scores were mostly stable; more pts had worsening in body image and systemic therapy side effects than had improvement and more pts had improvement in breast and arm symptoms than had worsening. Responders also had longer time to symptom deterioration.

Conclusions: In this study of first-line eribulin treatment for HER2- MBC, a majority of pts had stable or improvement in QoL scales. Responders to eribulin were more likely than nonresponders to have stable or improved QoL.
**Title:** Quality of life results from a phase 2, multicenter, single-arm study of eribulin mesylate plus trastuzumab as first-line therapy for locally recurrent or metastatic HER2+ breast cancer

Lee Schwartzberg¹, Sharon Wilks², Shannon Puhalla³, Joyce O’Shaughnessy⁴, Erhan Berrak⁵, James Song⁵, David Cox⁵ and Linda Vahdat⁶. ¹West Clinic, Memphis, TN; ²US Oncology-Cancer Care Centers of South Texas, San Antonio, TX; ³University of Pittsburgh Medical Center, Pittsburgh, PA; ⁴Baylor Charles A. Sammons Cancer Center, Texas Oncology, Dallas, TX; ⁵Eisai Inc, Woodcliff Lake, NJ and ⁶Weill Cornell Medical College, New York, NY.

**Body:** Introduction: Eribulin mesylate is a nontaxane microtubule dynamics inhibitor that has showed an overall survival benefit relative to other commonly used agents in patients with ≥2 prior MBC therapies. Primary data from a phase 2 trial for first-line eribulin + trastuzumab [TRAS] in HER2+ patients with MBC showed an objective response rate of 71%, clinical benefit rate of 84.6%, disease control rate of 96.2%, PFS of 11.6 months, and tolerability similar to known profiles for these agents. Here, we present prespecified QoL, efficacy, and safety/tolerability results.

Methods: Patients received eribulin mesylate 1.4 mg/m² IV on days 1 and 8 of each 21-day cycle and initial TRAS (8 mg/kg IV/day 1), followed by 6 mg/kg on day 1 of each subsequent cycle. Response, PFS, QoL as measured by EORTC QoL assessment tool (QLQ-C30) and QLQ-BR23, and tolerability were assessed. Percentage of patients with at least ±10-point change from baseline was calculated at each visit. Time to deterioration was defined as time from first dose to worsening in QoL score that reached minimally clinically important difference (MID) (ie, 10 points in global health status [GHS] in QLQ-C30) without further improvement of at least MID; this was estimated overall and by response status.

Results: At cycle 6 (n=44; completion rate=84.6% of 52 patients enrolled), more patients fell in the stable category (within +/-10 points change from baseline), except for pain (47.7% with improvement), cognitive functioning (45.5% worsening), fatigue and systemic therapy side effects (50% worsening for each), and arm symptoms (47.7% improvement) (Table). Median times to deterioration for GHS/QoL were 7.6 months overall (n=51), and 7.6 and 7.0 months for responders (n=36) and nonresponders (n=15), respectively (HR 0.73; 95% CI 0.32, 1.68; P=0.446). Mean symptom scores in EORTC QLQ-C30 were significantly correlated with corresponding AE rates for fatigue (r=0.31), nausea/vomiting (r=0.50), pain (r=0.41), dyspnea (r=0.49), insomnia (r=0.35), constipation (r=0.30), and diarrhea (0.40; P≤0.03 for all comparisons). The most common treatment-related AEs (all grade incidence ≥25%) were alopecia (88.5%), fatigue (69.2%), peripheral neuropathy (69.2%), neutropenia (59.6%), nausea (46.2%), diarrhea (32.7%), anemia (25%), constipation (25%), and decreased appetite (25%).

**Table. QoL EORTC QLQ-C30 Scores: Change from Baseline to Cycle 6 (n=44)**

<table>
<thead>
<tr>
<th>Symptom or Category</th>
<th>Improved</th>
<th>Stable</th>
<th>Worsened</th>
</tr>
</thead>
<tbody>
<tr>
<td>GHS/Qol</td>
<td>13 (29.5)</td>
<td>23 (52.3)</td>
<td>8 (18.2)</td>
</tr>
<tr>
<td>Physical</td>
<td>10 (22.7)</td>
<td>26 (59.1)</td>
<td>8 (18.2)</td>
</tr>
<tr>
<td>Role</td>
<td>13 (29.5)</td>
<td>20 (45.5)</td>
<td>11 (25.0)</td>
</tr>
<tr>
<td>Emotional</td>
<td>17 (38.6)</td>
<td>18 (40.9)</td>
<td>9 (20.5)</td>
</tr>
<tr>
<td>Cognitive</td>
<td>6 (13.6)</td>
<td>18 (40.9)</td>
<td>20 (45.5)</td>
</tr>
<tr>
<td>Social</td>
<td>12 (27.3)</td>
<td>18 (40.9)</td>
<td>14 (31.8)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>14 (31.8)</td>
<td>8 (18.2)</td>
<td>22 (50.0)</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>9 (20.5)</td>
<td>24 (54.5)</td>
<td>11 (25.0)</td>
</tr>
<tr>
<td>Pain</td>
<td>21 (47.7)</td>
<td>14 (31.8)</td>
<td>9 (20.5)</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>12 (27.3)</td>
<td>19 (43.2)</td>
<td>13 (29.5)</td>
</tr>
</tbody>
</table>
Conclusions: Given the improvements in pain and in arm and breast symptoms, long median time to deterioration in functioning/symptom scales in this analysis, and the tumor response rates and safety profile in the primary analysis, combination eribulin/TRAS may be an acceptable treatment option for locally recurrent or HER2+ MBC and merits further study in larger clinical trials.
Title: Long term hair loss in patients with early breast cancer receiving docetaxel chemotherapy

Nicola J Thorp¹, Felicity Swift¹, Donna Arundell¹ and Helen Wong¹. ¹Clatterbridge Cancer Centre, Wirral, Merseyside, United Kingdom.

Body: Background
There is increasing recognition that a small number of patients receiving docetaxel-containing regimes for early breast cancer (EBC), experience permanent alopecia. However, there is little data to inform discussions with patients regarding this serious late side effect. The aim of this study was determine the incidence, the site, the extent, and duration of the hair loss.

Methods
A postal questionnaire was sent (in October 2013) to patients who had received docetaxel during 2010, in the neo/adjuvant settings for EBC at our regional cancer centre. This comprised questions relating to scalp hair loss (using the Ludwig scale to provide a pictorial description of the pattern of hair loss), hair loss to other parts of the body, hair products used, and any comments that the respondents wished to add about their experience of hair loss. Univariate and multivariate analyses were undertaken to determine any other risk factors for persistent alopecia.

Results
134 of 189 (71%) questionnaires were returned. Of those responding, 72 patients were pre-, 10 were peri- and 52 post-menopausal. 26 patients were taking anastrazole, 14 letrozole, 74 tamoxifen and 20 no adjuvant hormones. Of the respondents, 99 (74.4%) patients had no significant scalp hair loss, and 21 (15.8%) had significant scalp hair loss. 13 (9.8%) of patients gave equivocal responses and 1 patient did not answer the scalp hair loss question. 16 patients in the study were using products such as wigs and hair extensions. 5 patients reported no regrowth of eyebrows, 2 patients reported no eyelash regrowth, 6 no regrowth of nostril hair and 14 no regrowth to other parts such as legs. Univariate and multivariate analyses showed no significant associations with other patient and treatment characteristics (eg adjuvant endocrine therapy). Patients’ observations regarding the social and emotional consequences of permanent hair loss confirmed a significant impact on quality of life.

Conclusions
This retrospective questionnaire study confirms that long term significant scalp alopecia (here lasting for up to 3.5 years following completion of chemotherapy) may affect 10-15% of patients following docetaxel for EBC (taking into consideration a potential bias for no hair loss in the non-responders). This rate is higher than previous estimates. Long term hair loss to other parts of the body was also widely reported. This appears to be unrelated to other patient and treatment characteristics. Long term hair loss had a significant impact on quality of survival. This is an important quality of life issue for patients which merits prospective study to confirm incidence, to identify effective preventive and management strategies. This risk should be discussed routinely (as part of the process of informed consent) with all patients embarking upon docetaxel as a component of management of EBC.
Title: It's as simple as ABC: Can we improve the provision of coordinated medical and supportive care to patients with advanced breast cancer?

Sally Greenberg¹, Meron Pitcher¹, Bruce Mann²,³, Kathleen Hendry¹, Kerry Shanahan²,³, Melanie K Fisher¹ and Jung H Foo⁴.
¹Western Hospital, Melbourne, Victoria, Australia; ²Royal Melbourne Hospital, Melbourne, Victoria, Australia; ³Royal Women's Hospital, Melbourne, Victoria, Australia and ⁴Western and Central Melbourne Integrated Cancer Service, Melbourne, Victoria, Australia.

Body: Background: International and local guidelines regarding advanced breast cancer (ABC) management recommend coordinated care, discussion in multi-disciplinary meetings (MDM), breast care nurse (BCN) involvement and supportive care screening (SCS). There was concern among clinicians, at three University affiliated hospitals in Melbourne Australian, that these were not routinely performed for all patients at our hospitals. A WCMICS service improvement grant was used to design a Model of Care (MOC) aiming to address these deficiencies.

Method: A 6 month retrospective audit of all new ABC patients was conducted at each institution. We reviewed timing and details of diagnosis, MDM discussions, BCN involvement, SCS and communication with primary care physicians (PCP). Surveys/interviews of PCP, patients and oncology clinicians were conducted. A best practice MOC was developed, piloted for three months and evaluated.

Results: The new MOC resulted in an increased proportion of patients discussed in MDM (from 33% to 88%), reviewed by a BCN (from 30% to 80%), and improved provision (from 61% to 90%) and timing (median 20 down to 16 days) of communication with PCP. 50% of patients interviewed in both the baseline and subsequent evaluation surveys did not feel their care was well coordinated between specialists and PCP.

Conclusions: A best practice MOC for patients with newly diagnosed ABC is possible to implement across a number of institutions with improvements in the provision of coordinated medical and supportive care. Further improvements can be made in coordination of care between PCP and specialists.
Title: Emotional/psychological characteristics of women with triple-negative breast cancer: Do socioeconomic, demographic, and provider variables impact emotional change from diagnosis to post-treatment?

Kathleen D Swiger¹, Jocelyn A Sendecki², Janine E Guglielmino³, Hope S Rugo, Carey K Anders⁴, Susan M Domchek⁵, Arin Ahlum Hanson³, Hayley Dinerman⁶ and Catherine Creme Henry³. ¹Consultant, Greensboro, NC; ²Thomas Jefferson University, Philadelphia, PA; ³Living Beyond Breast Cancer, Haverford, PA; ⁴University of North Carolina, Chapel Hill, NC; ⁵University of Pennsylvania, Philadelphia, PA and ⁶Triple Negative Breast Cancer Foundation, Norwood, NJ.

Body: Purpose: To determine whether women with triple-negative breast cancer (TNBC) experience greater levels of emotional concern from diagnosis through post-treatment compared to women with other breast cancer subtypes.

Respondents and Methods: Women diagnosed with breast cancer responded to an 80-question online survey to identify education, information, and support needs. Respondents self-reported their breast cancer subtype and rated the emotions they experienced at diagnosis, during, and after treatment on a scale of "none," "low," "moderate," and "high." The responses of 656 women with TNBC (25.1%) were compared to 1,954 non-TNBC women (74.9%). Differences between TNBC and non-TNBC women were assessed using logistic regression at each time point. Using generalized logistic modeling, differences in score changes were categorized as decreased, same, or increased in relation to cancer subtype and other covariates.

Results: At all time points in an unadjusted analysis, TNBC women reported more fear, anxiety, and worry than non-TNBC women, although this finding was only statistically significant for fear and anxiety at diagnosis (high fear: 67% vs. 62%, p=0.046, high anxiety 68% vs. 64%, p=0.046 respectively). Change in emotions between diagnosis and treatment phase was not significantly different between cancer types. Between treatment and post-treatment, women with TNBC were significantly less likely than non-TNBC patients to report a decrease in negative emotion (fear: 58% vs. 66%; anxiety: 54% vs. 65%; worry: 53% vs. 63%, p<0.001 for all). TNBC women with young children were less likely to report a decrease and more likely to report an increase in worry than non-TNBC women (decrease: 61% vs. 70%; increase 8% vs. 4%, p=0.09). A similar pattern was seen in TNBC women with income <$50K annually with respect to fear (decrease: 47.3% vs. 68%, increase 11% vs. 6%, p=0.06). Cancer stage was significantly associated with emotional change. Women with TNBC stage >=2 showed greater increases in negative emotion and lesser decreases in positive emotion than non-TNBC women with similarly staged cancers (p<0.001). Race/ethnicity, age, education, children, living situation, or use of a mental health professional did not influence this relationship.

Conclusion: Women with TNBC experience greater fear, anxiety, and worry than women with non-TNBC subtypes at all points from diagnosis though post-treatment. While women with all breast cancer subtypes report a reduction in negative emotion over time from treatment to post-treatment, this change is less profound in TNBC women and appears to be driven nearly entirely by concern about the disease. The marginal effect on change in fear with respect to income may reflect concerns about paying for care, and increased worry in women with small children may reflect concerns about prognosis. Most strikingly, cancer stage was the strongest modifier of emotional change: TNBC women at cancer stage >=2 showed the least decline in negative emotion compared to corresponding non-TNBC women. These data support the development of TNBC-specific interventions focused on these patients’ emotional needs during and after treatment.
Title: TransHERA: The cell cycle regulator p27 predicts benefit from trastuzumab treatment in HER2-positive early breast cancer patients treated within the HERA trial

Martin Filipits¹, Michael Gnant¹, Urania Dafni², Varvara Polydoropoulou², Margaret J Hills³, Brian Leyland-Jones⁴, Martine Piccart-Gebhart⁵ and Mitch Dowsett³. ¹Medical University of Vienna, Comprehensive Cancer Center, Austria; ²Frontier Science Foundation-Hellas, Greece; ³Royal Marsden Hospital, London, United Kingdom; ⁴Stanford University and Avera Cancer Institute and ⁵Jules Bordet Institute, Belgium.

Body: Purpose: Predictive biomarkers may help predicting adjuvant trastuzumab response and thus optimize the treatment of patients with HER2-positive breast cancer. The aim of the present study was to assess the prognostic/predictive value of various biomarkers involved in cell cycle regulation or proliferation.

Methods: Expression of p27, cyclin D1, TOP2a, and Ki67 was immunohistochemically determined in tissue micro arrays of specimens from 862 patients randomized to the trastuzumab (1 or 2 year; N=561) and observation (N=301) arms of the HERA trial. The primary endpoint of the analysis was disease-free survival (DFS). Biomarker expression status was determined as continuous variable or by pre-defined categories. The interaction terms between the four biomarkers and treatment were assessed in multivariate Cox proportional hazards regression models adjusted for variables of clinical interest. Associations were considered significant only if the false discovery rate (FDR) adjusted p-values remained significant.

Results: Baseline characteristics were well balanced between the two study arms. A total of 249 DFS events (28.9%) were observed in the TransHERA cohort, with an overall 8-year DFS of 70.5% (95% CI 67.2%-73.5%). None of the four biomarkers was significantly associated with DFS in the total study population. When biomarkers were categorized according to pre-defined cut-off levels, only p27 turned out to be highly predictive: Expression data for p27 were available in 753 TransHERA patients. A highly significant interaction was detected between p27 and treatment when adjusting for clinical parameters and the remaining three biomarkers (p=0.0039). For patients classified as p27 low (≤70% p27-positive tumor cells; N=318), a significant treatment effect was observed, with the hazard of a DFS event being greater for the observation group compared to patients treated with trastuzumab (HR_{Trast vs Obs}=0.43, 95% CI 0.29-0.64, p<0.001). In contrast, no statistically significant effect of trastuzumab treatment was detected in the p27 high group (N=435; HR_{Trast vs Obs}=0.97, 95% CI 0.66-1.44, p=0.89), indicating that p27 high patients derived little or no benefit from trastuzumab treatment. Cyclin D1, TOP2a, and Ki67 used as categorical variables were not predictive, while cyclin D1 used as continuous variable was predictive of adjuvant trastuzumab benefit.

Conclusion: HER2-positive early breast cancer patients with low p27 expression in their tumors appear to benefit from trastuzumab treatment, whereas patients with high p27 expression do not.

This study was funded by Roche.
Title: Trastuzumab interruption for treatment-induced cardiotoxicity in HER2 positive early breast cancer

Anthony F Yu1, Nandini U Yadav1, Betty Y Lung1, Anne A Eaton1, Howard T Thaler1, Clifford A Hudis1, Chau T Dang1 and Richard M Steingart1. 1Memorial Sloan Kettering Cancer Center, New York, NY.

Body: Background
Trastuzumab (H) improves outcomes among patients with HER2-positive breast cancer but is associated with a risk of treatment-induced cardiotoxicity (TIC), especially when administered after an anthracycline (A). H interruption is recommended for significant asymptomatic or symptomatic declines in left ventricular ejection fraction (LVEF). It is unclear how frequently TIC leads to H interruption, and the subsequent management is variable in clinical practice.

Methods
Patients (pts) with HER2-positive early breast cancer receiving adjuvant H with chemotherapy between January 2005 and October 2010 were studied (n=608). Tumor characteristics, chemotherapy regimen, cardiac risk factors, LVEF (at baseline and during treatment), and treatment interruption were obtained from the medical record. We evaluated the incidence, time of occurrence, management, and associated risk factors of H interruption due to TIC.

Results
Median age was 51 years (range 26-81); 488 (80%) pts had A prior to H administration. H was interrupted in 108 (18%) pts. Cumulative dose of H was lower among pts in the interrupted group (median 86 vs. 108 mg/kg, p < 0.0001). The most common reason for interruption was TIC (66 of 108 pts): 20 (30%) had symptomatic congestive heart failure and 46 (70%) had asymptomatic decline in LVEF. Of the 66 pts, 55 (83%) were referred to a cardiologist and 36 (55%) were prescribed a new cardiac medication. The mean LVEF at baseline, at time of cardiotoxicity diagnosis, and at follow-up after interruption of H was 63%, 45%, and 55%, respectively. Pts with H interruption for TIC were older (54 vs. 50 years, p=0.014) with lower LVEF before A (63 vs. 67%, p<0.0001) and before H therapy (62% vs. 67%, p<0.0001), compared to those with continuous H treatment. Thirty-three of 66 pts were re-treated with H, and 5 pts had a significant recurrent decline in LVEF.

Conclusion
At our institution, interruption of H therapy is common and most often due to TIC, from both A and H, with the majority of pts receiving A prior to H. Risk factors associated with H interruption were older age and lower LVEF prior to A and H administration. Cardiac dysfunction improved after interruption of treatment but did not fully recover to baseline. Strategies to prevent cardiotoxicity and minimize H treatment interruption should be investigated to prevent persistent LV dysfunction in affected pts.
**Title:** The Promher Study: An observational Italian study on HER2+ve, pT1a-b, pN0, M0 breast cancer (BC) patients (pts)

Stefania Gori, Monica Turazza, Simona Duranti, Elena Fiorio, Jennifer Foglietta, Marcella Gulisano, Ilaria Marcon, Marta Gubbiotti, Maria Giovanna Cavazzini, Simon Spazzapan, Valeria De Simone, Giancarlo Bisagni, Chiara Saggia, Luigi Cavanna, Emilio Bria, Laura Iezzi, Elisabetta Cretella, Patrizia Vici, Daniele Santini, Alessandra Fabi, Ornella Garrone, Antonella Ferro, Silvana Saracchini, Lucia Evangelisti, Sandro Barni, Lucia Mentuccia, Lucio Laudadio, Alessandro Inno, Gianluigi Lunardi, Francesca Coati, and Luca Boni.

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**Body:**

Background. The management of small (≤ 1 cm), node-negative, HER2+ve BC is controversial, since data from randomized clinical trials specifically addressing the benefit of adjuvant systemic treatment with or without Trastuzumab in this setting are still lacking. The aims of this retrospective study are to assess how pts are managed in routine clinical practice in Italy, whether clinical or biological features may influence the choice of adjuvant systemic therapy and if there is any difference in the outcome between treated and not treated pts.

Patients and methods. Data of 268 consecutive pts who underwent surgery from January 2007 to December 2012 for HER2+ve, pT1a-b pN0 BC, were collected from 25 Italian centres. Descriptive statistical analyses and multivariate logistic regression models were used, with the aim of investigating the relationship between the baseline clinical and biological features and the adjuvant treatment strategy.

Results. Pts characteristics were: median age 57, 69% postmenopausal status, 77% had conservative surgery, 32% pT1a, 68% pT1b, 48% G3, 66% ER+ve, 75% Ki67 ≥14%. Ninety percent of pts received adjuvant systemic therapy: 19% hormone therapy (HT) alone, 3% chemotherapy (CT) +/- HT, 64% Trastuzumab + CT +/- HT and 4% Trastuzumab + HT. At the multivariate analysis, the odds of being treated with adjuvant systemic therapy with or without Trastuzumab, resulted higher in presence of conservative surgery (p=0.002), pT1b (p<0.001) and positivity of hormone receptors status (p<0.001). Among the patients treated with adjuvant systemic therapy, the administration of Trastuzumab appeared to be more frequently associated with pT1b (p=0.010) and negative hormone receptors (p=0.004). After 37 months of median follow-up, local and/or distant recurrence were 4/29 (14%) for pts who did not receive any systemic treatment, 2/59 (4%) for pts receiving systemic treatment without Trastuzumab and 2/180 (1%) for pts receiving Trastuzumab.

Conclusion. This preliminary analysis shows that in Italy the majority of these pts received systemic adjuvant treatment and about 2/3 were treated with Trastuzumab. Pathological tumor size (pT1b) and negative hormone receptor status represent the main factors influencing the choice of including Trastuzumab in the adjuvant treatment. Survival data are still not mature to drive definitive conclusions about outcome.
Title: Trastuzumab can be safely administered concurrently with anthracycline for adjuvant treatment of HER2-positive breast cancer

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Body: Background Anthracycline and trastuzumab are the preferential choices in the treatment of HER2-positive breast cancer. Due to the unacceptably high rates of cardiotoxicity observed in the metastatic breast cancer, concurrent administration of anthracycline and trastuzumab (A+H) is contraindicated. However, in the neoadjuvant setting, A+H has shown high rates of pathological complete response and very low cardiotoxicity. So far, all the large adjuvant trials have only evaluated the sequential strategy of administration anthracycline and trastuzumab (A-H), whereas the safety and efficacy of A+H has never been evaluated prospectively. Thus, we conducted a prospective study to evaluate the cardiac safety and efficacy of A+H regimen in the adjuvant treatment of HER2-positive breast cancer.

Methods This is a prospective, randomized and controlled trial. Participants, with HER2-positive, operable breast cancer, but without previous neoadjuvant treatment, were randomized to receive adjuvant A+H or A-H. If anthracycline was administered alone or sequentially to taxane, the dose of doxorubicin and epirubicin was 60mg/m² and 90-100mg/m², respectively. If anthracycline was given concurrently with taxane, the dose of doxorubicin and epirubicin was 50mg/m² and 75mg/m², respectively. Trastuzumab was given every 3 weeks (loading dose of 8mg/kg, followed by 6mg/kg) for one year. Left ventricular ejection fraction (LVEF) was monitored by echocardiogram (ECHO) at baseline (before chemotherapy) and 3, 6, 9, 12 and 24 months after the initial dose of trastuzumab. The primary endpoint was cardiac safety. The second endpoints were disease-free survival (DFS) and overall survival. ClinicalTrials.gov ID: NCT01413828.

Results Between August 2011 and March 2014, 196 HER2-positive breast cancer patients (98 in the A+H group and 98 in the A-H group) were enrolled and randomized. Women in the two groups had similar baseline characteristics including age, tumor stage, hormonal receptor status, chemotherapy regimen, radiation therapy and endocrine therapy. Trastuzumab was well-tolerated in both groups and the primary cardiac event was asymptomatic decrease in LVEF. In the A+H group, there were 11 (11.2%) patients showed more than 10% but less than 20% reduction in LVEF (NCI-CTC Grade I). In the A-H group, there were 14 (14.3%) patients showed NCI-CTC Grade I LVEF reduction and 1 (1.0%) patient showed more than 20% reduction in LVEF (NCI-CTC Grade II). There was no case of congestive heart failure. The difference of the rates of cardiac events between the two groups was not significant (P=0.400). The mean LVEF of the baseline and 3, 6, 9, 12 and 24 months after initial dose of trastuzumab also showed no difference between the two groups. Patients in both groups had excellent disease control at a median follow up of 16 months. There were numerically more DFS events in the A-H group (6/98, 6.1%) than the A+H group (10/98, 10.2%), but the difference of DFS did not reach the statistical significance (P=0.485). There was no death in both groups.

Conclusions Trastuzumab administered concurrently with anthracycline is a safe adjuvant regimen and might improve the survival of patients with HER2-positive breast cancer.
Title: Randomized Phase III trial of afatinib plus vinorelbine versus trastuzumab plus vinorelbine in patients with HER2-overexpressing metastatic breast cancer who had progressed on one prior trastuzumab treatment: LUX-Breast 1

Nadia Harbeck1, Chiun-Sheng Huang2, Sara Hurvitz3, Dah-Cherng Yeh4, Zhimin Shao5, Seock-Ah Im6, Kyung Hae Jung7, Kunwei Shen8, Jungsil Ro9, Jacek Jassem10, Qingyuan Zhang11, Young-Hyuck Im12, Marek Wojtkiewicz13, Qiang Sun14, Shin-Cheh Chen15, Rainer-Georg Goeldner16, Annick Lahogue17, Martina Uttenreuther-Fischer16, Binghe Xu18, Martine Piccart-Gebhart19 and on behalf of the LUX-Breast 1 Study Group. 1Brustzentrum Frauenklinik der Universität München, Munich, Germany; 2National Taiwan University Hospital, Taipei, Taiwan; 3David Geffen School of Medicine at UCLA/Translational Research in Oncology, Los Angeles; 4Taichung Veterans General Hospital, Taichung, Taiwan; 5Fudan University Shanghai Cancer Center, Shanghai, China; 6Seoul National University Hospital, Seoul, Korea; 7Asan Medical Center, University of Ulsan College of Medicine, Seoul, Korea; 8Comprehensive Breast Health Center, Ruijin Hospital, Shanghai, China; 9National Cancer Center, Goyang, Korea; 10Medical University of Gdansk, Gdansk, Poland; 11Third Affiliated Hospital of Harbin Medical University, Heilongjiang, China; 12Samsung Medical Center, Seoul, Korea; 13Comprehensive Cancer Centre, Medical University, Bialystok, Poland; 14Peking Union Medical College Hospital, Beijing, China; 15Chang Gung Medical Foundation-Linkou Branch, Taoyuan County, Taiwan; 16Boehringer Ingelheim Pharma GmbH & Co. KG, Biberach and der Riss, Germany; 17SCS Boehringer-Ingelheim Comm.V, Brussels, Belgium; 18Cancer Institute and Hospital, Chinese Academy of Medical Sciences, Beijing, China; 19Institut Jules Bordet, Brussels, Belgium and 20none.

Body: Background: Afatinib is an oral, irreversible ErbB family blocker with anti-tumour activity in patients (pts) with HER2-positive metastatic breast cancer (MBC) after failure on trastuzumab.1 Preclinically, afatinib + vinorelbine (AV) showed an additive effect; clinically, the AV combination had a manageable safety profile and showed activity in two Phase I trials.2,3 This randomized, open-label, Phase III trial (LUX-Breast 1) compared AV with trastuzumab + vinorelbine (TV) in pts with HER2-positive MBC who had progressed on a prior T-based regimen.

Methods: Pts with HER2-positive MBC and failure of one T-based regimen (adjuvant/first-line) were randomized 2:1 to AV (40 mg/day oral + 25 mg/m²/week iv) or TV (2 mg/kg/week iv after 4 mg/kg loading dose + 25 mg/m²/week iv). Treatment continued until progressive disease (PD) or unacceptable adverse events (AEs). The primary endpoint was progression-free survival (PFS) by investigator review; secondary endpoints included objective response rate (ORR), overall survival (OS) and safety. Planned accrual was 780 pts.

Results

Between August 2010 and April 2013, 508 patients were randomized (AV:339, TV:169). Baseline characteristics were balanced in both arms (mean age 52 yrs, Asian 50.6%, White 41.6%, ER/PR positive 28.7%). 41.1% of pts failed on prior adjuvant and 58.9% on 1st line T-based treatment. A pre-planned risk/benefit assessment was found unfavorable by the DMC and recruitment was stopped. Pts ongoing on AV therapy were switched to TV, received A or V monotherapy, or stopped treatment. Primary endpoint analysis was performed with 307 of the originally 484 planned PFS events (211 [62.2%] AV arm; 96 [56.8%] TV arm). Median PFS was 5.5 months with AV vs 5.6 months with TV (HR 1.10; 95% CI 0.86, 1.41; P=0.4272). ORR was 46.1% with AV and 47.0% with TV (OR 1.04; 95% CI 0.71, 1.51; P=0.8510). OS analysis was based on 144 (28.4%) OS events (108 [31.9%] in AV arm; 36 [21.3%] in TV arm). Median OS was 19.6 months with AV and 28.6 months with TV (HR 1.76; 95% CI 1.20, 2.59; P=0.0036).

The most common drug-related AEs were diarrhea (80.1%), neutropenia (75.1%) and rash (45.1%) with AV, and neutropenia (78.7%), leukopenia (37.3%) and anemia (27.8%) with TV. Rate of infections (53.0% vs 40.5%) was higher with AV vs TV. More AV than TV pts discontinued due to AEs (15.4% vs 7.1%). Fatal AEs were reported for 18 (5.3%) in the AV vs 5 (3.0%) pts in the TV arm, and were mainly associated with PD (9 pts in AV and 1 in TV arm). Three AV pts died due to treatment-related causes (sepsis/multi-organ failure; septic shock; pulmonary fibrosis).

Conclusions: AV and TV demonstrated similar PFS and ORR, but OS diverged and was shorter for AV compared to TV in pts with HER2-positive MBC. The safety profile of AV was consistent with the individual monotherapies, but its tolerability compared unfavorably to TV. Analyses are ongoing to elucidate potential factors (e.g. impact of follow up treatments) contributing to the diverging PFS and OS outcomes.
2. Bahleda R et al. J Clin Oncol 2011;29; abs 2585
Body: Background:
Despite progress, a large number of breast cancer patients experience metastatic relapse and death. Hyperactivation of the mTOR pathway has been observed in patients (pts) with BC progressing on endocrine therapy. The BOLERO-2* trial demonstrated significant doubling of PFS obtained via dual blockade with everolimus (EVE) and exemestane (EXE), versus EXE alone in pts refractory to NSAIs.

Methods:
BALLET is a European multi-center open-label, single-arm, expanded-access study to evaluate the safety of EVE (10 mg/day) and EXE (25 mg/day) in postmenopausal women with hormone receptor-positive HER2 negative locally advanced or metastatic BC progressing on prior NSAIs. Study treatment continued until disease progression, unacceptable toxicity, death, drug locally reimbursed, discontinuation from the study for any other reason or last patient last visit (June 30th, 2014), whichever occurred first. Here we report an ad hoc-analysis that includes all pts recruited from May 12th 2012 till Dec 31st 2013 with cut-off date March 17th 2014 (final database will be available on October 31st 2014).

Results:
2,133 pts were recruited in 269 sites across 14 European countries. Baseline characteristics were median age: 64 yrs; PS (ECOG) 0/1/2: 64%/30%/3%; median time from first diagnosis: 8 yrs; Stage IV pts: 99%. At the data cut off, a total of 1795 pts (84%) had discontinued the treatment. Reasons for discontinuation were: disease progression (38%), drug reimbursement (35%), adverse events (15 %), consent withdrawn (4%), death (1.5%) and others (6.5%). EVE and EXE were administered as first line treatment in 10% of pts, as second line in 23%, as third line in 22% and as fourth line or beyond in 45% of pts. 74% of pts received more than 1 line of chemotherapy in the metastatic setting. 80% of pts experienced at least one adverse event (AE) referred by the investigators as related to EVE [45% stomatitis, 7% non-infectious pneumonitis (NIP)]. The most frequent grade 3-4 drug related AEs were stomatitis (8.9%), asthenia (3.2%), GGT increase (2.4%), hyperglycemia (2.4%), and NIP (1.8%).The median time to onset of stomatitis and NIP was 3-4 weeks and 2-3 months respectively.

Conclusions:
These results confirm that the combination of EVE + EXE is a tolerable treatment in a real world setting even in pts more heavily pretreated by chemotherapy compared to BOLERO 2. The better understanding of side effects leading to treatment discontinuation in this large European study where investigators frequently administered the drug for the first time, will allow defining priority actions for better management of side effects including patient education and early interventions. Longer follow up with mature data (expected in October 2014) will give additional information on the safety profile, stratified by line of treatment.

A phase I/II study of neratinib plus temsirolimus in HER2+ metastatic breast cancer reveals ongoing HER2 pathway dependence in many patients despite several lines of HER2 targeted therapy

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Targeting a single node in the HER2/PI3K pathway has been associated with benefit but frequently results in acquired resistance in the metastatic setting. Direct inhibition of HER2 even with combinations may be limited by mutational activation of the downstream PI3K pathway. Conversely, inhibition downstream results in feedback upregulation of receptor tyrosine kinase signaling which can diminish efficacy. We hypothesize that dual targeting of HER2 and mTOR will be tolerable while overcoming these limitations. We conducted a phase I/II trial examining the tolerability and efficacy of temsirolimus (T), an mTOR inhibitor, and neratinib (N), a HER1/2 kinase inhibitor, in patients with HER2+ metastatic breast cancer (MBC). Tumor biopsies were obtained to ascertain if PI3K pathway activation is frequent and to identify biomarkers of response.

Methods: The phase I study utilized a 3+3 dose escalation design to determine the maximum tolerated dose (MTD) of T (IV weekly) with N (fixed dose 240 mg oral daily) in patients with HER2+ trastuzumab-refractory MBC. Loperamide prophylaxis (4 mg daily) was initiated at Day 1 and then left to patient/physician discretion. The phase II study utilized a Simon two-stage design to assess the overall response rate by RECIST in HER2+ trastuzumab-refractory MBC. An expansion cohort has been subsequently initiated to investigate the benefit of more aggressive loperamide prophylaxis (16 mg daily) and temsirolimus dose escalation. All patients on the initial Ph I/II study underwent biopsy of metastatic disease for biomarker assessment. Activating mutations in the PI3K pathway were assayed using the Sequenom MassARRAY system or as part of Next Generation Sequencing of panel of ~250 cancer related genes along with PTEN immunohistochemistry.

Results: Eight patients enrolled in the phase I trial and the MTD was determined to be T at 8 mg IV weekly with neratinib at 240 mg daily with grade 3 diarrhea as the dose limiting toxicity. The phase II trial enrolled 34 patients. Seventy percent of patients on the Ph I/II studies had progression of disease on prior lapatinib, pertuzumab, or T-DM1. The most frequent treatment-related grade 2/3 events at the MTD (n=40) were diarrhea (gr 2 35%/gr 3 25%), mucositis (23%/10%), and leukopenia (28%/5%). Thirty-five patients treated at the MTD are evaluable for response; 13 patients had PR (9 cPRs) and 2 patients had SD ≥6 months. Most of the 15 responders had prior T-DM1 (6), pertuzumab (2), or lapatinib (8). Molecular analyses of pretreatment biopsies is ongoing and thus far has revealed PI3K pathway activation (PIK3CA or AKT mutation or PTEN low) in >70% of the analyzed tumors (24/33). Responses were frequent in this cohort but not in a group of tumors that had lost HER2 overexpression.

Conclusions: Temsirolimus and neratinib has clinical activity in the setting of HER2+ MBC with mutational activation of PI3K pathway and among patients exposed to multiple prior HER2 targeted agents. Final results of the efficacy, safety, and biomarker data will be presented along with preliminary data from the ongoing expansion study.
**Title:** Combination vorinostat and lapatinib reverses epithelial-mesenchymal transition, inhibits the cancer stem cell population of HER2+ breast cancer cells and is effective in heavily pretreated advanced tumors

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**Body:** Although strides have been made in the treatment of breast cancer, patients often relapse due to recurrence or metastasis. The targeting of these cellular processes has become essential in the quest for a cancer cure. Previously, we have demonstrated the efficacy of histone deacetylase inhibitors (HDACi) in inducing differentiation and inhibiting metastasis in triple negative breast cancer cells. In addition, we have shown that HER2 regulates the cancer stem cell (CSC) population in AI resistant cells. Based on these previous results, we hypothesize that the combination of both HDACi and HER2 inhibition may be synergistic and further reduce CSC. In this study, we treated a panel of HER2+ breast cancer cell lines with the combination of 1µM vorinostat (HDACi) and 1µM lapatinib (a dual EGFR/HER2 inhibitor) for 72 hours and examined its effects on cell number, mesenchymal and epithelial markers, migratory potential, and cancer stem cell characteristics. The combination reduced cell number when compared to either agent alone (p<0.01 compared to vehicle). Cells treated with the combination exhibited a more epithelial morphology when compared to vehicle treated cells. Furthermore, when compared to vehicle treatment, the combination downregulated the expression of mesenchymal proteins: Twist (p<0.001), Snail (n.s.), and Vimentin (p<0.001) and concurrently upregulated epithelial proteins: cytokeratin 18 (p<0.001) and E-cadherin (n.s.), suggesting that the combination may reverse EMT (epithelial-mesenchymal transition). The combination was also able to inhibit the migratory potential of cells as measured using xCELLigence. In addition to its effects on EMT and migration, the combination also significantly reduced markers of the CSC population. Cellular markers of CSC, including mammary stem cell markers CD49f (p<0.01), CD24lo/CD44hi (p<0.01), and aldehyde dehydrogenase activity (p<0.001) were decreased following treatment with the combination. The self-renewal capability of cells was also affected by the combination, as evidenced by decreased expression of pluripotency proteins BMI-1 (p<0.05) and β-catenin (n.s.), as well as reduction of both primary (p<0.05) and secondary (p<0.05) mammosphere formation. We further conducted a phase I/II clinical trial of vorinostat in combination with lapatinib. There were 12 patients enrolled (9 in phase I and 3 in phase II). The treatment was fairly well tolerated with no DLT observed in phase I. PK analysis showed no evidence of drug-drug interaction. Among 8 patients with HER2-positive (HER2+) breast cancer, the clinical benefit rate (CR, PR, and SD) was 37.5% (1 PR, 2 SD). Intriguingly, we observed that none of the HER2+ breast cancer patients developed metastasis at a new site during the treatment. Their disease progression was due to the enlargement of previously existing lesions. Taken together, these results suggest that the combination of vorinostat and lapatinib may target both metastasis and CSC and that the combination is well tolerated and effective in patients with advanced HER2+ disease.
Title: 4EVER - Final efficacy analysis of the phase IIIb, multi-center, open label study for postmenopausal women with estrogen receptor positive locally advanced or metastatic breast cancer (BC) treated with everolimus (EVE) in combination with exemestane (EXE)

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Body: Introduction:
The phase III BOLERO-2 trial showed a significant doubling of PFS benefit with EVE + EXE over EXE alone in postmenopausal women with hormone receptor positive advanced BC progressing after non-steroidal aromatase inhibitor (NSAI) therapy. The 4EVER trial further evaluated the combination of EVE+EXE with regard to efficacy and safety, quality of life and health resources utilization in a broader patient population, i.e. without limitations as to the number of previous chemotherapy lines, the time point of progression after NSAI therapy, and the previous EXE therapy.

Methods:
From May 2012 to November 2012 a total of 299 postmenopausal women with metastatic or locally advanced, hormone receptor positive, HER2 negative breast cancer, refractory to NSAI were recruited to this phase IIIb study. Here we report the results of the planned analysis of the primary and secondary endpoints. The primary endpoint was the overall response rate (ORR) at week 24. The secondary endpoints included: Progression-free survival (PFS), ORR at week 48, overall survival (OS), and quality of life. This study includes a broad exploratory translational research program e.g. changes in serum bone turnover biomarkers, the correlation of Interleukin-6 with anxiety and depression, presence and molecular characteristics of circulating tumor cells, the correlation of response to EXE+EVE with pharmacogenomics.

Results:
Trial database lock will occur in late June 2014, therefore, the final data concerning the primary and secondary efficacy and safety endpoints will be presented at SACBS 2014.

The preliminary baseline analysis included 299 patients (data cut off 15 Nov 2013):
HR status: ER+/PgR+ 78.1%, ER+/PgR- 20.9%, 0.7% ER-/PgR+, 0.3% ER-/PgR-. Tissue for receptor status analysis: 71.0% primary tumor, 29.0% metastasis. The mean time since initial diagnosis was 9.6 years, the mean time since first relapse/metastasis was 4.3 years. The mean time since last relapse/metastasis was 2.8 months. 68.1% of patients had bone lesions. Last anti-neoplastic therapy had been administered in the adjuvant (23.9%) and metastatic setting (73.0%). 25.9% of patients had no prior antineoplastic therapy in the metastatic setting, 16.3% had one, 12.2% two and 47.4% three or more prior therapies.

Conclusion:
The final analysis of the 4EVER study provides more important information on disease patterns and benefits of the combined treatment with EVE and EXE.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P5-19-07  
**Average Grade:** 6.00

**Title:** Randomized Phase II trial of afatinib alone or with vinorelbine versus investigator’s choice of treatment in patients with HER2-positive breast cancer with progressive brain metastases after trastuzumab and/or lapatinib-based therapy: LUX-Breast 3


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**Body:**

**Background:** Over one-third of patients (pts) with HER2-positive (+) advanced breast cancer (BC) develop brain metastases (BM), which often leads to short survival. Afatinib (A), an irreversible ErbB family blocker, demonstrated activity in pts with heavily pretreated, HER2+ metastatic BC (MBC) progressing after trastuzumab (T) therapy, with partial responses (PR) in 10% and clinical benefit in 46% of pts (Lin 2012a). We evaluated the activity of A alone or in combination with vinorelbine (V), versus investigator’s choice of therapy for MBC (IC), in pts with HER2+ BC with BM after prior T and/or lapatinib (L) therapy.

**Methods:** Eligible pts had at least one measurable and progressive lesion in the central nervous system (CNS; ≥10 mm on magnetic resonance imaging) after prior systemic and/or radiation therapy. Pts were randomized to receive A (40 mg/day oral), AV (40 mg/day oral + 25 mg/m²/week i.v.) or IC in 3-week cycles. Stratification factors were: ECOG performance status (PS, 0–1 vs 2), number of BM (≤3 vs >3) and prior exposure to L (yes/no). The primary endpoint was pt benefit at 12 weeks (i.e. absence of CNS and extra-CNS disease progression per RECIST 1.1, and no tumor-related worsening of neurological signs/symptoms or increase in steroid dosage). Secondary endpoints included progression-free survival (PFS), overall survival (OS), objective response rate (ORR) in CNS/extra-CNS lesions, and safety.

**Results:** 121 pts were randomized (2 were not treated): median age, 53 years; ECOG PS 0–1, 83%; >3 BM, 59%; prior L therapy, 78.5%. The IC treatment consisted of: T+ chemotherapy (CT) (22 pts); T+L+CT (3 pts); L+CT (10 pts); L alone (1 pt); or CT alone (6 pts). Results for efficacy endpoints are shown (Table).

<table>
<thead>
<tr>
<th></th>
<th>A (n=40)</th>
<th>AV¹ (n=38)</th>
<th>IC¹ (n=43)</th>
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<tr>
<td><strong>Pt benefit rates at 12 weeks, n (%)</strong></td>
<td></td>
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<tr>
<td>Median PFS, weeks</td>
<td>11.9</td>
<td>12.3</td>
<td>18.4</td>
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<tr>
<td>Median OS, weeks</td>
<td>57.7</td>
<td>37.3</td>
<td>52.1</td>
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<tr>
<td><strong>ORR (PR), n (%)</strong></td>
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<tr>
<td>CNS lesions</td>
<td>0</td>
<td>3 (8)</td>
<td>6 (14)</td>
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<tr>
<td>Extra CNS lesions</td>
<td>0</td>
<td>3 (8)</td>
<td>2 (5)</td>
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<tr>
<td><strong>Disease control, n (%)</strong></td>
<td></td>
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<tr>
<td>CNS lesions</td>
<td>27(68)</td>
<td>27 (71)</td>
<td>31 (72)</td>
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<tr>
<td>Non CNS lesions</td>
<td>17 (43)</td>
<td>19 (50)</td>
<td>26 (61)</td>
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</tbody>
</table>

¹One pt randomized but not treated

In the treated set (n=119), the most frequent treatment-related adverse events (AEs) in the A and AV arms were diarrhea (90% and 84%) and rash (38% and 54%); neutropenia (51%) was also common in the AV arm. Diarrhea (33%), neutropenia (21%) and...
asthenia (21%) were the most frequent related AEs in the IC arm. Grade (G) 3/4 treatment-related AEs were observed in 50%/3% (A), 57%/24% (AV; G4 AEs were mainly neutropenias) and 14%/7% (IC) of pts; there were no treatment-related G5 events. Conclusions: Approximately one third of pts with HER2+ MBC benefited from the assigned treatments and two thirds had CNS lesions controlled per RECIST in each group. Objective response in CNS was infrequent (0 to 14%) with all treatments. Overall, AEs were manageable in this heavily pretreated pt population.

Title: IMMU-132, a potential new antibody-drug conjugate (ADC) for the treatment of triple-negative breast cancer (TNBC): Preclinical and initial clinical results

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Body: Despite major advances in breast cancer treatment, TNBC, which comprises approximately 15% of all breast cancer cases, continues to have a poor prognosis, with an increased risk of recurrence and mortality. IMMU-132 is a new ADC targeting Trop-2, an antigen found in high prevalence in many epithelial cancers, including TNBC, and conjugated to SN-38, a topoisomerase inhibitor and active metabolite of irinotecan, at a high drug:antibody ratio (7.6:1). Studies in mice bearing human pancreatic tumor xenografts (Capan-1) have shown IMMU-132 remains intact in the serum until internalized within the tumor cell where SN-38 is released (pH dependent), resulting in selective cancer cell death. IMMU-132 was found to deliver >120-fold higher amount of SN-38 to xenografted tumors than irinotecan. In animals bearing established MDA-MB-468 TNBC xenografts, IMMU-132 at well-tolerated doses improved responses (more robust regression and delayed progression), compared to irinotecan at a maximum therapeutic dose regimen or to an irrelevant antibody conjugate control. Animals that progressed after receiving the control responded to IMMU-132 therapy, even after those tumors had more than doubled from their initial size. These encouraging pre-clinical data led to the development of the ongoing phase I/II clinical trial in patients (pts) with diverse relapsed/refractory epithelial cancers, including TNBC (NCT01631552). In dose escalation (8 to 18 mg/kg given on days 1 and 8 of a 3-week treatment regimen), dosing was limited primarily by neutropenia, with IMMU-132 being tolerated best for multiple cycles at 8 to 10 mg/kg. Since most (26/30) archival tumors from pts with TNBC expressed Trop-2 (30% ≥2+) and many cancer cell lines express abundant (i.e., >100,000) copies of Trop-2, no enrichment strategies for TNBC were employed to treat TNBC pts with IMMU-132. As of June 1, 2014, 10 pts with TNBC have completed their first response assessment by CT, with 6 having disease shrinkage as their best response; 2 pts had a partial response (-32 and -51% shrinkage of target lesions, with time to progression of 18+ and 30 wks, respectively), and 4 had stable disease (-3, -14 and -19 and -27% shrinkage for 18+, 14, 45, and 24 wks, respectively), according to RECIST. For all pts with diverse cancers treated to date at the phase II dose levels of 8 and 10 mg/kg, 6/25 (24%) had G3 neutropenia; G4 febrile neutropenia occurred in 1 pt. Alopecia, diarrhea, fatigue, nausea, and vomiting are the more common related non-hematological toxicities, but most occur at Grade ≤2. There has been no evidence of immunogenicity to the antibody or the drug, even over multiple cycles of treatment. These promising results in pts who failed multiple prior therapies (median, 4; range, 1-8) suggest IMMU-132 is a safe and potentially effective drug for pts with TNBC, justifying continued evaluation of IMMU-132 in less refractory pts and also in combination with other agents.
Title: Stage 1 results from MDV3100-11: A 2-stage study of enzalutamide (ENZA), an androgen receptor (AR) inhibitor, in advanced AR+ triple-negative breast cancer (TNBC)

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Body: Background
TNBC is a heterogeneous disease with many subtypes that share one commonality; "triple-negative" breast tumors lack sufficient expression of a target (ER, PgR, HER2) associated with a therapeutic agent. AR expression occurs in a subset of TNBC and could identify patients (pts) that respond to AR inhibition. Preclinically, AR+ TNBC cell lines grow in response to AR stimulation and this growth is inhibited by ENZA. A phase 2 clinical study investigating bicalutamide in AR+ TNBC demonstrated a 19% clinical benefit rate at 24 weeks (CBR24) with no objective responses in 24 pts. ENZA is a potent AR inhibitor that improves overall survival in men with metastatic castration-resistant prostate cancer and is being evaluated in pts with advanced AR+ TNBC.

Methods
MDV3100-11 is an open-label, Simon 2-stage study evaluating ENZA (160 mg daily) in pts whose breast cancer expresses AR (>0% by IHC) but not ER, PgR or HER2 amplification (NCT01889238). There was no limit to prior therapies; bone-only non-measurable disease was allowed. Pts with CNS metastases or seizure history were excluded. Tissue was required; prescreening for AR was allowed. The primary endpoint is CBR at 16 weeks (CBR16), defined as complete response (CR), partial response (PR), or stable disease (SD) ≥16 weeks per RECIST 1.1 in Evaluable pts. Evaluable pts were prespecified as those with tumors expressing ≥10% AR by central review and who had assessment for response. ITT analyses include all pts. Secondary endpoints include CBR24, response rates, safety and tolerability. The analysis plan specified progression to Stage 2 if CBR16 is ≥3 of 26 Evaluable pts in Stage 1, and the null hypothesis (true CBR16=8%) is rejected if overall CBR16 is ≥9 in 62 Evaluable pts.

Results
Complete data on all Stage 1 pts (N=42) are reported herein; 16 were not evaluable (10 had AR <10%, 6 had AR ≥10% but no response assessment). In the 26 Evaluable pts, median age was 62.5 years, 77% had measurable disease, 69% had ≥3 sites of metastases, 62% had visceral involvement, and 42% received ENZA in ≥3rd line. CBR16 was 42% (11 of 26; 95%CI 24, 62) and CBR24 was 35% (9 of 26; 95%CI 18, 54), with 1 PR and 1 CR. Related adverse events (AEs) of any grade ≥10% in the ITT were fatigue (29%), nausea (26%), decreased appetite (19%), diarrhea (14%), hot flush (12%) and vomiting (10%). Fatigue (7%) was the only Grade ≥3 related AE in ≥5%. Go to Stage 2 criteria were met, and enrollment is complete at 118. As of Sept 2014, 13 additional pts had clinical benefit at week 16 (including 3 PRs); data continue to mature.

Conclusion
The 42% CBR16 observed in Stage 1 alone was sufficiently high to reject the null hypothesis for the whole study. Data beyond Stage 1 are not mature; however, responses continue to be observed. AEs from ENZA in women with TNBC were generally mild and consistent with other studies of ENZA. These encouraging results suggest ENZA may provide meaningful benefit to pts with AR+ TNBC. Ongoing IHC and genomic analyses on 400 collected tissue samples will further inform how best to identify pts most likely to derive benefit from ENZA.
Tolerability and anti-tumor activity of the oral PI3K inhibitor GDC-0941 in combination with paclitaxel, with and without bevacizumab or trastuzumab in patients with locally recurrent or metastatic breast cancer

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Body: Background:
GDC-0941 is a potent and selective oral inhibitor of class I PI3K kinases that demonstrates broad activity in diverse xenograft cancer models and increases the anti-tumor activity of taxanes, associated with increased apoptotic cell death, in multiple BC xenografts.

Methods:
In the Phase Ib dose-escalation study, GDC4629g, GDC-0941 was administered in combination with paclitaxel dosed at 90 mg/m2 intravenous [IV] weekly for 3 out of 4 weeks, bevacizumab at 10 mg/kg IV biweekly, and trastuzumab 2−4 mg/kg IV weekly in 28-day cycles. The study was conducted in 2 parts: Part 1 evaluated oral doses of 60 and 100 mg GDC-0941 on a "21+7" (21 days on/7 days off) dosing schedule in combination with paclitaxel with and without bevacizumab. Part 2 evaluated GDC-0941 on a "5+2" schedule (5 days on/2 days off in a repeating pattern), at doses of 165 - 330 mg, in combination with paclitaxel (Arm A), paclitaxel plus bevacizumab (Arm B) or paclitaxel plus trastuzumab (Arm C). The primary objective was to evaluate safety, tolerability, and pharmacokinetics (PK), and to determine the maximum tolerated dose (MTD) for each combination. Anti-tumor activity was evaluated by RECIST v1.0 and available archival tumor samples were analyzed to assess PI3K related biomarkers.

Results:
63 patients were enrolled: 20 in Part 1 and 43 in Part 2 (18 in Arm A, 16 in Arm B, and 9 in Arm C). The most frequent grade ≥3 drug-related adverse events (AEs) were: Part 1, neutropenia (45%), peripheral neuropathy and nail disorder (15% each); Part 2, Arm A, neutropenia (38%), pneumonia and rash (11% each); Arm B, neutropenia and rash (25% each); Arm C, rash and hypertension (11% each). The most common AEs assessed as being related to GDC-0941 were as follows:

<table>
<thead>
<tr>
<th>Adverse Event</th>
<th>All Grades</th>
<th>Grade ≥ 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DIARRHEA</td>
<td>45 (71.4%)</td>
<td>2 (3.2%)</td>
</tr>
<tr>
<td>NAUSEA</td>
<td>33 (52.4%)</td>
<td>0</td>
</tr>
<tr>
<td>NEUTROPENIA</td>
<td>29 (46.0%)</td>
<td>17 (27.0%)</td>
</tr>
<tr>
<td>RASH</td>
<td>29 (46.0%)</td>
<td>7 (11.1%)</td>
</tr>
<tr>
<td>FATIGUE</td>
<td>28 (44.4%)</td>
<td>0</td>
</tr>
<tr>
<td>STOMATITIS</td>
<td>19 (30.2%)</td>
<td>0</td>
</tr>
<tr>
<td>DECREASED APPETITE</td>
<td>15 (23.8%)</td>
<td>1 (1.6%)</td>
</tr>
<tr>
<td>VOMITING</td>
<td>15 (23.8%)</td>
<td>0</td>
</tr>
</tbody>
</table>

In Part 1, the MTD was not reached, the maximum administered dose (MAD) of GDC-0941 was 100 mg, a dose-limiting AE was subclavian vein thrombosis. In Part 2, Arm A, the MTD was 250 mg, Grade 3 febrile neutropenia, bacteremia, and rash were
DLTs. In Arm B and Arm C, the MAD was 260 mg, Grade 3 rash was a DLT in both arms. No differences in the PK of either GDC 0941 or paclitaxel were observed when administered in combination compared to historical single-agent data. Best tumor response by RECIST was as follows: Part 1, 1 (5%) complete (CR) and 4 (21%) partial responses (PR); Arm A, 1 (6%) CR and 3 (17%) PR; Arm B, 7 (47%) PR; Arm C, 1 (11%) PR.

Conclusions:
GDC-0941 was generally well-tolerated in combination with paclitaxel with or without bevacizumab or trastuzumab at dose ranges of up to 330 mg at the “5+2” dosing schedule. Anti-tumor activity has been observed in combination with paclitaxel with and without bevacizumab or trastuzumab. Updated biomarker data and associations with clinical outcomes will be presented.
Title: Noninterventional study HELENA – Advanced (metastatic or locally recurrent, inoperable) HER2-positive breast cancer: First-Line trEatmeNt with pertuzumAb after adjuvant trastuzumab therapy

Marc Thill1, Katja Ziegler-Löhr2, Harald Wagner3, Gertrud Helling-Giese4, Jasmin Greinemann5, Otto Schmalhofer6 and Dietmar Reichert7.

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Body: Background: The CLEOPATRA trial (808 patients) demonstrated a significant benefit for overall survival (HR=0.66; 95% CI 0.52–0.84; p<0.0008) and progression free survival (HR=0.62; 95% CI 0.51–0.75; p<0.0001) in the first line treatment of patients with HER2-positive metastatic breast cancer (MBC) receiving pertuzumab in addition to trastuzumab plus docetaxel. An exploratory subgroup analysis was performed for patients who had received prior neoadjuvant and/or adjuvant trastuzumab therapy (88 patients). The observed hazard ratios of 0.68 (95% CI 0.30–1.55) and 0.62 (95% CI 0.35–1.07; 16.9 months) indicate a benefit in overall and progression free survival for this subpopulation.

Methods & Aims: HELENA is a multicentre, noninterventional study recruiting patients with advanced HER2-positive breast cancer, who relapsed after receiving trastuzumab in the adjuvant setting. All patients receive first line treatment with pertuzumab in combination with trastuzumab and docetaxel in clinical routine in Germany according to local SmPC. The main objective of HELENA is to confirm the progression free survival of 16.9 months observed in the CLEOPATRA trial in this specific patient population in a real world setting.

Parameters of interest:

- Time-based efficacy parameters of pertuzumab in combination with trastuzumab and docetaxel, in particular PFS in female patients with prior trastuzumab treatment in the adjuvant setting and in clinically relevant subgroups (hormone receptor-positive and -negative, visceral and non-visceral metastatic disease, patients < 65 years and ≥ 65 years as well as < 75 years and ≥ 75 years)
- Interval between the last documented dose of the adjuvant trastuzumab treatment and the onset of pertuzumab first-line treatment
- Effective treatment duration, reason for treatment discontinuations and treatment modifications
- Demographic characteristics and medical history of the patient
- Evaluation of potential prognostic variables: ECOG performance status, co-morbidity, tumor stage, type of histological classification, histologic grading, location of metastases, primary surgery method, residual tumor burden
- Extension and localization of metastasis or locally recurrent, inoperable tumor before onset of pertuzumab first-line treatment
- Safety of pertuzumab: Incidence, management and outcome of (S)AEs and pregnancies
- Outcome of (neo)adjuvant (DFS) trastuzumab therapy

Recruitment: HELENA started recruitment in June 2013 and will enroll 478 patients in 150 sites, being suitable in number, type and geographic distribution for providing a representative picture of first-line treatment of advanced (metastatic or locally recurrent, inoperable) HER2-positive breast cancer in Germany.

Results: First interim results of HELENA will be presented at the SABCS 2014 meeting.
Title: Subgroup analysis on efficacy in the routine treatment - Results of the 2nd interim analysis of BRAWO, the non-interventional trial “Breast Cancer Treatment with Everolimus and Exemestane for HR+ Women”

Body: Introduction
BRAWO is a German non-interventional study of 3000 patients (pts) with advanced or metastatic, hormone-receptor-positive, HER2-negative breast cancer treated with everolimus (EVE) and exemestane (EXE). We report results of the 2nd preplanned interim analysis (IA) with a data cut-off on 8th July 2014.

Methods
BRAWO collects data on the routine clinical treatment with EVE and EXE at about 400 sites. Main objectives are to extend the knowledge on a) the efficacy in the clinical routine and the impact of physical activity on efficacy and quality of life, b) prophylaxis and management of stomatitis, and c) the sequence of therapy in the clinical routine. The 2nd IA was defined to take place 12 months after the inclusion of the 500. patient into the documentation.

Results
Efficacy data will be reported for the first 500 documented pts. Apart from data on the total study population we will present the PFS results for subgroups including pts with or without prior EXE therapy, with or without prior chemotherapy for the advanced setting, with or without visceral metastasis and with regard to the line of treatment in the advanced setting and the extent of physical activity. The respective summary of adverse events for these patients will be presented as well as results on treatment compliance and dosing. Baseline characteristics and medical history as well as insights into treatment sequences before EVE and EXE will further be shown for all enrolled patients with valid baseline documentation (approx. 1200).

Conclusion
This subgroup analysis will provide insights into the treatment efficacy in the clinical routine and will add to a more comprehensive understanding of the treatment with EVE/EXE.
2014 San Antonio Breast Cancer Symposium

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Average Grade: 4.50

Title: Clinical activity of abemaciclib, an oral cell cycle inhibitor, in metastatic breast cancer

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Body: Background: Abemaciclib, a small molecule inhibitor with selectivity against cyclin-dependent kinases 4 and 6 (CDK4/6), induces G1 arrest in Rb-proficient human breast cancers. In an early phase clinical trial, the safety and antitumor activity of abemaciclib (LY2835219) were evaluated in 2 cohorts of patients with metastatic breast cancer (mBC). One cohort evaluated single-agent abemaciclib in an unselected population of patients with mBC [Part D], while the combination of abemaciclib plus fulvestrant was evaluated in patients with hormone receptor positive (HR+) mBC [Part G]. We previously reported early results for these 2 cohorts of patients with mBC treated with either single-agent abemaciclib or the combination of abemaciclib plus fulvestrant (Patnaik et al, ASCO 2014). In the single-agent cohort, 47 patients with previously treated mBC were enrolled (36 HR+). All patients with >30% tumor reduction had HR+ mBC (13 of 36 patients). In this group of 13 patients with HR+ mBC, 9 patients had confirmed response for an objective response rate of 25%, and 4 patients had unconfirmed response. This study was ongoing with 14 of 36 HR+ mBC patients on treatment at time of analysis (range 238-471 days). Patients continuing on single-agent abemaciclib included 4 patients with unconfirmed response and 6 patients with confirmed response. For the combination of abemaciclib plus fulvestrant, 18 patients with HR+ mBC enrolled and 13 patients (72%) were still on treatment (range 31-143 days) at the time of analysis.

Methods: In the single-agent cohort, patients with mBC were treated with abemaciclib at 150 or 200mg orally every 12 hours on a continuous schedule. In the combination cohort, patients with HR+ mBC (n=18) were treated with the combination of abemaciclib plus fulvestrant. Patients received abemaciclib at 200mg orally every 12 hours on a continuous schedule. Patients also received fulvestrant at 500mg intramuscularly every month. NCI CTCAE v4.0 was used to grade adverse events (AEs) and RECIST v1.1 was used to assess tumor response.

Results: In the single-agent cohort, patients began enrolling in May 2012 with the last patient enrolled in March 2013. Patients had a median of 7 prior systemic therapies and 81% of patients had ≥2 metastatic sites. In the combination cohort, patients began enrolling in September 2013 with the last patient enrolled in January 2014. Patients in the combination cohort had a median of 4 prior systemic therapies and 67% of patients had ≥2 metastatic sites. An updated analysis will be presented for objective response rate, duration of treatment and clinical benefit rate and will include an additional 6 months of information for both the single-agent and combination cohorts. New analyses will include time to response, duration of response, change in tumor size over time, and characteristics of responders. In addition, safety data will include longer term follow-up through approximately September 2014.

Conclusions: Abemaciclib is an oral cell cycle inhibitor that demonstrates single-agent activity against mBC, especially for HR+ disease. Based on its safety and efficacy profile, abemaciclib warrants further clinical investigation in confirmatory studies, both as a single agent and in combination with endocrine therapy.
Title: A phase 1b study of trebananib plus paclitaxel and trastuzumab or capecitabine and lapatinib in patients with HER2+ locally recurrent or metastatic breast cancer

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Body: Background: The angiopoietin axis is distinct and critical for angiogenesis. Trebananib suppresses tumor angiogenesis by binding to angiopoietin-1 and -2 (Ang1/2), thereby inhibiting their interaction with the Tie2 receptor. This interim analysis evaluated the tolerability and efficacy of trebananib plus paclitaxel and trastuzumab or capecitabine and lapatinib in HER2+ locally recurrent or metastatic breast cancer (MBC).

Methods: Patients (pts) in cohorts A1 and A3 (no prior 1st-line trastuzumab, lapatinib, or chemotherapy for MBC) received trebananib (A1, 10 mg/kg; A3, 30 mg/kg) IV QW plus paclitaxel 80 mg/m\textsuperscript{2} IV QW and trastuzumab 8 mg/kg loading dose, then 6 mg/kg Q3W. Pts in cohorts B1 and B3 (history of failed 1st-line MBC treatment, no prior lapatinib or capecitabine) received trebananib (B1, 10 mg/kg; B3, 30 mg/kg) IV QW plus capecitabine 1000 mg/m\textsuperscript{2} PO Q12 h, days 1-14 Q21D and lapatinib 1250 mg PO QD. Cohorts were expanded to n = 20 if \( \leq \)1 of 6 or \( \leq \)2 of 9 pts had dose-limiting toxicities (DLTs). Cohort B3 was closed early due to poor enrollment. Endpoints were treatment-emergent adverse events (AEs) and DLTs (primary); and efficacy, pharmacokinetics (PK), and incidence of anti-trebananib antibodies (secondary).

Results: All pts received \( \geq \)1 dose of trebananib. Across all cohorts, two DLTs occurred (A1: grade 3 transient ischemic attack, n = 1; A3: grade 3 increased gamma-glutamyltransferase, n = 1). Across A1 and A3, AEs \( \geq \)50% were peripheral edema, diarrhea, alopecia, fatigue, nausea, nail disorder, and rash; AEs grade \( \geq \)3 in \( \geq \)10% of pts were peripheral/sensory neuropathy and dyspnea. No pt in A1 or A3 died during treatment. Across B1 and B3, AEs \( \geq \)50% were diarrhea, nausea, palmar-plantar erythrodysesthesia syndrome (PPES), and peripheral edema; AEs grade \( \geq \)3 in \( \geq \)10% of pts were diarrhea, PPES, and neutropenia. One pt in B1 died from sepsis considered to be possibly related to administration of trebananib and capecitabine. Peripheral edema was a frequently reported AE for cohorts A1, A3, B1, and B3 (n = 13, 15, 10, 3); most were grade 1 and 2 and appeared to be manageable. No AEs of gastrointestinal perforation, ascites, or proteinuria were reported. Efficacy endpoints are summarized in Table 1. No apparent PK drug-drug interaction was observed. No pt developed neutralizing binding antibodies during treatment.

Table 1

<table>
<thead>
<tr>
<th>TUMOR RESPONSE\textsuperscript{a}</th>
<th>1ST LINE-TREATMENT</th>
<th>( \geq )2ND-LINE TREATMENT\textsuperscript{b}</th>
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<tr>
<td></td>
<td>A1 (n = 20)</td>
<td>A3 (n = 20)</td>
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<tr>
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<td>17</td>
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<tr>
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<td>Objective response rate, %</td>
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### PROGRESSION-FREE SURVIVAL

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### DURATION OF RESPONSE

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<th>Evaluable pts, n</th>
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<td>8.2–not estimable</td>
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<td></td>
<td>7</td>
<td>8.3</td>
<td>4.1–16.8</td>
</tr>
</tbody>
</table>

*Tumor response was assessed only in pts with measurable disease at baseline †Efficacy results for B3 are not reported due to the small sample size (n = 5)

**Conclusion:** Interim results from this phase 1b study of pts with HER2+ locally recurrent or MBC suggest that adding trebananib to paclitaxel and trastuzumab or capecitabine and lapatinib is tolerable and may improve antitumor activity.
Title: Prescribing preferences of US-based medical oncology physicians for patients with hormone receptor positive, HER2 negative metastatic breast cancer following prior endocrine therapies

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Body: Background: Non-steroidal aromatase inhibitors (NSAI) are a standard first-line therapy (Rx) for post-menopausal pts with hormone receptor positive (HR+), HER2 negative (HER2-) advanced and recurrent metastatic breast cancer (mBC). When studied in phase III clinical trials, combination hormonal Rx regimens have not consistently produced significant improvements in time to event measures compared to sequential single agents. However recent phase II and III studies combining hormonal and targeted agents have shown significant extensions in PFS and potential increases in OS. Due to the emergence of several targeted agents, standards of care for treatment after failure on a NSAI are evolving.

Methods: Prescribing preferences (PPrefs) of 187 U.S.-based medical oncology physicians (MOPs) were studied using a validated, proprietary, live, case-based market research tool (Challenging Cases®). Data were acquired using blinded, audience-response iPad technology and acquisition events took place in March and May 2014. A core case was constructed and PPrefs for three 1st, three 2nd, and one 3rd failure scenario were assessed. Core case: 60-year-old female; History of mild hypertension well-controlled by medication; diagnosed with stage 2B HR+, HER2-, grade 3, invasive ductal carcinoma; Primary Rx included lumpectomy+ sentinel node biopsy/axillary dissection, chemotherapy (CT) and radiotherapy (RT) followed by anastrozole with plans for 5 years (yrs) of Rx. Question posed: What systemic Rx would you recommend now?

Results:

Figure 1: PPrefs for Rx for first recurrent disease during or after 5 yrs of adjuvant anastrozole

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Endocrine Rx</th>
<th>Targeted Strategy</th>
<th>CT</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to Recurrence(REC) Met sites</td>
<td>Another NSAI, Fulvestrant (F), Exemestane (EXE)</td>
<td>EXE + Everolimus (EVE)</td>
<td>Single agent(SA)/ combo</td>
<td>Other/ Clinical Trial (CLT)/Continue NSAI and add F</td>
</tr>
<tr>
<td>S1a, N=187</td>
<td>18 mths while on NSAI Non-Visceral (N-VIS) and Visceral (VIS)</td>
<td>10%</td>
<td>28%</td>
<td>6%</td>
</tr>
<tr>
<td>S1b, N=97</td>
<td>18 mths while on NSAI N-VIS, Unproven VIS</td>
<td>7%</td>
<td>46%</td>
<td>5%</td>
</tr>
<tr>
<td>S2, N=187</td>
<td>2 yrs post 5 yrs NSAI N-VIS</td>
<td>25%</td>
<td>35%</td>
<td>18%</td>
</tr>
</tbody>
</table>

Figure 2: PPrefs for next Rx following adjuvant anastrozole and then letrozole (LET) at first failure.

<table>
<thead>
<tr>
<th>Scenario</th>
<th>Endocrine Rx</th>
<th>TS</th>
<th>CT</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time to REC after TX with LET Met sites</td>
<td>F</td>
<td>EXE</td>
<td>EXE + EVE</td>
<td>CLT/RT to rib + no Rx change, Continue current NSAI + add F</td>
</tr>
<tr>
<td></td>
<td>Time to REC on F</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>-----------------</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>S3</td>
<td>N=187</td>
<td>5 mths N-VIS</td>
<td>42%</td>
<td>6%</td>
</tr>
<tr>
<td>S4</td>
<td>N=186</td>
<td>12 mths N-VIS</td>
<td>46%</td>
<td>6%</td>
</tr>
<tr>
<td>S5</td>
<td>N=94</td>
<td>18 mths N-VIS</td>
<td>65%</td>
<td>5%</td>
</tr>
<tr>
<td>S6</td>
<td>N= 186</td>
<td>6 mths N-VIS, VIS</td>
<td>N/O</td>
<td>3%</td>
</tr>
</tbody>
</table>

N/O - not offered

**Conclusion:**
MOPs PPrefs for management of pts with ER+, HER2- mBC are dictated by time to REC, number of RECs, and presence/absence of VIS disease. EXE and EVE has substantial traction in all scenarios studied. Since these PPref data were acquired, OS findings from the BOLERO-2 trial of EXE alone or EXE + EVE have been presented. PPrefs using these case scenarios will be studied at 2 additional events prior to SABCS, allowing more robust data and insights into the early impact of the BOLERO-2 survival data on PPrefs to be available at the meeting.
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Average Grade: 4.50

Title: Effect of palbociclib concentration on heart rate-corrected QT interval in patients with cancer

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Body: Background: Palbociclib is a selective cyclin-dependent kinase 4/6 inhibitor that blocks G1/S cell cycle progression. The current population analysis assessed the effect of palbociclib exposure on QT interval and heart rate (via evaluation of RR interval) in cancer patients.

Methods: Plasma palbociclib concentration (C) and electrocardiogram (ECG) data were pooled from 3 studies of once-daily oral palbociclib: (Study 1) phase 1 dose-escalation study in advanced cancer (Schedule 1: 25, 50, 75, 100, 125, and 150 mg on a 3 wk on/1 wk off cycle [3/1]; Schedule 2: 100, 125, 200, and 225 mg [2/1]); (Study 2) phase 2 study in mantle cell lymphoma (125 mg [3/1]); (Study 3) phase 1/2 study in advanced breast cancer (Cycle 1: 125 mg [2/1]; Cycle 2+: 125 mg [3/1] + letrozole 2.5 mg daily). Blood samples were collected predose, after first palbociclib dose, and at steady state, including the anticipated time of maximal palbociclib concentration. Triplicate (~2 min apart) ECGs were obtained at baseline; 3 h postdose (Study 1); predose on Day (D) 1 and D21 (Study 2); predose on D1 and predose, 2, 4, 8, 24, 48, and 96 h postdose on D14 of Cycle 1, predose and 4 h postdose on D1 and D14 of Cycle 2, and end of treatment (Study 3, phase 1); and predose on D1 of Cycle 1&3, D14 of Cycle 1&2, and end of treatment (Study 3, phase 2). Baseline singlet ECG data (from time closest to first palbociclib dose) were used to estimate a study-specific heart rate correction factor [β], using a linear mixed effect model of log(QT) vs log(RR/1000) with intersubject variability only on intercept. The averaged triplicate ECG data with time-matched palbociclib concentration data were used to explore RR–C and corrected QT interval (QTc)–C relationships. A linear mixed effects model was used to assess RR–C and QTc–C with intersubject variability on both intercept and slope; sex was tested as a covariate on QTc interval intercept.

Results: 184 patients supplied 569 matched pharmacokinetic and ECG assessments. Estimated β values for Studies 1, 2, and 3 were 0.367, 0.369, and 0.363, respectively. Compared with QT correction by Fridericia (QTcF) and Bazett (QTcB) methods, the study-specific correction (QTcS) best minimized the apparent QT–RR correlation and therefore was selected for use in subsequent analyses. Palbociclib had no effect on RR interval in RR–C analysis but increased QTcS in a concentration-dependent manner. The average (90% CI) QTcS increase at the mean and median maximum steady-state concentrations for patients on palbociclib 125 mg (C\text{max,ss} = 107 and 112 ng/mL, respectively [Study 3]) were 5.60 (2.48–8.72) and 5.88 (2.61–9.16) ms. Sex was not a significant covariate for intercept by analysis of variance. A similar effect of palbociclib on QTcF was observed.

Conclusion: In a pooled population analysis of patients with cancer, palbociclib had no concentration-dependent effect on heart rate. There was a slight positive linear relationship between palbociclib concentration and QTcS; however, the upper bound of the 1-sided 90% CI for the increase in QTcS at C\text{max,ss} did not exceed the threshold of 10 ms. Therefore, QT prolongation is not a major safety concern for palbociclib at the 125-ng/mL recommended therapeutic dose.
Title: Final results of the phase I "HIT" study: A multicenter phase I-II study evaluating trastuzumab administered by intrathecal injection for leptomeningeal meningitis of HER2+ metastatic breast cancer (MBC)

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Body: Metastatic breast cancer (MBC) is the leading non hematologic cause of leptomeningeal metastases (LM), associated with a poor prognosis. Amongst breast cancer (BC) subtypes, HER2+ tumors present a high LM incidence. Intravenous (IV) trastuzumab (T) has improved survival of MBC patients but better control of central nervous system (CNS) disease is needed. The main physiopathological hypothesis for intracranial recurrences is the low level of T in cerebrospinal fluid (CSF) due to his high molecular weight (148kD). Intraventricular (via Ommaya port) or intrathecal (IT) T administration would permit LM progression control through high therapeutic T concentrations in the CSF. This prospective trial, sponsored by Institut Curie, was the first Phase I-II study to investigate the safety and efficacy of IT T administration for HER2+ LM in BC. The final results of the Phase I cohort are reported herein. Methods: LM diagnosis was based on either CSF with HER2+ cytology or clinical/MRI imagings of meningitis. Adequate organs functions for all patients were required. The T IT (or via Ommaya port) injections were given on weekly basis during 8 weeks. We used a Fibonacci dose escalation design, 4 dose levels (DL) (30-150mg). The objectives of this phase I were to investigate the maximum tolerated dose (MTD) and the recommend dose (DR) of T. A pharmacokinetic (PK) data analysis was performed. We targeted a concentration in the CSF close to the residual serum concentration (30µg/mL) achieved with standard IV schedule.

Results: Starting May 2011, 19 patients were included, with 16 evaluable for toxicity (three had not received the treatment) treated in 4 DL (30-150mg). Twelve patients were evaluable for PK and 210 samples T concentrations available (103 in the LCR and 107 in the serum). The MTD was not reached and the treatment appears to have been well tolerated by the patients. Neither Grade ≥3 toxicity nor neurological toxicity related to IT T was observed. The T target-concentration in the CSF was reached at DL4 and the recommended T dose for the Phase II trial is 150 mg. Five patients have experimented a evident clinical benefit and received more than 8 weekly injections with an average of 23 [12-40]. Extended data on clinical outcome and PK will be presented. Conclusion: Intraventricular or IT injections appears to be safe at the dose of 150 mg and possibly effective. The Phase II study will investigate the efficacy of the recommended dose on neurological progression-free survival at 2 months in 19 patients. This trial was performed with Roche funding.
Title: A phase II study of Foretinib for triple negative metastatic breast cancer: NCIC CTG IND 197

Daniel Rayson¹, Sasha Lupichuk⁵, Ted A Vandenberg³, Dhesy-Thind Sukbinder⁶, Susan Ellard⁷, Shailendra Verma⁴, Stephen Chia⁸, Catherine Prady⁹, Susan Dent³, Kylea R Potvin³, Muhammad Salim¹⁰, Tamara N Shenkier⁸, Hao Xu², Allison Allan⁴, Ming Tsao¹², Ghassan Allo¹², Linda Hagerman³, Elizabeth Eisenhauer⁶ and Penny Bradbury². ¹Queen Elizabeth II Health Sciences Centre, Halifax, NS, Canada; ²NCIC Clinical Trials Group, Queen’s University, Kingston, ON, Canada; ³London Regional Cancer Centre, London, ON, Canada; ⁴Ottawa Hospital Research Institute, Ottawa, ON, Canada; ⁵Tom Baker Cancer Centre, Calgary, AB, Canada; ⁶Juravinski Cancer Centre, Hamilton, ON, Canada; ⁷British Columbia Cancer Agency, Southern Interior, Kelowna, BC, Canada; ⁸British Columbia Cancer Agency, Vancouver Cancer Centre, Vancouver, BC, Canada; ⁹Hopital Charles LeMoyné, Greenfield Park, QC, Canada; ¹⁰Allan Blair Cancer Centre, Regina, SK, Canada; ¹¹Kingston General Hospital, Kingston, ON, Canada and ¹²University Health Network/Princess Margaret Hospital, Toronto, ON, Canada.

Body: Background
Foretinib is an oral, multikinase inhibitor (RTK) inhibitor targeting MET, RON, AXL, TIE-2, VEGFR and VEGF. Antitumor activity has been demonstrated in papillary renal cell carcinoma and is thought to be due to dual effects on tumour cell endothelium and survival via simultaneous inhibition of MET and VEGFR2/KDR. Triple negative breast cancer (TNBC) remains a disease subtype with a paucity of targeted therapeutic options and has been demonstrated to preferentially overexpress MET compared to other breast cancer subtypes. MET expression in archival tumor specimens has been correlated with poor outcomes in metastatic TNBC. We conducted a multi-centered phase II trial of foretinib for locally recurrent or metastatic, incurable, TNBC.

Patients and Methods
Foretinib 60 mg po daily was administered on a continuous schedule in 4 week treatment cycles. All patients had to have available archival primary tumor specimens for central confirmation of triple negative status and correlative studies. We employed a multi-nomial 2-stage design with 29 unselected patients entered on stage 1 and stage 2 proceeding if ≤17 early progressions were observed. Stage 2 accrued 18 further patients all undergoing fresh biopsy of an accessible metastatic site and having circulating tumor cells (CTC) collected. Patients had to have measurable disease with an ECOG PS of 0-2 and could have received adjuvant and 1 prior line of palliative chemotherapy. The protocol-defined threshold for drug activity was ≥5 responses or ≤17 early progressions in the response-evaluable population.

Results
Forty seven patients (pts) were registered with 45 evaluable for safety (at least 1 dose of foretinib). Forty-one pts were eligible with centrally confirmed TNBC with 37 being evaluable for response. Partial responses (PR) were observed in 2 pts (5.4%) with a median duration of 4.4 months (m). Fifteen pts (40.5%) experienced stable disease (SD) as best response with a median duration of 5.4 months (range 2.3-9.7 m). Overall PR + SD rate was 46%. Most common grade 3 non-hematological toxicities at least possibly related to study drug included hypertension (49%) and diarrhea (6.7%). Two cases of grade 3 nausea, fatigue, dyspnea and thromboembolism and single cases of grade 3 heart failure, QTc prolongation, anorexia, dehydration, proteinuria and pleural effusion were observed. Four pts went off study due to adverse events (8.9%). Correlative work examining archival primary tumor specimens for MET, PTEN and EGFR expression, as well as assessment of second stage CTC and fresh tumor biopsies, is ongoing.

Conclusions
Although the primary endpoint of this trial was not met, foretinib 60 mg po daily on a continuous dosing schedule elicited encouraging clinical benefit (PR + SD = 46%) amongst a MET unselected TNBC patient population with an acceptable and manageable toxicity profile. Our data suggests that foretinib merits further investigation in a concurrent or sequential treatment strategy for metastatic TNBC. Ongoing correlative work may identify predictive molecular markers for benefit.
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Average Grade: 6.25

Title: Impact of adding palbociclib to letrozole on pain severity and pain interference with various activities of daily life in patients with ER+, HER2- metastatic breast cancer as first line treatment

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Body: Background
Palbociclib, a selective inhibitor of CDK-4/6, prevents DNA synthesis by blocking cell cycle progression. A randomized Phase 2 study of palbociclib (P) 125 mg QD for 3 weeks followed by 1 week off plus letrozole (L) 2.5 mg QD continuously compared to L alone was conducted. A secondary objective of the study was to assess the impact of adding P in combination with L compared to L alone on pain severity and pain interference with various activities of daily life.

Methods
This Phase 2 trial was designed to evaluate P+L in front-line ER+/HER2- metastatic breast cancer (MBC) compared to L alone. The primary endpoint was investigator assessed progression-free survival (PFS) defined as time from randomization to objective progression or death. Patient reported outcomes of pain severity (PS) and pain interference (PI) with various activities of daily life were assessed using the Brief Pain Inventory (BPI) at baseline and day 1 of each cycle thereafter. The PS and PI scores were summarized by cycle using observed values as well as changes from baseline.

Results
The final analysis of primary endpoint showed a statistically significant improvement in PFS for the P+L arm (20.2 months) vs. L arm (10.2 months) with hazard ratio (HR)=0.488 (95% CI: 0.319, 0.748) and 1-sided p=0.0004. The most common adverse events in the P+L arm were neutropenia, leukopenia, fatigue, and anemia.

For the PS scale, patients in the P+L arm showed numerically lower scores and greater reductions from baseline than the L arm, until the later cycles, when the number of patients had decreased to a small number. The difference between treatment arms in the mean change from baseline was statistically significant at some of the earlier cycles (Cycles 5, 6, 7, 8, 10, 12; p<0.05; no adjustments were made for multiplicity) representing a numerically greater decrease in the pain experienced by patients in the P+L arm compared with the L arm.

For the PI score, observed mean scores for both treatment arms appeared to be stable over time until the later cycles, when the number of patients had decreased to a small number. Patients in the P+L treatment arm also generally showed a consistently greater numeric reduction from baseline in pain interference (ie, a decrease in PI on daily activities) until later cycles although without reaching statistical significance at any cycle.

Conclusions
The addition of palbociclib to letrozole was associated with increased efficacy without negatively impacting pain severity or pain interference on daily activities.
Title: A phase I study of MK-2206 in combination with lapatinib in patients with advanced solid tumors followed by dose-expansion in advanced HER2+ breast cancer

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Body: Background: AKT mediates signaling in the human epidermal growth factor receptor-2 (HER2) pathway. HER2 inhibition can result in feedback regulation of signaling, leading to high AKT activity. Preclinical studies demonstrate activity of combined HER2 and AKT inhibition. MK-2206 is an oral selective inhibitor of AKT. This study was designed to determine the maximum tolerated dose (MTD), dose limiting toxicities (DLTs), adverse events (AEs), clinical activity, pharmacokinetic (PK) parameters and explore potential biomarkers of the combination of MK-2206 with lapatinib.

Methods: The dose escalation cohort included adult patients (pts) with solid tumors treated with MK-2206 (30-60 mg qod) and lapatinib (1000-1500 mg qd) continuously. Cycles were 28 days, except cycle 1 (35 days), due to a 1 week MK-2206 lead-in to evaluate for PK interactions. MK-2206 plasma concentrations were evaluated on day 1, 9 (steady state) and 15 (steady state in combination with lapatinib). Lapatinib plasma concentrations were evaluated on day 9 (first dose) and day 15 (steady state in combination with MK-2206). Both used a validated LC-MSMS assay. Pharmacokinetic parameters were calculated using non-compartmental methods with WinNonLin Phoenix version. The dose expansion cohort included women with advanced HER2+ breast cancer treated at the MTD. Peripheral blood mononuclear cells (PBMCs) and archived tumor samples were collected for all pts.

Results: In the dose escalation cohort, 23 pts (median age 59 [range 22-72]; 15 female:8 male) were enrolled. Cancers were colorectal (8 pts), lung (4 pts), breast (3 pts) and other (8 pts). 19 evaluable pts were treated a median of 8 weeks (range 3-35). At dose level four, 1 pt had grade 4 hyponatremia, grade 3 rash and hypocalcemia and 1 pt had intolerable grade 2 mucositis with delivery of <75% of drug. The MTD was 45mg po qod of MK-2206 with 1500 mg po qd of lapatinib. The most common AEs at least possibly related to therapy included diarrhea (grade 3-4 in 3 pts; grade 1-2 in 16 pts), nausea (grade 3 in 2 pts; grade 1-2 in 14 pts) and rash (grade 3 in 2 pts; grade 1-2 in 12 patients). PK analyses demonstrated lapatinib half-life was approximately 18 hours on both day 9 and 15. MK-2206 half-life was 65±119 hours on day 1, 104±84 on day 9 and 59.1±25 on day 15. Dose adjusted MK-2206 AUC (hr*ng/mL/mg) was 26.6±31 of day 1, 100.6±78 on day 9 and 62.8±58 on day 15. In the dose expansion cohort, five HER2+ women were enrolled (median age 43 yrs [range 33-56]). The majority were heavily pretreated: all had prior progression on trastuzumab and 2 with prior lapatinib. They were on study for a median of 8 weeks (range 4-24 weeks). One pt had stable disease for 6 months and another pt had clinical benefit with non-measurable skin disease and she remains on study. Exploration of PBMCs for evidence of target inhibition in HER2-PI3K-AKT signaling and tumor tissue for PTEN loss, PI3K mutations will be presented.

Conclusions: Continuous dosing of MK-2206 in combination with lapatinib is well-tolerated. Preliminary results of pharmacokinetic analysis indicate a potential for a drug interaction. Clinical benefit was seen in patients with HER2+ breast cancer.
Title: TRASTYVERE study: A retrospective analysis of HER2-positive metastatic breast cancer (MBC) patients treated in Spain with lapatinib (L) plus trastuzumab (T)

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Body: Background: Vertical dual blockade with L and T in heavily pretreated HER2+ MBC patients has shown consistent survival gain in a phase III trial (Blackwell KL et al. 2012), justifying an EMA approval for the hormone-negative subgroup. However, there is very limited information about the futility of the combination in clinical practice, mostly in patients progressing also on prior L regimens.

Methods: We conducted a retrospective analysis among patients treated in Spain by compassionate uses for the combination of T-L. The study was approved by the regulatory authorities and ethics committees from the 14 participating centers. Major inclusion criteria were (1) HER2+ MBC; (2) progression on at least one prior line of T for advanced disease; and (3) T-L treatment started between JAN/2005 and DEC/2012. Concomitant endocrine therapy for HR+ patients as well as prior exposure to L was allowed. Chemotherapy combinations were excluded. A total of 111 patients were predefined for the primary outcome: clinical benefit rate (CBR). Secondary endpoints included time to progression (TTP), overall survival (OS) and toxicity. 114 women were included and externally monitored.

Results: The median age was 60 years (34 - 89); 64% HR+; 77% visceral disease; 32% CNS disease (37 patients); 47% with ≥3 organs involved. Mean number of prior T lines 4 (range 0-13); 64% previously treated with L. A total of 40 patients (35%) achieved a CBR (95%CI 26–44%); 6 CR, 19 PR and 15 SD lasting >24 weeks. The median time to progression was 3.8 months (95%CI 3.3–5.1) and the median overall survival 21.6 months (95%CI 17.1–27.3). CBR, median TTP and median OS achieved in patients with CNS disease were 32.4% (95%CI 17.3–47.5%), 3.6 (95%CI 2.8–5.9) and 15.4 (95%CI 10.9–27.3) months, respectively.

The CBR was independent of L treatment (41.5% L naïve vs. 31.5% L pretreated, p=0.285) and HR status (39% HR- vs. 32.9% HR+, p=0.509). Patients with <3 metastatic sites showed higher CBR than patients with ≥3 (45 vs. 24.1%, respectively, p=0.019).

No significant trends were observed in any pre-specified condition for TTP and OS. Grade 3/4 toxicities were reported in 20 patients (17.5%). Only 2 patients report asymptomatic cardiac toxicities.

Conclusions: The combination of T-L seems safe and active in heavily pretreated patients. The combination remains active among patients progressing on prior L. Future research may focus on the ability of endocrine therapy to increase activity among HER2+/HR+ patients.

REFERENCES:
**Body:** 

**Background:** Our preclinical data show that entinostat enhances the efficacy of lapatinib in HER2 positive (HER2+) breast cancer cell lines via FOX-03-mediated Bim1 expression. In-vitro and in- vivo, the combination of entinostat and lapatinib enhanced apoptosis in lapatinib-resistant cells. We conducted a phase I study with the primary objective to determine the RP2D of entinostat plus lapatinib in patients with HER2+ metastatic breast cancer with progressive disease (PD) after trastuzumab treatment.

**Methods:** This was a single-center, open-label study to evaluate the dose limiting toxicity (DLT) and determine the MTD of every-other-week entinostat plus daily lapatinib in 28-day cycles. Patients with locally recurrent or metastatic breast cancer in whom trastuzumab had failed were enrolled. DLT was defined as: any toxicity resulting in 14 or more days of treatment delay, febrile neutropenia (NTP), grade 4 NTP for over 7 days, any grade 3 or higher non-hematologic toxicity, grade 3 nausea, vomiting, diarrhea or electrolyte imbalance lasting over 48 hours despite adequate supportive care, or a platelet count less than 10,000. Grading was assigned according to common toxicity criteria (CTC 4). A standard 3+3 dose escalation design was used. Entinostat was given at 10 mg (level 0), 12 mg (level 1), or 15 mg (level 2) by mouth every 14 days. Lapatinib was given at 1250 mg by mouth daily. Toxicity was evaluated on day 15 and day 28 for C1 and C2, and at the end of each cycle thereafter.

**Results:** Fifteen patients with HER2+ metastatic breast cancer were enrolled. Median age was 52 years (range 26-69), median of all prior systemic treatment was 4 (range 1-12), and median prior trastuzumab-based regimens was 2 (range 1-6). Eight patients had prior lapatinib exposure. Seven patients had ER+/HER2+ tumors. Median number of cycles completed was 2 (range 1-13). DLT was observed in 0 of 3 patients at level 0, 1 of 6 at level 1, and 1 of 6 at level 2. 3 patients at level 1 had lapatinib dose reductions because of grade 3 rash (n=2) or grade 3 dyspnea (n=1) or grade 3 abdominal pain (n=1). 2 patients at level 2 had entinostat dose reductions because of grade 4 NTP in cycle 1 (n=1) or grade 3 NTP in cycle 5 and grade 4 neutropenia in cycle 6 (n=1). Five patients had SD (defined per 2009 RECIST guidelines): two at dose level 1 for 6 months and 13 months, three at level 2 for 3, 4, and 8 months. Median time to progression was 2 months (range 1-13). The most common treatment related adverse events were fatigue (n=15), myalgia (n=14), nausea (n=14), diarrhea (n=13), anemia (n=11), and rash (n=11).

**Conclusion:** MTD was not reached. Cumulative toxicity of entinostat plus lapatinib was fairly well tolerated. The combination therapy suggests there was clinical activity in at-least 5 patients who had SD in this cohort of heavily pretreated trastuzumab-resistant MBC. Data from the EG104900 clinical trial showed that lapatinib plus trastuzumab significantly improved median overall survival compared with lapatinib alone. Therefore, this study was modified after 15 patients to add trastuzumab to the combination. We are currently conducting a phase 1b trial to determine the MTD of entinostat, trastuzumab and lapatinib.
**Title:** A phase I clinical trial of ganetespib (heat shock protein 90 inhibitor) in combination with paclitaxel and trastuzumab in human epidermal growth factor receptor-2 positive (HER2+) metastatic breast cancer

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**Body:** Introduction: Targeted therapies in HER2+ metastatic breast cancer (MBC) have significantly improved survival, however efficacy is limited by development of therapeutic resistance. HSP90 is a molecular chaperone involved in the stability and function of multiple signaling onco-proteins. HER2 is an acutely sensitive HSP90 client and HSP90 inhibition can overcome trastuzumab resistance. Ganetespib is a novel, synthetic HSP90 inhibitor with increased potency and tolerability compared with earlier agents. Our group has conducted a single agent ganetespib trial in unselected patients which showed anti-tumor activity in HER2+ and triple negative breast cancer. In addition, preclinical data suggests HSP90 inhibition is synergistic with taxanes with potential for significant clinical activity. Ganetespib has been combined with docetaxel in non-small cell lung cancer, it has not previously been combined with paclitaxel and trastuzumab. This study will define the maximum tolerated dose (MTD) and recommended phase II dose (RP2D) of ganetespib when given with paclitaxel and trastuzumab for patients with HER2+ MBC.

Methods: In this 3+3 phase I dose escalation study, patients with trastuzumab resistant HER2+ MBC receive trastuzumab (4mg/kg loading dose, then 2mg/kg) and paclitaxel weekly (80mg/m²) with ganetespib day 1, 8, 15 of 28 day cycle. Patients are required to have prior pertuzumab and T-DM1 (prior pertuzumab and T-DM1 are not mandated if heavily pretreated prior to their respective FDA approvals). Hormone receptor positive patients are required to have at least one prior line of endocrine therapy. The single agent dose limiting toxicity (DLT) of ganetespib is diarrhea and therefore patients receive prophylactic anti-motility agents. The anticipated MTD of ganetespib in this combination has been informed by experience with docetaxel and based on this only three dose levels of ganetespib are being explored 100mg/m², 150mg/m² and a third intermediate cohort of 125mg/m², if needed. Secondary endpoints include evaluation of effects of ganetespib on the pharmacokinetics of paclitaxel and preliminary assessment of efficacy of the combination (scans at 8 weeks and every 12 weeks thereafter, RECIST 1.1).

Results: The first dosing cohort has fully enrolled and there were no significant toxicities or DLTs reported. Median age was 48 years (range 39-49), median prior lines of chemotherapy were 4 (range 3-7) and included prior pertuzumab and T-DM1 in all 3 patients. 5 adverse events have been defined as possibly/probably related to ganetespib – grade 2 anemia and leukopenia, grade 1 diarrhea (2 patients), fatigue, and rash. Enrollment to the second and potentially final cohort is underway.

Conclusion: This study will define the RP2D of ganetespib in combination with paclitaxel and trastuzumab. Final safety, pharmacokinetic and preliminary response data for all patients will be presented. This combination, with a novel anti-HER2 agent, has encouraging potential for activity in HER2+ breast cancer which is refractory to other HER2 targeting agents.
Phase Ib/II study of LEE011 and BYL719 and letrozole in ER+, HER2– breast cancer: Safety, preliminary efficacy and molecular analysis

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Body: Background: Activation of the PI3K/AKT/mTOR and cyclin D–CDK4/6–INK4–Rb pathways, including through alteration of PIK3CA and CCND1, has been implicated in resistance to aromatase inhibitors. BYL719 (BYL), an α-isoform selective PI3K inhibitor, demonstrated clinical activity as a single agent and in combination with hormone therapy in pts with advanced HR+ breast cancer (BC), while the CDK4/6 inhibitor LEE011 (LEE) showed evidence of clinical activity as a single agent in pts with advanced solid tumors and in combination with letrozole (LET) in pts with heavily pretreated advanced ER+ BC. In ER+ BC models, the triple combination of LEE, BYL, and LET had enhanced activity vs each agent alone. A Ph Ib/II, 3-arm study is currently investigating the combination of LEE, BYL, and LET in pts with ER+ BC. Here we report on safety, preliminary efficacy, and molecular analysis from Arm (A)1 (LEE + LET) and A2 (BYL + LET) of the Ph Ib part of the study.

Methods: Postmenopausal pts with advanced ER+, HER2– BC receive daily oral LEE (3-wks-on/1-wk-off; A1) or BYL (continuous; A2), plus fixed, daily LET (2.5 mg, continuous) in 28-day cycles. Primary objective: determine the MTD and/or RP2D of each combination. A Bayesian Logistic Regression Model using the escalation with overdose control principle and real-time PK guide dose escalation. Secondary objectives: safety, PK, and preliminary efficacy. Potential biomarkers that are predictive of response are also being assessed by next-generation sequencing of tumor samples.

Results: As of March 28, 2014, 10 pts received 600 mg LEE plus LET (A1), and 7 pts 300 mg BYL plus LET (A2). At study entry, all pts had stage IV disease; number of prior endocrine regimens for advanced disease was: 0–1 (47%); 2–3 (35%); 4–5 (18%); 35% of pts had previously received PI3K/AKT/mTOR pathway inhibitors for advanced disease. Of the 15 pts evaluable for dose determination (10 in A1 and 5 in A2), 1 dose-limiting toxicity was observed (A1: Grade [G]4 neutropenia; data cut-off: May 15). Most common (all grade >30%) study drug-related AEs (all grade/G3–4) were: A1: neutropenia (90%/50%) and nausea (40%/0%); A2: hyperglycemia (57%/14%), decreased appetite, diarrhea, and nausea (43%/0% each). PK of LEE and BYL on Days 1 and 21, and LET on Day 1, are comparable with historic single-agent data. PK of LET at steady state is being evaluated. In A1, of 6 pts with known response, 1 pt had a PR, 2 pts had SD, 1 pt without measurable disease had NCRNPD, and 2 pts had PD. In A2, of 5 pts with known response, 2 pts had SD, and 3 pts had NCRNPD. Biomarker analysis showed that 2 pts in A2 with SD who are still on study (1 with 25% tumor shrinkage) had PIK3CA mutations.

Conclusion: LET plus LEE or BYL had an acceptable safety profile and preliminary clinical activity in pts with ER+/HER2– advanced BC. Dose escalation continues, and upon determination of the MTD/RP2D in A1 and A2, enrollment into A3 (LEE + BYL + LET) will begin to determine the MTD/RP2D. Genetic alterations in PIK3CA were observed in A2. Analysis of baseline aberrations in the cyclin D–CDK4/6–INK4–Rb pathway in A1 and A3 are ongoing and will be updated. The Ph II part of the trial will compare LET plus LEE or BYL with the triple combination.
Title: Multi-institutional retrospective analysis of clinical and pathological factors predicting resistance to lapatinib-based therapy in HER2 positive metastatic breast cancer (HER2+ MBC)

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Body: Background
The combination of the dual HER1/HER2 inhibitor lapatinib (L) and capecitabine (C) is a therapeutic option for patients (pts) with HER2+MBC whose disease progresses after treatment with the monoclonal antibody trastuzumab. At present time, no clinical or pathological factors except HER2 status are clearly recognized as predictors of the activity of LC. We conducted a retrospective analysis of pts with HER2-positive metastatic breast cancer receiving LC after trastuzumab failure to identify factors associated with resistance to LC.

Materials and methods
We collected clinical and pathological data from 151 pts with HER2+ MBC receiving LC after failing a prior trastuzumab-based treatment (either adjuvant or for metastatic disease) treated at 13 Italian Institutions between March 2007 and December 2013. Time to progression (TTP) and overall survival (OS), calculated by the Kaplan Meier (KM) method, were from LC treatment beginning to disease progression or to death in the absence of progression (TTP), and to the date of death or to the date of last follow-up (OS), respectively. LC resistance was defined as TTP from treatment initiation lower or equal to the median TTP for the overall population. KM curves were compared by the Log-rank test. Logistic regression analysis was used to study predictors of TTP below the median value for patients receiving LC. Analyses were performed using SPSS version 17.0 (SPSS Inc., Chicago, IL).

Results
At a median follow-up of 41 months (IQR 23-62), median TTP to LC therapy was 7 months (IQR 5.5-8.5) and median OS was 18 months (IQR 10-28). Fifteen pts were excluded because of short follow-up (i.e. on LC treatment and <7 months of fu). Of the remaining 136, a total of 74 pts with a PFS ≤ 7 months were defined LC-resistant (LC-R) and a total of 62 pts were defined LC-sensitive (LC-S). All clinical and pathological variables analyzed resulted evenly distributed between the two groups, except best tumor response (CR+PR) to LC, which was higher in patients with LC-S disease (72% vs 29%, p<0.001). Conversely, best tumor response in LC-R patients showed higher rates of PD (43% vs 2%, p<0.001). Median OS was 14 months (IC 95% 11.4-22.6) and 26 months (IC 95% 22.5-29.5) in LC-R and LC-S pts, respectively (p<0.001). Although we could not find independent predictors of LC-R, factors indicating failure of the first-line trastuzumab based therapy, as PD as best tumor response and short duration of first-line trastuzumab, were associated to LC-R.

Conclusions
A short time to progression during capecitabine and lapatinib (LC-R) is associated with reduced OS in patients failing prior trastuzumab based therapy for HER2+ MBC. Patients who had modest clinical benefit from previous trastuzumab-based therapy could experience LC-R indicating the possibility of primary resistance to anti HER2-treatment. For these patients, alternative targeting strategies are urgently needed.
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Title: Efficacy and tolerance of everolimus in 123 consecutive very advanced luminal breast cancer patients. A multicenter retrospective study

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Body: Background: The online publication in December 2011 of the Bolero-2 study (Baselga et al, NEJM) establishing the added value of everolimus (Eve) in endocrine resistant breast cancer (BC) patients (pts) has triggered its immediate use in current practice. We evaluated our practice 2 years (y) after the French marketing authorization (July 2012).

Patients and Methods: We retrospectively reviewed the medical charts of 123 consecutively treated pts in two Unicancer Institutions (Institut Curie & Institut Bergonié). All pts had luminal (ER positive, HER2 negative) BC. Median age at diagnosis was 48 y (29-78) and median delays to first met event and to everolimus therapy were 6.0 y (0-25) and 12.6 y (1.3-34.8), respectively. PS at inclusion was 0 (47.6%), 1 (50.8%) or 2 (1.6%). Pts had received a median number of 2 lines of chemotherapy (0-8) and of 2 lines of endocrine therapy (0-6) for metastatic disease. Visceral disease was present in 56% of the pts. Pts had either 1 (25.4%), 2 (32.2%) or 3+ (42.4%) involved sites.

Results: Eve based therapy. Initial dose of Eve was 10 mg (76.4%) or 5 mg (23.6%) and was combined with exemestane (79.9%), anastrozole/letrozole (14.0%) or tamoxifen (6.1%). GnRH agonists were used in 10 premenopausal women. Dose adjustments of Eve were necessary in 41.7% of the 10 mg pts, and in 33.3% of the 5 mg pts (p=0.426). Overall, grade 2 or grade 3 side effects were experienced by 47.7% and 34% of the pts, respectively. Most frequent side effects were grade 2/3 mucositis (32.5%/11.2%), grade 1/2 decreased appetite (42.8%/24.3%), grade 1/2 rash (46.7%/22.7%), and grade 2/3 fatigue (33.3%/7.1%). Grade 2/3 weight loss was observed in 27.2%/7.6% of the pts. Only 5 cases of grade 3 pneumonitis were recorded. One patient had a grade 4 hypercholesterolemia which quickly resolved after cessation of therapy. No toxicity related death was observed.

Response and Survival. Out of 116 evaluable/measurable pts, the best observed response was disease improvement (RECIST and non RECIST objective response) in 47 pts (40.5%), stable disease in 28 pts (24.1%) and progressive disease in 41 pts (35.4%). From onset of Eve based therapy and after a median follow up of 10 mo, overall survival was 21 mo (0.4-26+), median progression free survival was 9 mo (0.4-26+), and time to treatment failure was 5.7 mo (0.4-16+). Eve was stopped for progression, toxicity or both in 64 pts (52%), 36 pts (29.2%) and 8 pts (6.5%) respectively. Multivariate analysis showed that more than 2 lines of previous chemotherapy was an independent predictor for PFS (HR for progression=2.28 – p=0.01), and that 2 or more involved sites was an independent predictor for OS (HR for death=2.7 – p=0.021).

Conclusion: We evaluated a multicenter population in routine practice of very advanced, slowly evolving luminal BC patients, which appears very close to the Bolero-2 population although more heavily pretreated. Eve based therapy appears feasible with dose reduction in more than 40% of the population and side effect rates are very similar to those reported in the pivotal Bolero-2 trial. Efficacy is highly encouraging and deserves a further evaluation of everolimus in this population.
PTEN status dictates the roles of PI3K isoforms p110α and p110β in modulation of AKT/mTOR and response to growth factor signaling in ER+ breast cancer

Lloye M Dillon1, Stephanie J Bouley1 and Todd W Miller1. 1Geisel School of Medicine at Dartmouth and Norris Cotton Cancer Center, Lebanon, NH.

Class 1A phosphatidylinositol 3-kinases (PI3Ks) regulate cell growth, survival, and metabolism. PI3Ks are heterodimeric lipid kinases composed of a p85 regulatory subunit and a p110 catalytic subunit (p110α, p110β, or p110δ). p110α and p110β play distinct roles in PI3K signaling in carcinoma cells. p110α is frequently activated by growth factor receptor kinase signaling. In contrast, p110β was shown to play a role in insulin metabolic action, G protein-coupled receptor (GPCR) and Rac1 signal transduction, and oncogenic transformation. Cancer cells deficient in PTEN, the tumor suppressor phosphatase that antagonizes PI3K signaling, are often sensitized to pharmacological inhibitors of p110β. As a result, early clinical studies with p110β inhibitors are often restricted to patients with PTEN-deficient cancers. However, analysis of data from the Genomics of Drug Sensitivity in Cancer database revealed that genetic lesions in PTEN or PIK3CA (encodes p110α) were significantly and independently associated with increased sensitivity to the p110β inhibitor AZD6284. Among 668 cancer cell lines evaluated, 61 lines had AZD6482 IC50 values ≤2 mM, but only 25 lines harbored an alteration in PTEN and/or PIK3CA. Thus, a significant fraction of cancer cell lines (and tumors) without PTEN/PIK3CA alterations are likely to be sensitive to p110β inhibition.

To explore the role of p110β in PI3K signaling in ER+ PTEN-deficient breast cancer, we treated cells with the p110β inhibitor GSK2636771 and the p110α inhibitor BYL-719, alone or in combination, and assessed effects on steady state and growth factor-induced activation of AKT and MEK/ERK activation, and cell growth. p110β inhibition reduced the viability of PTEN-deficient cells; however, combined inhibition of p110α and p110β was more effective at reducing AKT and ERK phosphorylation and increasing apoptosis in ER+ PTEN-deficient cells. Furthermore, anti-estrogen treatment potentiated the anti-proliferative effects of PI3K inhibition. p110β inhibition reduced insulin-like growth factor 1 (IGF-1)-induced pAKT levels, and delayed AKT phosphorylation in both PTEN-deficient and PTEN-wild-type cells. In contrast, p110β inhibition sensitized both PTEN-wild-type and PTEN-deficient cells to heregulin stimulation, and promoted PI3K (p85)/HER3 interaction. These results indicate that p110β inhibition desensitizes cells to IGF-1 stimulation, hypersensitizes cells to heregulin, and modulates downstream AKT and MEK/ERK activation in response to growth factor receptor activation. Our findings suggest that the anti-tumor efficacy of p110β inhibitors may be related to growth factor dependence and PTEN status.
Title: Understanding pharmacodynamics and consequences of PI3K inhibition in ER+ breast tumors

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Body: PI3K inhibitors have shown promise for the treatment of anti-estrogen-resistant breast cancers. Current PI3K inhibitor treatment regimens incompletely and transiently inhibit the pathway in carcinomas, and are accompanied by adverse effects in patients. We found that different periods of PI3K inhibition (12, 24, 36 h) potentiated anti-estrogen-induced apoptosis and inhibition of proliferation to similar extents in cultured ER+ cells. We thus hypothesized that short-term, complete inhibition of PI3K will have a greater anti-tumor effect and reduced systemic toxicity than chronic, partial inhibition.

Pharmacokinetic analysis of the orally available pan-PI3K inhibitor GDC-0941 at low (100 mg/kg) and high (800 mg/kg) doses in mice revealed that plasma levels peaked after 15-30 min. (18.6 uM and 20.7 uM, respectively), and decreased to a plateau phase after 1 h that was maintained for 8 h with low dose (6.8-10.7 uM) and 23 h with high dose (7.9-15 uM). We performed MCF-7 tumor pharmacokinetic analyses with low and high doses, and with 2 low doses administered 12 hours apart. Tumor GDC-0941 levels peaked after 9 h (1.6 uM with low-dose; 16.9 uM with high-dose). The second low dose increased tumor drug concentrations to 3.2 uM at 9 h after the second dose, compared to 1.6 uM at 9 h after the first dose. After 48 h, tumor drug concentrations decreased to 0 uM with low dose, and to 4.5 uM with high dose.

Mice bearing MCF-7 tumors were treated with fulvestrant (5 mg/wk). Three days later, GDC-0941 was administered to assess pharmacodynamic effects. Phospho-AKT and -S6 levels (markers of PI3K and mTORC1 activities, respectively) were maximally suppressed after 1 h and 3 h of high- and low-dose treatments, respectively, returned to baseline within 16 h after low-dose treatment, and remained suppressed for 36 h following high-dose treatment. PARP cleavage (marker of apoptosis) occurred within 1 h and 3 h of high- and low-dose treatments, and increased over time. Re-treatment of mice with low-dose GDC-0941 after 12 h induced continued inhibition of PI3K and mTORC1 for 9-12 h, suggesting that BID low-dose treatment may be sufficient to continually inhibit PI3K. Comparison of high-dose and low-dose BID tumors showed that these treatments induced similar amounts of PI3K inhibition and PARP cleavage at 21-24 h.

Mice bearing MCF-7 or fulvestrant-resistant T47D/FR tumors were treated with vehicle, fulvestrant, GDC-0941 (100 mg/kg QD 5 d/wk; 100 mg/kg BID 3 d/wk, 800 mg/kg QW), or combinations of fulvestrant and GDC-0941. Drug combinations induced tumor regression, fulvestrant did not affect tumor growth, high-dose GDC-0941 QW slowed tumor growth, and low-dose GDC-0941 QD or BID appreciably inhibited tumor growth. However, there was no significant difference among doses and schedules of GDC-0941 in the context of a fulvestrant backbone in either tumor model. These data suggest that transient/metronomic (QD, BID) and chronic/infrequent (QW) PI3K inhibition may provide similar anti-tumor efficacy in combination with an anti-estrogen. However, these tumor growth data conflict with cell fate data indicating that high-dose GDC-0941 induced much more apoptosis than low-dose GDC-0941. Ongoing studies will reveal how different schedules of PI3K inhibition shape tumor biology.
**Title:** Autophagy promotes escape from PI3K inhibition in ER+ breast cancer

Wei Yang¹ and Todd W Miller¹. ¹Norris Cotton Cancer Center, Geisel School of Medicine at Dartmouth, Lebanon, NH.

**Body:** PI3K inhibitors have shown promise for the treatment of ER+ breast cancers. Autophagy has been suggested to promote resistance to PI3K inhibitors. Interestingly, cancer cells often exhibit basal levels of autophagy, which is thought to serve as a mechanism to manage oxidative stress and remove damaged organelles (e.g., mitochondria). Pharmacodynamic analysis of the orally available pan-PI3K inhibitor GDC-0941 at low (100 mg/kg) and high (800 mg/kg) doses, and 2 low doses administered 12 hours apart (BID), in combination with fulvestrant in mice bearing MCF-7 xenografts revealed that treatment induced accumulation of LC-3 II (marker of autophagy) after 24-48 h. In ER+ breast cancer cells cultured in hormone-depleted medium, PI3K inhibition gradually induced apoptosis while increasing LC-3 II. Autophagy promotes glucose metabolism, and PI3K activation is a major driver of glucose uptake. We thus hypothesized that inhibition of autophagy will potentiate the anti-tumor effects of PI3K inhibition by suppressing glucose metabolism.

Chloroquine (CQ) is an anti-malarial drug that inhibits autophagy in mammalian cells by an unknown mechanism. CQ inhibited proliferation and autophagy in ER+ breast cancer cells in vitro. CQ treatment potentiated GDC-0941-induced inhibition of cell proliferation and promotion of apoptosis in growth conditions (10% FBS) with or without fulvestrant, and in hormone-depleted conditions (10% DCC-FBS). CQ treatment increased PI3K inhibitor-induced mitochondrial membrane depolarization, suggesting that this drug combination engages an intrinsic apoptotic pathway. PI3K inhibition also induced autophagy in fulvestrant-resistant MCF-7/FR and CAMA-1/FR cells. CQ increased GDC-0941-induced apoptosis in MCF7/FR and CAMA-1/FR cells, offering combined targeting of PI3K and autophagy as a promising therapeutic strategy for the treatment of anti-estrogen resistant breast cancer.

Mice bearing ZR75-1 xenografts are currently being treated with vehicle, CQ (2 mg/day via drinking water), 800 mg/kg GDC-0941 QW, or the combination. Thus far, single-agents treatments significantly inhibit tumor growth. However, the drug combination has not shown synergistic effects in vivo. Analysis of different doses and schedules of GDC-0941 plus CQ, with or without anti-estrogen treatment, is ongoing.
Title: Effect of adjuvant systemic therapy in reducing rates of loco-regional recurrence in early-stage breast cancer: Results from nine NSABP randomized phase III trials

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Body: Background
Adjuvant systemic therapy reduces risk of distant recurrence (DR) and breast cancer death. In addition, adjuvant systemic therapy reduces risk of loco-regional recurrence (LRR). We examined the magnitude of the effect of adjuvant systemic therapy (tamoxifen, chemotherapy, and chemotherapy + trastuzumab) in reducing incidence rates and cumulative incidence rates of LRR as first event in nine recent NSABP randomized trials that were conducted from 1981 to 2005 and included a total of 21,815 patients.

Methods
Nine NSABP clinical trials of adjuvant (or neoadjuvant) systemic therapy, in which a reduction in LRR or DR was observed, were included in the analysis (NSABP B-13, B-14, B-19, B-20, B-21, B-27, B-28, B-30, and B-31). The cumulative incidence rates of LRR as the first disease-free survival (DFS) event were estimated and compared across treatment arms via log-rank tests. The sub-distribution proportional hazards models were applied to estimate the reduction in incidence rate of LRR from adjuvant systemic therapies. The corresponding magnitude of reduction in the incidence rate of any DFS event was estimated from Cox proportional hazards models.

Results
Across all nine clinical trials, adjuvant systemic therapy resulted in reductions in LRR that were comparable to or greater than the reductions in DFS events (Table). The observed reductions in LRR with adjuvant chemotherapy were of greater magnitude in trials of node-negative patients (35-58%) than in trials of node-positive patients (13-15%). Reductions in LRR were of similar magnitude with adjuvant chemotherapy as with adjuvant tamoxifen. In B-27, the sequential addition of neoadjuvant or adjuvant docetaxel to neoadjuvant AC reduced LRR rates by 27%. The addition of trastuzumab to adjuvant chemotherapy decreased LRR rates by 34%.

Conclusions
Rates of LRR have steadily declined over time in NSABP adjuvant clinical trials. This decline can be attributed to improvements in surgical and radiotherapy techniques but is also the result of the use of increasingly effective adjuvant systemic therapy.

<table>
<thead>
<tr>
<th>NSABP Trial</th>
<th>Population</th>
<th>Treatment Comparison</th>
<th>HR(95%CI)DFS</th>
<th>HR(95%CI)LRR</th>
<th>10-yr Cum Incidence of LRR(%)</th>
<th>Log rank p-value</th>
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<tbody>
<tr>
<td>B-13 (n=1,084)</td>
<td>N(-) / ER(-)</td>
<td>MF v No Adj Rx</td>
<td>0.66 (0.55,0.79)</td>
<td>0.42 (0.29-0.62)</td>
<td>5.9 v 13.5</td>
<td>&lt;0.001</td>
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<td>B-14 (n=4,028)</td>
<td>N(-) / ER(+)</td>
<td>TAM v Placebo</td>
<td>0.70 (0.65,0.77)</td>
<td>0.54 (0.45-0.66)</td>
<td>5.2 v 11.2</td>
<td>&lt;0.001</td>
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<tr>
<td>B-19 (n=1,074)</td>
<td>N(-) / ER(-)</td>
<td>CMF v MF</td>
<td>0.69 (0.57, 0.84)</td>
<td>0.48 (0.31-0.73)</td>
<td>5.3 v 10.0</td>
<td>&lt;0.001</td>
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<td>B-20 (n=2,299)</td>
<td>N(-) / ER(+)</td>
<td>CMF/MF+TAM v TAM</td>
<td>0.76 (0.66, 0.88)</td>
<td>0.57 (0.41-0.78)</td>
<td>3.7 v 7.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Study</td>
<td>Node Status</td>
<td>Treatment Comparison</td>
<td>Hazard Ratio (95% CI)</td>
<td>5-Year OS (95% CI)</td>
<td>p-Value</td>
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<tr>
<td>B-21</td>
<td>N(-) / ≤1cm</td>
<td>TAM v Placebo</td>
<td>0.93 (0.71, 1.21)</td>
<td>4.7 v 8.8</td>
<td>0.10</td>
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<tr>
<td>B-27</td>
<td>Operable T1-3 N0-1</td>
<td>AC→T v AC (neoadj)</td>
<td>0.92 (0.81, 1.05)</td>
<td>9.1 v 12.2</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td>B-28</td>
<td>N(+)</td>
<td>AC→P v AC</td>
<td>0.90 (0.81, 1.00)</td>
<td>8.6 v 10.0</td>
<td>0.24</td>
<td></td>
</tr>
<tr>
<td>B-30</td>
<td>N(+) / HER2(-)</td>
<td>AC→T v AT/TAC</td>
<td>0.84 (0.76, 0.93)</td>
<td>4.6 v 5.4</td>
<td>0.23</td>
<td></td>
</tr>
<tr>
<td>B-31</td>
<td>N(+) / HER2(+)</td>
<td>AC→P+H v AC→P</td>
<td>0.59 (0.50, 0.69)</td>
<td>5 v 7.4</td>
<td>0.02</td>
<td></td>
</tr>
</tbody>
</table>

N: Node; M: Methotrexate; F: 5-FU; TAM: Tamoxifen; C: Cyclophosphamide; A: doxorubicin; T: Docetaxel; P: Paclitaxel; H: trastuzumab
Title: Final results of the VitaCal randomized phase III study evaluating the impact of a tailored oral vitamin D supplementation regimen on serum 25-hydroxyvitamin D levels in early breast cancer patients

William Jacot¹, Nelly Firmin¹, Lise Roca¹, Delphine Topart², Sophie Gallet¹, Anna Durigova¹, Simone Mirr¹, Stéphane Poudroux¹, Jean-Pierre Bleuse¹, Pierre-Jean Lamy¹ and Gilles Romieu¹. ¹ICM Val d'Aurelle â– Montpellier Cancer Institute, Montpellier, France and ²Montpellier University Hospital, Montpellier, France.

Body: Purpose: Only a minority of patients with early breast cancer (EBC) treated with adjuvant or neoadjuvant chemotherapy have sufficient baseline vitamin D. The current recommendations regarding daily vitamin D supplementation appears too low to correct this deficiency in this population. Optimal vitamin D dosing has yet to be determined in this setting. The current randomized phase III study address the issue of the effectiveness and safety of a tailored high dose oral vitamin D supplementation as a means for restoring normal 25-hydroxy vitamin D (25OHD) levels in a large population of chemotherapy-treated EBC patients.

Methods: Chemotherapy-treated EBC patients were stratified according to the degree of Vitamin D deficiency, time between chemotherapy initiation and inclusion (0 to 6 months versus 6 to 12 months), hormone receptors status and menopausal status. Participants were randomly assigned to receive a 6-months conventional (C) vitamin D and calcium supplementation or a 6-months high dose oral vitamin D regimen tailored on the degree of deficiency (T) associated with a conventional calcium supplementation. Primary endpoint was the efficacy (6-months percentage of 25OHD serum levels normalization) in the T arm compared with the C arm. Patients without vitamin D normalization from the C arm were allowed to switch to the T arm after 6 months. Statistical analyses were performed on an intent to treat basis.

Results: The trial accrued 215 patients, among which 197 patients presented with vitamin D deficiency, and randomized 195 patients (T, 100; C, 95) from July 2011 to January 2013. The groups were well balanced in regard to the stratification characteristics, as well as in regard of median weight and neoadjuvant or adjuvant chemotherapy status. Compliance to the daily oral supplementation was low in both arms, 64% of the patients in both arms taking less than 80% of the planned oral supplementation dose. Compliance to the tailored high dose vitamin D schedule appeared better (78%). After 6 months of treatment, at the primary endpoint analysis time, significantly more patients in the T arm presented with normalized serum vitamin D levels compared to the C arm (30% vs. 12.6%; p=0.003). Vitamino-calcic supplementation was well tolerated, with no difference in the treatment-related toxicity between the 2 arms. 52 patients without vitamin D normalization from the C arm switched to the T arm after 6 months. At the 12 months endpoint, 44% of these patients achieved vitamin D normalization.

Conclusion: In this randomized phase III study, a tailored high dose oral vitamin D supplementation allowed a statistically higher percentage of serum 25OHD levels normalization compared to a conventional regimen, without any increase in side effects, in a large population of chemotherapy-treated EBC patients. Observance of a daily oral supplementation remains poor in this setting, advocating for an adaptation of the schedule and dosage of this supplementation in a population of patients subject to chemotherapy-induced emesis.

Clinical trial number NCT01480869.
Title: Delay in trastuzumab initiation leads to decreased overall survival in patients with HER2+ early stage breast cancer

Christopher M Gallagher¹, Kenneth More², Anthony Masaquel³, Tripthi Kamath³, Annie Guerin⁴, Raluca Ionescu-Ittu⁴, Marjolaine Gauthier-Loiselle⁶, Roy Nitulescu⁴, Nicholas Sicignano⁵, Brian Barnett³ and Eric Wu⁶. ¹Walter Reed National Military Medical Center, Bethesda, MD; ²Naval Medical Center Portsmouth, Portsmouth, VA; ³Genentech, San Francisco, CA; ⁴Analysis Group, Inc, Montreal, QC, Canada; ⁵Health ResearchTx, LLC, Trevose, PA and ⁶Analysis Group, Inc, Boston, MA.

Body: Background
Trastuzumab reduces the risk of relapse in women with HER2+ early stage breast cancer. Yet, little information exists on the timing of trastuzumab initiation and its association with relapse and survival outcomes in these patients. The study aimed to investigate the impact of delaying the initiation of adjuvant trastuzumab treatment for >6 months on time to relapse, overall survival, and relapse-free survival among patients with HER2+ early stage breast cancer who did not receive neoadjuvant therapy.

Methods
Adult women initiating trastuzumab adjuvant therapy within 1 year of breast cancer surgery who did not receive neoadjuvant therapy were selected from the US Department of Defense health claims database from 01/2003 to 12/2012 (N = 2,749). By design, participants had to be alive and relapse-free at the time they initiated adjuvant trastuzumab. Patients were classified into two groups based on the time from breast cancer diagnosis to trastuzumab initiation: ≤6 months and >6 months. An algorithm based on secondary neoplasm ICD9 codes along with treatment gaps and initiations was used to identify relapses. Percent relapses and/or deaths were reported by study groups and compared using χ² tests. The impact of delaying trastuzumab initiation on time to relapse, overall survival, and relapse-free survival was estimated from Cox regression models adjusted for age, overall comorbidity profile at the time of the BC diagnosis (Charlson index), type of surgery (breast conserving vs. breast removing), and radiotherapy (prior to the initiation of trastuzumab). In all three Cox models the follow-up started at adjuvant trastuzumab initiation.

Results
Of 2,749 women who met the selection criteria, 79.3% initiated adjuvant trastuzumab ≤6 months of diagnosis and 20.7% initiated adjuvant trastuzumab >6 months after the diagnosis (Table). Patients who delayed the initiation of trastuzumab for >6 months were younger (57.2% aged <65 years vs. 50.9%, p = .008) and a higher proportion of them received radiotherapy prior to the initiation of trastuzumab compared to those who initiated trastuzumab earlier (77.2% vs. 53.1%, p < .001). There were no significant differences between the two groups in overall comorbidity profile and type of surgery. Patients who initiated trastuzumab >6 months after diagnosis had a higher risk of relapse, death, or relapse/death than those who initiated trastuzumab ≤6 months of diagnosis in both unadjusted and adjusted analyses (Table).

<table>
<thead>
<tr>
<th>Outcome</th>
<th>N events (% events)</th>
<th>Hazard Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤6 months group N =</td>
<td>&gt;6 months group N =</td>
</tr>
<tr>
<td></td>
<td>2,180</td>
<td>569</td>
</tr>
<tr>
<td>Relapse outcome</td>
<td>333 (15.2%)</td>
<td>134 (24.3%)</td>
</tr>
<tr>
<td>Death outcome (overall survival)</td>
<td>138 (6.3%)</td>
<td>64 (11.6%)</td>
</tr>
<tr>
<td>Relapse or death outcome (Relapse-free survival)</td>
<td>386 (17.6%)</td>
<td>148 (26.8%)</td>
</tr>
</tbody>
</table>

*p-value < .05

Conclusions
The results of this population-based study among patients with HER2+ early stage breast cancer who did not receive neoadjuvant therapy suggest that delays of over 6 months in the initiation of trastuzumab among HER2+ early stage breast cancer patients are associated with a higher risk of relapse and shorter overall survival and relapse-free survival.
Disclaimer
Research derived from an IRB approved protocol at Naval Medical Center Portsmouth, VA. The views expressed in this abstract are those of the authors and do not necessarily reflect the official policy or position of the Department of the Army, Department of the Navy, Department of Defense or the United States Government. Dr. C.G. and Dr. K.M. are members of the U.S. military. This work was prepared as part of their official duties. Title 17 U.S.C. 105 provides that 'Copyright protection under this title is not available for any work of the United States Government.' Title 17 U.S.C. 101 defines a United States Government work as a work prepared by a military service member or employee of the United States Government as part of that person's official duties.
Title: Clinical intervention trial with physical activity during chemotherapy for primary breast cancer: Different effects of endurance and resistance training on physical fitness and quality of life

Christoph Mundhenke¹, Weisser Burkhardt², Walter Jonat¹, Juliane Dürkop¹, Lisa Keller¹, Arne Falk² and Thorsten Schmidt¹.
¹OB/GYN, Breast Unit, UKSH, University of Kiel, Kiel, Germany and ²Sports Science, University of Kiel, Kiel, Germany.

Body: Aim: Previous findings suggest that physical activity during breast cancer treatment can reduce side effects and improve clinical outcome. However, physical training is not performed to the same degree as e.g. for coronary heart disease. In part, this is due to the lack of studies comparing different forms of exercise on their effectiveness, which aggravates the composition of exercise-guidelines. As a contribution for this goal, this intervention study compares the effects of moderate endurance and moderate resistance training on physical fitness, fatigue, concentration and the quality of life.

Methods: In a randomized, controlled intervention trial 12-week supervised endurance (ET) or resistance training (RT) were compared with standard usual care (UC) in patients with primary breast cancer during adjuvant or neoadjuvant chemotherapy. 78 female patients were enrolled. Endpoints were muscular strength (NM), endurance (Watt/kg/bodyweight in kg) and well during endurance stress test (Borg scala), quality of live (QL) (EORTC QLQ C30+BR23) before and after 12 weeks of treatments. 14 out of 81 patients dropped out (due to chemotherapy related side effects or withdrawal of consent). 67 patients are fully evaluable.

Results: RT (vs. UC) was superior for improving strength (p: 0.015). A trend towards improvement in strength was observed in the (ET vs. UC) (p:0.149; n.s.).All groups decrease in the endurance stress test /Watt/kg/bodyweight in kg) after 12 weeks (RT: p: n.s; ET: p: n.s; UC: p: n.s), however the maximal endurance lost in Watt was greatest in UC (p: 0.001). The subjective perceived exertion at 100 watts remained stable in the RT (p: n.s.) and decreased in ET (p:0.02) and in UC (n.s.).

In the RT group quality of life score improved significantly during 12 weeks of intervention (p: 0.011). There is also a trend for improvement of QL in the ET group (p: >0.05; n.s.). The UC group showed a decreased QL.

Conclusion: Important improvements in strength, endurance and quality of life from exercise training in breast cancer patients receiving chemotherapy are demonstrated in this trial. RT showed a superior improvement in physical strength, in subjective perceived exertion and quality of life over ET and UC. The beneficial results suggest that physical intervention (including a resistance intervention) should be implemented into standard of care during adjuvant chemotherapy for breast cancer. A combined intervention of endurance and resistance intervention may be optimal and needs to be further prospectively evaluated.
**Title:** ERALOP study: Post adjuvant FEC - docetaxel chemotherapy for early breast cancer: Hair regrowth in the real life

Hugues Pierre Bourgeois\(^1,2\), Aurélie Jamet\(^3\), Françoise Grudé\(^2\), Carole Adounkpe\(^3\), Pierre Kerbrat\(^4\), Remy Delva\(^5\), Hélène Simon\(^6\), Philippe Deguiral\(^7\), Bertrand Diquet\(^3\), Pascale Lainé\(^3\) and Anne-Lise Septans\(^5\). \(^1\)Centre Jean Bernard, Clinique Victor Hugo, Le Mans, Pays de la Loire, France; \(^2\)Observatoire dédié au Cancer Bretagne Pays de la Loire, Angers, Pays de la Loire, France; \(^3\)CHU Angers, Angers, Pays de la Loire, France; \(^4\)CRLCC Eugène Marquis, Rennes, Bretagne, France; \(^5\)Institut de Cancérologie de l'Ouest Paul Papin, Angers, Pays de la Loire, France; \(^6\)CHRU Brest Morvan, Brest, Bretagne, France and \(^7\)Clinique Mutualiste de l'Estuaire Cité Sanitaire, Saint Nazaire, Pays de la Loire, France.

**Body:** **Background:** during 2008 we have collected one hundred observations of persistent significant alopecia (PSA). FEC 100-docetaxel 100 mg/msq regimen was mostly concerned. We therefore decided to evaluate exact incidence of this relevant side effect through women points of view.

**Methods:** ERALOP is a retrospective study using a self-questionnaire targeting patients (pts) treated with this sequential regimen from 2008 to 2009. The primary objective was to estimate the incidence of a PSA at 6 months after last course of docetaxel with CTCAE 4.0 classification: grade 1: hair loss of up to 50% not obvious from a distance, a different hair style may be required to cover the hair loss, grade 2: hair loss > 50% with a psychosocial impact. The sample size calculation of 635 patients took into account: PSA incidence of 3.2% (TAC regimen), precision of 0.015, \(\alpha\) risk at 0.05, 20% patients lost for follow up. ERALOP study was approved by local ethic comitee.

**Results:** from July 2012 to October 2012, 829 pts received a self-questionnaire. 176 pts (21%) did not answer and were considered as without PSA. 653 (79%) answers with medical data fully documented were collected. Median age of patients was 56 years. Six months after last docetaxel course, PSA incidence grade 2: 8.6% (71 pts), grade 1: 32.6% (271 pts), grade 0: 56% (466 pts), NA: 2.5% (21 pts). 73% of pts with PSA received hormonotherapy. At the time of the inquiry (median follow up of 3.7 years), PSA incidence grade 2 was 3.5% (29 pts), grade 1: 30% (248 pts), grade 0: 63.8% (529 pts), for a global PSA incidence of 33.4%. Between 6 months after last course of docetaxel and time of the inquiry, it appears slight or total regrowth for 40 pts with PSA grade 2 and 187 with PSA grade 1. Three pts still wore a wig, and many pts had suboptimal regrowth of eyelash (31%), eyebrow (47%), pubic hair (27%), and nail disorders (27%). A multivariate analysis was performed to look for PSA risk factors. All the oral treatments, including dexpanthenol, biotin, methionine cysteine and cystin-vitamin B6 proved to have no efficacy. Treatment by topical minoxidil could have a little efficacy. Impairment of health-related quality of life will be assessed by Dermatology Life Quality Index (DLQI) and alopecia grade will be evaluated at the end of hormonotherapy.

**Conclusions:** Physicians and patients should be aware of this new distressing side-effect. This high level of PSA lead us to conduct ALOPREV trial to investigate, in spite of FEC induced alopecia, the properties of cooling cap prevention trial during docetaxel infusion. Preliminary results are encouraging and this option could be considered.
**Title:** Clinical outcomes according to pathological complete response (pCR) and proliferation index of residual tumor (RT) after neoadjuvant chemotherapy (NC) in invasive breast cancer (IBC)

Antonella Ferro¹, Alessia Caldara¹, Mariachiara Dipasquale¹, Chiara Trentin¹, Renza Triolo¹, Mattia Barbareschi¹, Daniela Bernardi¹, Marco Pellegrini¹, Daniela Cazzolli¹, Gabriella Berlanda¹, Fabio Gasperetti¹, Francesca Maines¹, Paolina Tuttobene¹, Orazio Caffo¹ and Enzo Galligioni¹. ¹Santa Chiara, Trento, Italy.

**Body:**

**BACKGROUND:**
IBC is a heterogeneous disease with several subtypes molecularly identified by gene expression profile. Since subtypes defined by immuhistochemistry (IHC) panel are similar although not identical to molecular subtypes, IHC may represent an easier alternative to identify them.

**PURPOSE:**
To assess the clinical outcomes of pts who received NC for IBC and the differences by IHC-related subtypes.

**METHODS:**
We retrospectively reviewed the clinical records of the pts treated with NC for stage II-III IBC from 2000 to 2013. For each pt we recorded baseline tumor size, type of NC [which consisted of anthracyclines (A) + taxanes (T) in HER2- and T + trastuzumab (H) ± A in HER2+ pts], type of surgery, pathological response (pCR defined as the absence of invasive cells in the breast and the lymph nodes regardless of DCIS). IHC subtypes were defined according to ER and PgR expression, Ki-67 level, and HER2 status:

- **Luminal A (LA):** ER and PR+, neg HER2 and Ki67 < 14% (= 3%)
- **Luminal B (LB):** ER and/or PR+, neg HER2 and Ki67 ≥ 14% (=30%)
- **Luminal HER2 (LHER2):** ER and/or PR+, positive HER2 and any Ki67 (=27%)
- **HER2 positive (HER2+):** neg ER and PR, positive HER2 and any Ki67 (=12%)
- **Triple negative (TN):** neg ER and PR, neg HER2 and any Ki67 (13%)
- **Unknown subtype in 33 cases (15%)**

The loco-regional and distant RFS and OS were evaluated according to pCR. pCR and survival outcomes were also assessed on the basis of both pre- and post- NC Ki67 levels.

**RESULTS:**
In the consecutive series of 213 pts who received NC median age was 50 yrs (r. 25-75). The NC consisted of an A+T based regimen in HER2 negative (145 pts) and of a T+ H with A (31 pts) or without A (34 pts) in HER2+ disease. Only 14 did not receive surgery: 10 for distant metastases development and 4 because still on NC. Quadrantectomy was performed in 120 pts (60%). Among all pts, pCR was achieved in 44 pts(22%) with further 4 pts showing a RT ≤1 mm.

<table>
<thead>
<tr>
<th>Subtype</th>
<th>LA (%)</th>
<th>LB (%)</th>
<th>LHER2 (%)</th>
<th>HER2+ (%)</th>
<th>TN (%)</th>
<th>Median Ki67 (%)</th>
<th>Recurrence Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pCR</td>
<td>0</td>
<td>12.5</td>
<td>42.5</td>
<td>27.5</td>
<td>17.5</td>
<td>48</td>
<td>4.5</td>
</tr>
<tr>
<td>No pCR</td>
<td>100</td>
<td>42.3</td>
<td>29.2</td>
<td>8.8</td>
<td>14.6</td>
<td>37</td>
<td>31.5</td>
</tr>
<tr>
<td>p Value</td>
<td></td>
<td>&lt;0.001</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>=0.001</td>
</tr>
</tbody>
</table>

All but 19 HER2+ pts (84) received H obtaining pCR in 38% of cases regardless chemotherapy type (A-based 35% vs Not A-38%)

The median follow-up was 45 months (range 1-166 ms).

The 4y-RFS and OS were better in which achieved pCR than those did no (RFS 92 vs to 74%; p=0.0014 and OS 95 vs 78%; p=0.0074).
Median Ki67 in pretreated core biopsy was 40 compared to 27% in post-NC RT. Patients with high (>30%) post-NC Ki67 levels showed significantly higher risk for disease relapse (4 y-RFS 60%; p=0.0019) and death (4y OS 71%; p=0.018) compared with patients with <15% (4y-RFS 93 and OS 88%) or >15-30 Ki67 levels (4y-RFS 83 and OS 82%).

CONCLUSIONS:
According to literature data, pts achieving pCR after NC showed better RFS and OS compared to no pCR pts. The pCR rate was significantly higher in aggressive subtypes (HER2 and TN). In HER2 disease, pCR was achieved by using chemo + H, irrespective of A-addition. Interestingly high pre-NC Ki67 levels seem to predict the possibility obtaining pCR, while post-NC Ki67 levels seem to be of prognostic value in pts who do not receive pCR.
2014 San Antonio Breast Cancer Symposium

Publication Number: P5-21-07
Average Grade: 6.00

Title: Unexpected low incidence of cardiovascular related events in women with primary breast cancer; population derived retrospective cohort

Antroula Papakonstantinou¹, Laila Hubbert², Rasmus Mikiver³, Jonas Bergh¹ and Elham Hedayati¹. ¹Karolinska Institutet, Stockholm, Sweden; ²Linkoping University, Linkoping, Sweden and ³Regional Cancer Centre Southeast Health Region, Linkoping, Sweden.

Body: Background
Many studies and even a meta-analysis of the EBCTCG found that, compared to methotrexate containing chemotherapy (non-ACT) or no chemotherapy (no-CT), anthracycline-containing chemotherapy (ACT) decreases overall mortality after breast cancer (BC). However, increased cardiac mortality was observed but did not outweigh improvement in BC survival. The incidence of cardiotoxicity after adjuvant BC treatment is reported with varying frequencies and still remains unknown.

Methods
Women younger than 60 years and diagnosed with lymph node positive BC between 1998 and 2002 were identified through the Cancer Registry of the Regional Cancer Centre in the southeast health region of Sweden. Data on patient, tumor and treatment characteristics were registered from the Cancer Registry and medical records. Information on cardiovascular toxicity (CVT) was collected from the National Patient Registry, the Cause of Death Registry and the Prescribed Drugs Registry. ICD diagnoses for cardiac diseases and hypertension were used. A predefined Kaplan Meier and Cox regression analysis and a post hoc multivariate analysis to investigate confounders were performed. CVT was registered as event only if it occurred prior to BC relapse.

Results
Of 524 eligible women, 329 were analyzed (mean age, 50 years; range, 28 to 60 years) and 195 were excluded due to missing medical records (n=153), primary metastatic disease (n=18), trastuzumab treatment (n=6) and known heart disease at BC diagnosis (n=13). Of those analyzed, 176 received ACT, 64 non-ACT and 89 no-CT. The cumulative CVT was 16 %. CVT was observed in 12 % of those that received ACT, 14 % of those that received non-ACT and 25 % of those that received no-CT. CVT was significantly higher among women that received no-CT (p=0.024) meanwhile BC relapse and BC mortality was significantly higher among women treated with ACT compared to no-CT (p=0.02). According to Kaplan Meier curve, the risk for CVT among women treated with ACT was higher than the rest the first 5 years after diagnosis. 13 out of 85 patients that received epirubicin <450mg/m² and 5 out of 54 that received epirubicin >450 mg/m² developed CVT (p=0.302). A multivariate analysis adjusted for age, treated side, obesity, smoking and received chemotherapy showed that age between 51 and 60 year was a significant risk factor for developing CVT (p=0.035).

Conclusion
Clinically overt CVT 10-14 years after adjuvant BC treatment was diagnosed in 16% of women, but the subclinical incidence remains unclear. Women that did not receive chemotherapy showed increased incidence of CVT, probably due to lower risk for BC relapse and better overall survival. Age seems to be an independent risk factor for CVT. The incidence of clinical ACT CVT was unexpected low, however, CVT was observed already in epirubicin < 450 mg/m². BC relapse still remains the most important risk factor for mortality among women with high risk BC and nowadays high-dose chemotherapy and dose dense treatments are given, increasing the risk of CVT. It is essential to co-operate with cardiologists and investigate methods to identify patients that are at risk of developing chemotherapy-related CVT and find suitable measures to prevent CVT influencing overall survival.
One-tenth of patients younger than 40 years develop a permanent chemotherapy-induced ovarian function failure after receiving adjuvant anthracycline-based chemotherapy with or without taxanes

Vivianne C Tjan-Heijnen¹, Ingeborg J Vriens¹, Ashley J Beijers¹, Maureen J Aarts¹, Maaike de Boer¹, Joyce H Royen¹ and Ron J van Golde¹. ¹Maastricht University Medical Centre, Maastricht, Netherlands.

**Body**

**Background**

To assess the incidence and predictors of (permanent) chemotherapy-induced ovarian function failure (COFF) in premenopausal women with hormone receptor positive breast cancer treated with adjuvant chemotherapy.

**Patients and methods**

In our university hospital, patients with COFF and hormone-receptor positive breast cancer are monitored for ovarian function recovery by 3-monthly FSH and estradiol blood levels (serum estradiol is measured by direct immunoassay). In this present study, we collected data from the medical records of all premenopausal women with hormone-receptor positive breast cancer treated with anthracycline-based chemotherapy, with or without the addition of taxanes, who were diagnosed in the years 2005-2012. To meet the definition of COFF, the amenorrhea and ovarian function suppression had to last ≥24 weeks since the last menstruation before or during chemotherapy. Patients with hormone-receptor negative breast cancer were excluded.

**Results**

We identified 135 eligible women. Initial oral hormonal therapy consisted of tamoxifen (n= 116) or aromatase inhibitors (AI, n=16, of whom 1 patient younger than 40 years). Median follow-up of the included patients was 25 months (range 3-69 months). The majority of women was older than 40 years (80%). Permanent or temporary COFF was present in 95.6% of patients; that is, in 97.2% of patients of ≥ 40 years versus in 88.8% of patients < 40 years of age, which was not different between age-groups. However, permanent COFF was significantly more often present in women ≥ 40 years (75%) as compared with 11.1% of women < 40 years ( P < 0.03). Patients who developed a permanent COFF had a mean age of 47.4 (SD 3.9) years, whereas patients who developed a temporary COFF had a mean age of 38.0 (SD 6.5).

In 57% of the patients, premenopausal hormone levels were the first evidence of ovarian function recovery. The second-last FSH and estradiol values of patients who had an ovarian function recovery were still clearly in postmenopausal range (Figures will be shown at the meeting).

**Conclusion**

COFF is seen in 89% of patients < 40 years, but in the majority it was reversible. This is reassuring for those with a childwish. As in a significant proportion of patients FSH and estradiol values are the first sign of ovarian function recovery, close monitoring of ovarian function is required if ovarian function suppression is considered an additional effective hormonal treatment, and with respect to indication of non-hormonal contraceptive devices. We would not recommend AI as single hormonal treatment in young patients with COFF.
Title: Oncologist treatment choices in patients with early stage invasive lobular breast carcinoma - a survey

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Body: Introduction: Invasive lobular carcinoma (ILC) is common and accounts for 5-15% of all breast cancers. ILC has distinct clinical and histological features that separate it from invasive ductal carcinoma (IDC) with regards to its breast imaging characteristics, patterns of recurrence and sensitivity to systemic therapy. ILC presents challenges to the physician in many aspects of local-regional and systemic therapy choices. We surveyed breast cancer physicians on their beliefs and practice patterns on issues around the management of ILC.

Methods: A questionnaire was developed and circulated electronically using a modified Dillman technique to surgical, radiation, and medical oncologists across Canada and Ireland.

Results: The questionnaire was completed by 91 of 429 physicians (21% response rate). Response rate by specialty was 25/69 (36%), 21/54 (41%) and 45/306 (13%) for surgical, radiation and medical oncologists respectively. Most surgeon responders (77%) would feel "uncomfortable making treatment decisions for ILC with a mammogram alone" and 100% would be "more comfortable with an MRI". Although 55% reported treating ILC as they would IDC, 22% of surgeons will purposefully obtain larger gross margins intra-operatively. Some radiation oncologists believe ILC is an independent risk factor for local-regional recurrence after breast conserving surgery (49%), and after mastectomy (29%). 33% of radiation oncologists would offer radiation therapy after mastectomy specifically because of the ILC subtype even in the absence of usual indications for radiotherapy. Most medical oncologists treat ILC comparably to IDC with the factors having the largest influence on systemic treatment decisions being tumour stage, hormone receptor status and HER-2 status. 51% of medical oncologists treat ILC with adjuvant chemotherapy as they do for IDC at least ‘most of the time’, while 40% use neoadjuvant chemotherapy as frequently as they do for IDC at least ‘most of the time’. 75% of medical oncologists manage the hormonal treatment of ILC as they do IDC ‘most of the time’ or ‘always’.

Conclusions: There remains significant clinical equipoise in the local-regional and systemic management of ILC. This survey has demonstrated wide variations in both beliefs and practices of management for ILC. Clinical guidance, developed on clinical trials specifically assessing the management of ILC, is required.
Title: RANK/RANKL expression by immunohistochemistry (IHC) in young breast cancer (BC) patients and during pregnancy: Association with clinicopathologic features, gene expression profiles and patient outcome

Hatem A Azim, Jr1, Fedro A Peccatori2, Sylvain Brohee1, Daniel Branstetter3, Giancarlo Pruneri2, Sherene Loi4, Giuseppe Viale2, Bill Dougall3 and Christos Sotiriou1. 1Jules Bordet Institute, Brussels, Belgium; 2European Institute of Oncology, Milan, Italy; 3Amgen Inc, Thousand Oaks and 4Peter MacCallum Cancer Center, Melbourne, Australia.

Body: Background & Objectives: RANKL is a major paracrine effector of the mitogenic action of progesterone in mammary epithelium via its receptor RANK. Increased mammary tumor formation following pregnancy was observed in transgenic mice with gain of function in RANK, a process that was arrested using a RANKL inhibitor. Based on epidemiological studies, pregnancy increases BC risk on the short term with pregnancy-associated BC associated with poor prognosis. Here, we report for the first time the expression of RANK/RANKL in young BC patients using IHC, and its association with diagnosis during pregnancy and prognosis. We also evaluate genes and pathways that are activated in RANK/RANKL expressing tumors.

Patients & Methods: 195 young BC patients were included; of whom 65 were diagnosed during pregnancy. All patients had central pathologic review and 85% had available gene expression data using Affymetrix. RANK/RANKL expression by IHC on the primary tumor and adjacent normal tissue was performed at Amgen laboratories, blinded for clinical data. IHC was performed with antibodies against human RANK (N-1H8) and human RANKL (M366) using the H-score. The difference in expression of RANK/RANKL between pregnant and non-pregnant patients and the association with clinicopathologic features were examined. We evaluated genes and pathways that are associated with RANK/RANKL expression as a continuous variable in a linear regression model. Finally, we tested the association between RANK/RANKL expression and disease-free survival (DFS).

Results: Median age was 36 years (range: 28-47). RANKL expression was more prevalent in the pregnant group independent of other pathologic features; both on the tumor (mean H score: 32 vs. 8) and adjacent normal tissue (mean H score: 87.3 vs. 32.9, both p<0.001). 18.7% of pregnant and 5.3% of non-pregnant patients had ≥10% of cells with RANKL expression 3+. RANKL expression was significantly higher in PgR+, well differentiated, and luminal-A tumors, with negative correlation with Ki-67 (all p<0.001). RANK expression was higher on normal compared to tumor (23.6 vs. 14.2, p=0.003), with no differences according to pregnancy status. RANK expression was higher in triple negative and poorly differentiated tumors (all p<0.001). Using FDR<0.05, 151 and 1207 genes were significantly correlated with RANKL and RANK expression by IHC, respectively. A positive correlation was observed between mRNA and IHC expression of RANKL (r=0.89, p<0.001) and RANK (r=0.19, p=0.01). High RANKL expression was associated with pathways related to mammary gland development, bone resorption, T-cell proliferation and regulation of chemotaxis, while RANK expression was associated with immune response and proliferation pathways. At a median follow-up of 65 months, neither RANK nor RANKL expression was associated with DFS.

Conclusions: RANKL expression is higher during pregnancy both in normal breast tissue and primary tumor and is associated with important biological processes. These results support the preclinical data suggesting RANKL as a key player in the crosstalk between pregnancy and BC guiding further development of RANKL-targeted therapy.
2014 San Antonio Breast Cancer Symposium

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Average Grade: 6.80

**Title:** Exploring biomarkers of response to zoledronic acid in breast cancer from clinical trial result of neoadjuvant chemotherapy with zoledronic acid: JONIE-1 study

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**Body:**

**Background:**
Preclinical and clinical data have shown that zoledronic acid (ZOL) increases antitumor effect of chemotherapy (CT) in breast cancer (BC). We previously reported that the addition of ZOL to neoadjuvant CT is potentially beneficial in postmenopausal patients with triple-negative BC in JONIE-1 Study (0% pCR rate in CT versus 50% in CT combing ZOL, p=0.029). Our data suggest that there might be subpopulation that responds to ZOL addition; however, there is lack of evidence to explain mechanisms, emphasizing the need for exploring biomarkers of response using clinical samples. We hypothesized three mechanisms of antitumor effect of ZOL.

1. ZOL inhibits Src signaling pathway directly in BC cell.
2. ZOL suppresses releasing Insulin-like growth factor-1 (IGF-1) from the bone and decreases chemoresistance in BC.
3. ZOL alters immune response to BC especially through TAM which induces cytotoxic T cell infiltration.

**Patients and Methods:** We investigated the relationship between clinicopathological features and tumor shrinkage in 178 Stage IIA-IIIB HER-2-negative BC patients from the JONIE-1 adjuvant phase III trial comparing CT (FEC100 q3w × 4 cycles followed by weekly paclitaxel for 12 cycles) versus CT combining ZOL (4mg q3-4w). To evaluate Src activation, IGF-1 receptor (IGF-1R) activation, M1/ M2 macrophage infiltration, and cytotoxic T cells infiltration, we performed immunohistochemistry and two pathologists were independently performed assessment. Formalin fixed, paraffin embedded core needle biopsy sample at diagnosis and surgical specimen after neoadjuvant CT were serially sectioned at 4 micrometer and processed for HE staining and immunohistochemistry using primary antibodies as follows; Rabbit anti-Src mAb (36D10, Cell signaling), Rabbit polyclonal anti-p-IGF-IR Ab (Tyr 1161) (Santa-Cruz Biotechnology), Mouse anti-CD80 mAb, (UMAB65, OriGene Technologies), Mouse anti-CD163 mAb (10D6, Lwica Biosystems), Mouse Anti-CD8 mAb (C8/144B, Dako).

**Results:** All immunohistochemistry were successfully performed. Src activation was recognized as peripheral membrane localization. Stained slides are now under evaluation by pathologist. Associations between clinicopathological features and the effect of CT with ZOL will be under investigation.

**Discussion:** Src activation was observed in more than 70% of triple negative BC. Multiple cellular functions of Src are mediated by Ras which is the main target of ZOL in osteoclasts. IGF-1R was overexpressed in approximately 50% of BC and IGF-1 signaling protects BC cell from CT by proliferative and anti-apoptotic effects. IGF-1 is the most released growth factor from the bone matrix during bone resorption and ZOL administration resulted in a significant decrease in IGF-1. Subclinical inflammation induced by macrophages in adipose tissue play an important role in promoting BC growth. It has been reported that ZOL reverted tumor-infiltrating macrophages (TAM) phenotype from M2 to M1. Therefore tumor infiltrating lymphocytes would be dominantly reverted to cytotoxic T cells for inhibiting tumor growth as well. We will present the results and discuss antitumor effects of ZOL at the meeting.
Body: Background: The incidence of change in HER2 status in primary breast cancer after neoadjuvant chemotherapy (NAC) and whether the prognosis is affected by the change in HER2 status is not well known.

Patients and Methods: Five hundred and eighty-eight patients who were treated with anthracycline- and/or taxane-based NAC and had non-pathologic complete response between 2001 and 2008 in our hospital were enrolled. Human epidermal growth factor receptor-2 (HER2) status was assessed in specimen by needle biopsy before NAC and on the residual tumor of surgical specimen. We determine the impact of change in HER2 status on recurrence-free survival (RFS). Twenty-eight patients had received trastuzumab with NAC, and 57 patients had received trastuzumab as adjuvant therapy. HER2-positive was defined as 3+ by immunohistochemistry and/or amplification by fluorescent in situ hybridization. Association between change in HER2 status after NAC and clinicopathologic factors, including age, clinical T stage, estrogen receptor (ER), progesterone receptor (PR), Nuclear grade (NG), lymphovascular invasion (LVI) and Trastuzumab usage (NAC and Adjuvant setting) were determined.

Result: A median follow-up period was 57 months (range, 3 to 131 months). Four hundred eighty-nine of the 588 patients (83.1%) had HER2-negative tumors and 99 patients (16.8%) had HER2-positive tumor before NAC. In 11 of the 489 patients (2.2%) HER2-negative changed to HER2-positive. In 33 of the 99 patients (30%) HER2-positive changed to HER2-negative. In clinicopathologic factors, ER and PR positive before NAC were associated with incidence of change in HER2 status after NAC. Receiving trastuzumab was not correlated with incidence of change in HER2 status. In terms of RFS, there was no difference between patients with and without change in HER2 status in both of the 489 patients with HER2-negative tumors and 99 patients with HER2-positive tumors before NAC (p=0.26, p=0.23, respectively).

Conclusion: We herein reported the incidence of change in HER2 status after NAC with the largest sample size. However, change in HER2 status did not seems to affect prognosis. Further prospective study is needed to confirm the prognostic impact of change in HER2 status.
Body: Background

Previous studies have shown that the menstrual cycle phase can influence PgR status of breast cancer. But data on whether the menstrual cycle phase affects Ki67 expression is inconsistent. This study aims to compare the Ki67 expression on ultrasonography guided vacuum-assisted breast biopsy (US-guided VABB) with matched breast cancer surgical specimens.

Materials and Methods

In 120 breast cancer patients without neoadjuvant chemotherapy who underwent US-guided VABB and surgical resection from April 2008 and March 2012 at Aichi Cancer Center Hospital, we examined the concordance of Ki67 level between US-guided VABB and surgical specimen. All the US-guided VABB were performed using 11-gauge Mammotome. In this study, the Ki67 cut-off level for positivity was defined at 20%.

Two phases of the menstrual cycle were pre-defined as indicated: phase 1 (low estrogen) days 27–35 or 1–6; phase 2 (high and intermediate estrogen) days 7–26 (Hayes BP, et al. Breast Cancer Res Treat 2013).

We defined the three groups as follows: the non-matching menstrual phase group (different menstrual cycle phase at the time of biopsy and surgery: n=18), the matching menstrual phase group (same menstrual cycle phase at the time of biopsy and surgery: n=25), and the post-menstrual group (n=77).

We evaluated the discordance of Ki67 expression between US-guided VABB and surgical specimens in the three groups.

Results

A differential expression of Ki67 was found in 13 patients and the concordance rate of Ki67 expression between US-guided VABB and surgical specimens was 89.2% with a Kappa statistic value of 0.78. (The concordance rate of ER, PgR, and HER2 status were 96.4%, 90.2%, and 97.0%, respectively.)

There were no major differences in tumor and patient characteristics (age, pathological tumor size, and number of biopsy specimens) between the non-matching menstrual phase group and the matching menstrual phase group.

The discordance rate of Ki67 expression for the non-matching menstrual phase group, the matching menstrual phase group, and the post-menstrual group were 22.2%, 4.0%, and 10.4%, respectively. In the patients with ER positive tumors, the discordance rate of Ki67 expression for the non-matching menstrual phase group, the matching menstrual phase group, and the post-menstrual group were 23.5%, 4.8%, and 10.9%, respectively. The discordance rate of Ki67 expression tended to be higher in the non-matching menstrual phase group than in the matching menstrual phase group. (p=0.11)

Conclusions

Though limited by the low number of patients, our study suggested that the menstrual cycle could affect Ki67 expression as patients with different menstrual cycle phase at the time of biopsy and surgery show discordant results. Prospective evaluation of Ki67 expression in premenopausal patients with ER positive tumor is needed.
Potential biomarkers of response to primary antiangiogenic and hormonal therapy in post-menopausal women with hormone-positive, HER2-negative primary breast cancer

Helena Verdaguer¹, Serafin Morales², Valentí Navarro¹, Alba Martinez Lopez¹, Anna Petit¹, Fina Climent¹, Oriol Casanovas¹ and Sònia Pernas¹. ¹Institut Català d’Oncologia, Hospital de Bellvitge, IDIBELL, Hospital del Llobregat, Barcelona, Spain and ²Hospital Arnau de Vilanova, Lleida, Spain.

**Body: Introduction**
The role of antiangiogenic therapy in primary hormonal therapy in HER2-negative ER-positive early breast cancer is unknown. Potential biomarkers of response to antiangiogenic therapy are lacking. A phase I clinical trial was conducted with sunitinib and exemestane given at conventional dose (25 mg/d) during 6 months, before surgery. 18 patients were enrolled, 15 in dose level 0 of sunitinib (25 mg/d) and 3 in dose level 1 (37.5 mg/d). Results were presented in SABCS 2011. Main toxicities were: asthenia (50%), leucopenia (28%, all grade 2), diarrhea (28%), mucositis (22%), and hypertension (22%). 10 patients achieved radiological partial response (56%) and 8 patients stable disease (44%). 7 patients (38.89%) obtained a pathological downstaging. Potential biomarkers of response to antiangiogenic therapy are presented.

**Materials and methods**
Tissue samples were obtained by fine-needle aspiration or core needle biopsy before starting treatment, one month after and at surgery. All samples were formalin-fixed paraffin-embedded. Ki67, phospho-ERK and mean vessel density (by CD34), were analyzed by immunostaining. At the same time points, plasma levels of angiopoietin 2 (ANG2), soluble VEGFR2 and VEGF were analyzed by ELISA. Basal levels and its changes over time were evaluated and correlated with clinical outcomes.

**Results**
Changes in Ki67 were observed, with a median value of 16.44% pre surgery and 12.78% post surgery (p=0.062). A significant decrease in mean vessel density was not observed.

Basal levels of plasmatic biomarkers are shown in the next table:

<table>
<thead>
<tr>
<th></th>
<th>SD</th>
<th>PR</th>
<th>p</th>
<th>Path Downst</th>
<th>No Path Downst</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANG2</td>
<td>2396+/-650</td>
<td>3455+/-1394</td>
<td>0.08</td>
<td>3184+/-1610</td>
<td>2818+/-768</td>
<td>0.6</td>
</tr>
<tr>
<td>VEGF</td>
<td>110+/-125</td>
<td>77+/-66</td>
<td>0.57</td>
<td>94+/-63</td>
<td>88+/-121</td>
<td>0.9</td>
</tr>
<tr>
<td>VEGFR2</td>
<td>10570+/-225</td>
<td>12110+/-4394</td>
<td>0.35</td>
<td>12140+/-3217</td>
<td>10980+/-3196</td>
<td>0.55</td>
</tr>
</tbody>
</table>

Values of biomarkers are average in ng/ml ± Standard Deviation. Differences analyzed by student’s t-test. Abreviations: SD= stable disease. PR= partial response. p = p value. Path. Downst.= Pathological downstaging. Furthermore, there was a significant decrease in VEGFR2 mean levels after one month of treatment (p=0.0046): 12284±3449 ng/ml at baseline; 8148±3216 ng/ml at one month and 7732±3052 ng/ml at 6 months. Differences between basal and one month determination were significant (p<0.01), but no differences were seen between 1 month and 6 months, showing a relevant early decrease of VEGFR2 plasma levels. In contrast, levels of VEGF did not change significantly over time and had no association with clinical outcomes.

**Conclusions**
Baseline ANG2 levels have a promising predictive value of response in this phase I trial of neoadjuvant combination of sunitinib plus exemestane. There is a significant early decrease in VEGFR2 with treatment with sunitinib. These results should be validated in further studies to improve the selection of patients for antiangiogenic+hormonal therapy.
Title: Feasibility of the PROSIGNA® multigene test in core biopsies and comparison to corresponding surgical breast cancer sections

Aleix Prat¹, Patricia Galván¹, Wesley Buckingham³, Maria Vidal¹,², Sherley Díaz², Paolo Nuciforo², Sean Ferree³, Barbara Adamo⁴, Santiago Ramon y Cajal² and Vicente Peg². ¹Vall d'Hebron Institute of Oncology (VHIO), Barcelona, Spain; ²Vall d’Hebron University Hospital; ³Nanostring Technologies and ⁴Hospital Clínic i Provincial.

Body: Background: The PROSIGNA® (PAM50) gene assay has been validated on formalin-fixed paraffin embedded (FFPE) surgical resection specimens (SRS) to identify the intrinsic subtypes of breast cancer and to estimate the 10-year risk of recurrence (ROR) in post-menopausal patients treated with adjuvant endocrine therapy. However, demonstration of the ability to perform PAM50 assay in diagnostic core biopsy specimens (CBS) before primary surgery and/or systemic therapy could be clinically useful. The objectives of this study were to 1) evaluate the feasibility of performing the PAM50 assay in CBS and 2) compare the PAM50 results from paired CBS and SRS.

Methods and materials: Baseline tissue surface area, cellularity and RNA yield (obtained after ~10 FFPE 10µm sections) were determined in CBS from 30 newly diagnosed breast cancer patients. The tissue volume requirements determined from these samples reflected the lower 95% confidence limits of a minimum RNA concentration of >20ng/µL. The RNA yield and assay pass rate of the established tissue volume requirements were then tested in 30 independent CBSs. Intrinsic subtype concordance, and ROR score variability, were determined from 1) multiple extractions of the same CBS (10 independent cases for a total of 84 extractions) and 2) multiple CBS of the same tumor (30 independent cases for a total of 79 CBS). To test the PAM50 assay concordance between paired CBS and SRS, the following PAM50 data were evaluated in an independent and retrospective set of 33 paired samples: 4-class subtype classification (Luminal A, Luminal B, HER2-enriched and Basal-like), 3-class subtype classification (Luminal A/B, HER2-enriched and Basal-like), ROR score (0-100), proliferation score and the correlation to each subtype centroid. Correlation and concordance between CBS and SRS were estimated using Pearson coefficients and multi-rater kappa values, respectively.

Results: Baseline median surface area, cellularity and RNA yield concentration were 10.2 mm², 45% and 155.3 ng/µL, respectively. Correlation of surface area and cellularity with RNA yield concentration was low (Person coefficient <0.25). Minimum tissue requirements were determined based on surface area: >12 mm² = 2 10-micron slides, 6-12 mm² = 4 slides; <6 mm² = 8 slides. Subtype calls on multiple extractions from the same CBS were 98% concordant (82/84) and the average ROR score standard deviation (SD) was 2.2 units. Subtype calls on different CBS of the same tumor were 94% concordant (74/79) and the average ROR score SD was 6.8 units. All 7 discordant cases were between Luminal A and B calls. Comparison between paired CBS and SRS revealed correlations of ≥0.90 (range 0.90-0.98) for ROR scores, proliferation scores and subtype centroid correlations. Intrinsic subtype concordance between paired CBS and SRS was 87% (kappa=0.81) and 97% (kappa= 0.91) for the 4-subtype and 3-subtype classifications, respectively. Finally, the overall PAM50 assay pass rate in CBS was >95%.

Conclusions: The PAM50 assay in CBS is feasible and measurements are comparable with surgical resections, which suggest that PAM50 can be performed on diagnostic core biopsy tissues.
Title: Association of baseline pro-inflammatory (IL-6, CRP) and coagulation (D-dimer) markers with baseline functional status in women with breast cancer (BC) undergoing chemotherapy

Yuan Yuan¹, Nilesh Vora², Tao Feng¹, Joanne Mortimer¹, Thehang Luu¹, George Somlo¹, Joseph Chao¹, Vivi Tran¹, Shu Mi¹, Tim Synold¹, James Waisman¹, Laura Zavala¹, Vani Katheria¹ and Arti Hurria¹. ¹City of Hope, Duarte, CA and ²Long Beach Memorial Medical Center, Long Beach, CA.

Body: Background: Pro-inflammatory and coagulation factors such as IL-6, CRP and D-dimer serve as biomarkers for aging. The utility of these markers as biologic correlates of physical function in patients with BC is not known. This study was performed to determine if baseline serum markers of inflammation (IL-6, CRP) and coagulation (D-dimer) correlate with baseline functional status in women with stage I-III BC requiring chemotherapy (chemo).

Methods: This is a prospective longitudinal study that enrolled 153 women across all age groups with BC who had pre-chemotherapy peripheral blood captured for IL-6, CRP, and D-dimer and a baseline assessment of the following functional status measures: activities of daily living (Medical Outcomes Study [MOS] Physical Health); instrumental activities of daily living (IADL); self-rated Karnofsky performance status (KPS); physician-rated KPS; number of falls in last 6 months; and Timed Up and Go (TUG). Peripheral blood samples were collected for measurement of IL-6, CRP and D-dimer. Quantitative IL-6 and CRP levels were obtained using NOVEX® immunoassay (Invitrogen) and D-dimer levels were measured with Nanopia® D-dimer(Sekisui). Univariate analyses were performed to describe correlations of these three biomarkers and 6 measures of physical function.

Results: 153 patients (mean age of 57.5 y, range 30-81 y) with stage I-III BC (Stages I [n=35; 23%], II [n=82; 54%], III [n=36; 24%]) were enrolled. Chemo regimens include: doxorubicin+cyclophosphamide/ paclitaxel(AC-T: 44%), docetaxel/cyclophosphamide (TC: 35%), docetaxel/carboplatin/trastuzumab (TCH: 7%) and other regimen(14%). Scores for the physical function measures are as follow: MOS (median 89, range 0-100); IADL (median 14, range 4-14); self-rated KPS (median 90, range 60-100); physician-rated KPS (median 100, range 80-100); TUG (median 9 seconds, range 5-18). Serum biomarkers measurements and distributions are listed in table 1. There were associations between decreased physical function by IADL and increased IL-6 (p<0.01); decreased MOS and increased D-dimer (p<0.01); increased number of falls and increased CRP (p=0.02) and D-dimer (p=0.04); increased TUG and increased IL-6 (p<0.01), CRP (p<0.01) and D-dimer (p=0.06) (table 2). Physician and patient-rated KPS did not correlate with IL-6, CRP and D-dimer level.

Conclusions: Baseline measures of inflammation and coagulation correlate with physical function measures among patients with breast cancer. Future analyses evaluating the association between aging biomarkers and measures of physical function with subsequent risk of chemotherapy toxicity is underway.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Spearman Coefficient</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MOS vs D-dimer</td>
<td>-0.21</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>IADL vs IL-6</td>
<td>-0.27</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Table 1. Serum biomarkers measurement at baseline prior to initiation of chemotherapy

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Median</th>
<th>Range</th>
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</thead>
<tbody>
<tr>
<td>IL-6 (pg/ml)</td>
<td>4.3</td>
<td>5.1</td>
<td>3.0</td>
<td>0-48.0</td>
</tr>
<tr>
<td>CRP(µg/ml)</td>
<td>5.7</td>
<td>7.9</td>
<td>2.8</td>
<td>0.1-48.4</td>
</tr>
<tr>
<td>D-dimer(µg/ml)</td>
<td>0.7</td>
<td>0.6</td>
<td>0.6</td>
<td>0.1-3.3</td>
</tr>
</tbody>
</table>

Table 2. Univariate analysis of measures of physical functions versus biomarkers
<table>
<thead>
<tr>
<th>Test</th>
<th>Correlation Coefficient</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of falls vs D-dimer</td>
<td>0.16</td>
<td>0.04</td>
</tr>
<tr>
<td>No. of falls vs CRP</td>
<td>0.19</td>
<td>0.02</td>
</tr>
<tr>
<td>TUG vs D-dimer</td>
<td>0.15</td>
<td>0.06</td>
</tr>
<tr>
<td>TUG vs IL-6</td>
<td>0.26</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TUG vs CRP</td>
<td>0.23</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Microarrays have shown that triple-negative breast cancers (TNBCs) are a heterogeneous group of disorders that have differential responses to chemotherapy. On the other hand, BRCA genes play an important role in DNA damage repair. Gene mutations and methylation of BRCA genes cause functional abnormalities, leading to defects in DNA repair capacity. This state is called "BRCAness." In this study, we addressed BRCAness, therapeutic effects, recurrence, and prognosis in patients with TNBCs who were treated with neoadjuvant chemotherapy.

We enrolled 40 patients with TNBC who were treated with neoadjuvant chemotherapy (anthracyclines alone in 3 patients and anthracyclines plus taxanes in 37 patients) at our hospital between April 2006 and October 2012. BRCAness was determined by preoperative core needle biopsy (CNB) specimens and surgical specimens. Genes from those specimens were amplified by multiplex ligation-dependent probe amplification (MLPA), and the amplicons were scored after separation by electrophoresis. With a cutoff value of 0.5, values of 0.5 or more were determined as the BRCA1-like Type (BRCAness) and those of less than 0.5 as the Sporadic Type to analyze clinical effects, pathological complete response (pCR) rate, recurrence, and prognosis.

With regard to therapeutic effects of neoadjuvant chemotherapy, pCR (ypT0/Tis/N0) was observed in 15 patients and non-pCR in 25 patients (pCR rate: 37.5%). Twelve patients had recurrence after surgery, and 8 of whom died of the original disease.

(1) The BRCA1-like Type accounted for 22 patients while the Sporadic Type accounted for 18 patients in CNB specimens. No major differences were observed between the BRCA1-like Type and Sporadic Type with regards to the pCR rate (7/22 vs. 5/18). Those two types had equivalent results in recurrence rate and prognosis.

(2) Among the 24 non-pCR patients whose BRCA status could be determined by surgical specimens, 9 were found to be of the BRCA1-like Type. Patients with a BRCA1-like tumor had more recurrences (7/9 vs. 5/15), and their relapse-free survival was also lower (p<0.05). No association with prognosis was found. Six patients whose BRCA status of CNB specimens was of the BRCA1-like Type and that of surgical specimens turned to be of the Sporadic Type were better in recurrence (p<0.01) and prognosis (p<0.05), compared with seven patients whose BRCA status of surgical specimens remained to be of the BRCA-1 like Type.

Patients with TNBC who achieved a pCR by neoadjuvant chemotherapy had a better prognosis even if the type is BRCA1-like. In contrast, the recurrence rate was higher when residual tumor remained after neoadjuvant chemotherapy and when the BRCA status became BRCA1-like. New clinical trials assessing the true recurrence (TR) rate of BRCA are expected since neither platinum-containing drugs nor poly (ADP-ribose) polymerase (PARP) inhibitors are effective against tumors with nonfunctional BRCA genes.
**Title:** Assessment of the prognostic and predictive ability of a gene signature compared to histological grade in estrogen receptor positive, HER2 negative breast cancer

Takayuki Iwamoto\(^1\), Catherine Kelly\(^2\), Giampalo Bianchini\(^3\), Takeo Mizoo\(^1\), Tomohiro Nogami\(^1\), Takayuki Motoki\(^1\), Tadahiko Shien\(^1\), Naruto Taira\(^1\), Naoki Hayashi\(^2\), Naoki Niikura\(^4\), Toshiyoshi Fujiwara\(^1\), Hiroyoshi Doihara\(^1\) and Junji Matsuoka\(^1\). \(^1\)Okayama University Hospital; \(^2\)Mater Misericordiae University Hospital; \(^3\)San Raffaele Hospital; \(^4\)St Luke's International Hospital and \(^5\)Tokai University Hospital.

**Body:**

**Background:** Genomic biomarkers have been widely adopted to assist in clinical decision making regarding chemotherapy use in estrogen receptor (ER)-positive, HER2-negative breast cancer. First generation genomic signatures (FGGS) serve predominantly as prognostic biomarkers and secondarily have a role in prediction of chemotherapy response. The majority of the FGGSs provide similar prognostic information which mainly capture in different ways tumor proliferation. While several studies have compared the prognostic value of FGGSs to clinico-pathological variables, few studies have performed a similar comparison for their predictive value. For this reason, we aimed to compare both the prognostic and predictive value of histological grade and the genomic marker.

**Methods:** We retrieved publicly available cDNA microarray data from 1,373 primary ER+/HER2- breast cancers (n=721 treated with various or unknown, n=350 untreated node negative, n=302 treated with neoadjuvant chemotherapy). We developed a genomic signature simulated from recurrence online (http://www.recurrenceonline.com/) to calculate recurrence score and recurrence risk using pre-defined sets of genes by cDNA microarray (B Gyorffy Breast Cancer Res Treat 2012). Breast cancers were categorized as low, intermediate or high risk for distant recurrence using grade and genomic signature. We compared the prognostic and predictive information provided by histological grade to the genomic signature. The outcome of interest in untreated patient was distant event free survival. The outcome of interest in the anthracycline-taxane treated patients was pathological complete response (pCR) in breast and axilla.

**Results:** Fifty five, 28 and 17% breast cancers were classified as low, intermediate and high risk by genomic signature and 22, 59 and 19% as grade I, II and III respectively. The genomic signature classified 11% of grade I/II cancers (126/1108) and only 42% of grade III cancers (112/265) as high risk, and 29% of Grade III (77/265) as low risk. Univariate analysis in the untreated cohort, showed both histological grade (overall p=0.007) and the genomic signature (p<0.001) could predict prognosis. In multivariate analyses for tumor size, age, grade and genomic signature, only the genomic signature remained statistically significant for prognosis. As expected a significantly higher rate of pCR was observed in histological grade III cancers (15.9%) compared to grade I (3.4%) and II (3.8%) after neoadjuvant chemotherapy (NAC). Results were similar using the genomic signature with pCR rates of 4.6%, 5.7% and 16.5% for low, intermediate and high risk, respectively. Grade I and II cancers (n=189) classified as high risk by the genomic signature had a pCR rate of only 2.8%. Instead, the grade III tumors which were also defined at high risk by the genomic signature had a pCR rate of 26.5%. Multivariate predictive models showed neither biomarker retained statistical significance in predictive response to NAC.

**Interpretation:** The genomic signature was better at identifying low risk cases compared to histological grade alone. There was no difference in prediction of NAC response between either biomarker. Better predictive biomarkers for NAC response are needed.
Title: Prognostic significance of breast cancer index (BCI) in node-positive hormone receptor positive early breast cancer: NCIC CTG MA.14

Dennis Sgroi¹, Paul Goss¹, Judy-Anne Chapman², Elizabeth Richardson¹, Shemeica Binns¹, Yi Zhang³, Cathy Schnabel³, Mark Erlander³, Kathy Pritchard⁴, Lei Han², Lois Sheperd² and Michael Pollack⁵. ¹Massachusetts General Hospital, Boston, MA; ²Queens University, Kingston, ON, Canada; ³bioTheranostics, Inc, San Diego, CA; ⁴Sunnybrook Odette Cancer Centre, University of Toronto, Toronto, ON, Canada and ⁵McGill University, Montreal, QC, Canada.

Body: Background: The continuous linear Breast Cancer Index (BCI) risk index combines the ratio of genes HOXB13 to IL17BR (H/I) and the molecular grade index (MGI) (Zhang et al, Clinical Cancer Research, 2013). The BCI signature was developed for node-negative breast cancer patients treated with tamoxifen. We examine here whether linear BCI is prognostic for node-positive hormone-receptor positive tamoxifen-treated patients.

Methods: MA.14 randomly assigned 667 hormone positive (HR+), postmenopausal women to 5 years of tamoxifen (TAM) +/- 2 years of octreotide LAR (TAM-OCT). A representative subgroup of 299 patients underwent gene expression profiling by RT-PCR for linear BCI. We performed exploratory analyses restricted to node positive patients. The primary objective was to assess the prognostic effect of BCI on relapse-free survival (RFS). RFS was defined as the time from randomization to the time of recurrence of the primary disease alone, including local and ipsilateral nodal recurrence and metastatic disease, and censoring at longest follow-up or death from another cause. With a median 9.8 years follow-up, the association of BCI with RFS was assessed by multivariate Cox regression including treatment, stratification factors (other than nodal status), and baseline patient and tumor characteristics. Patients were defined to be low risk based on BCI if the adjusted Cox survival was >95%, where adjustment was by trial treatment, stratification factors, and baseline patient and tumor characteristics, including IGF-1, IGFBP-3, and C-peptide.

Results: 292 of 299 patient samples passed internal analytical quality control; 116 node positive ER+ve patients had 34 (29.3%) relapses, with adjusted Cox survival at 9.6 years of 87.8%. Fifty-two of the 116 patients (45%) did not receive adjuvant chemotherapy, and experienced 11 (21%) RFS events. In the 116 patients, higher continuous BCI value was associated with shorter RFS (p=0.002): hazard ratio (HR) 1.49 (95% CI 1.16-1.91). Smaller pathologic T had significantly (p=0.03) better RFS HR=0.39, (95%CI 0.17-0.90). With MA.14 patient mean BCI of 5.09532, Cox survival at 4.1 years was 95.2%; 17/34 (50%) who recurred had failed by this time.

Discussion: In this subgroup analysis, we found that BCI and tumor size were significant prognostic factors for node-positive hormone-receptor positive patients who were treated with tamoxifen.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-01-11
Average Grade: 4.00

Title: The neuronal protein sortilin is expressed in aggressive breast cancers and participates in tumor cell growth and invasion

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Body: The membrane protein sortilin is involved in intracellular trafficking and has emerged as a key player in the regulation of neuronal viability and function. Few studies have suggested that sortilin may also be implicated in cancer, but its expression in human tumors and potential value as a therapeutic target is unknown. In this study, the level of sortilin was analyzed in a series of 318 clinically annotated breast cancers and 53 normal adjacent tissues by immunohistochemistry. Sortilin was specifically localized in epithelial cells and was detected in 65% of cancers compared to 46% of normal tissues (p=0.0088). Sortilin was detected in 79% of invasive ductal carcinomas compared to only 54% of invasive lobular carcinomas (p<0.0001). Interestingly, sortilin was associated with lymph node involvement (p=0.0093), suggesting a relationship with metastatic potential and poor outcome. In Log-Linear modeling, two-way analyses confirmed the association of sortilin with histological type (ductal vs. lobular invasive carcinomas) and lymph node invasion (p=0.002 and p=0.096 respectively). In vitro, sortilin was detected in a range of cancer cell lines using RTPCR and Western-blotting. Functional investigation using RNA interference revealed that a decrease in sortilin level in the highly metastatic breast cancer cell line MDA-MB-231 resulted in a reduced proliferation and invasiveness, but had no effect on cell viability or apoptosis. Together, these data reveal that sortilin is expressed in aggressive breast cancers and is a potential therapeutic target.
**Title:** Tumor-associated macrophages in tumor stroma as potential diagnostic and prognostic biomarkers in breast cancer

Zheli Xu¹, Wan Wang¹, Xinyu Feng¹, Yangao Man², Keren Wang¹ and Liang Sun¹. ¹Breast Surgery of the 3rd Clinical Medical College of Norman Bethune Health Science Center of Jilin University, Changchun, Jilin, China and ²Diagnostic and Translational Research Center, Henry Jackson Foundation for the Advancement of Military Medicine, Gaithersburg.

**Body:** Background: Tumor-associated macrophages (TAMs) within the tumor microenvironment directly affect tumor cell growth, extracellular matrix remodeling, and tumor angiogenesis. The majority of TAMs exhibit alternatively activated M2 properties, promote tumor development. In human breast cancer, TAMs are increasingly recognized as pivotal regulators, and high levels of TAMs are often correlated with poor prognosis and closely related to the process of metastasis. CD163 is regarded as a highly specific biomarker for M2 macrophages. In the present study, we evaluated the specificity M2 macrophage marker CD163 as a TAM marker and compared its prognostic value with clinicopathological parameters.

Methods: The breast cancer cohort analyzed consisted of 580 cases pathologically diagnosed with breast cancer from June, 2012 to May 2014, and all the chosen cases had not been accepted any chemotherapy or radiotherapy before operation. Mean age was 44.5 years (range from 19 to 84 years). CD163 was regarded as a specific biomarker for TAMs, and the extent of infiltrating CD163+ macrophages in tumor tissue was evaluated by immunohistochemistry. Meanwhile, Ki67, MMP-9, VEGF, HER2, estrogen receptor and progesterone receptor were also detected by immunohistochemistry in all these tumors. Spearman’s Rho and $\chi^2$ tests were used to examine the correlations between CD163+ macrophages and clinicopathological parameters.

Results: We found that CD163+ macrophages mainly infiltrated into tumor stroma, but not into tumor nest, were of clinical relevance. The density of CD163 was highly correlated with invasiveness status of breast cancer, CD163+ were positive in 93.8% (495/528) invasive breast cancer cases and were positive in 59.4% (19/32) carcinoma in situ cases. CD163+ macrophages in tumor stroma positively correlated with higher vascular grade, larger tumor size, Ki67 positivity, MMP-9 positively, higher VEGF expression, HER2 positively, estrogen receptor negativity, progesterone receptor negativity, triple-negative breast cancer and closely correlated with increased nodal metastasis. However, the relationships among CD163+macrophage and recurrence free survival, breast cancer specific and overall survival had not been comparatively analyzed in our study yet.

Conclusion: These findings highlight the importance of analyzing the density of TAMs as a diagnostic and prognostic biomarker for breast cancer patients. Generally, high density of TAMs predicts high tumor grade, HR-negatively, lymph node and distant metastasis. TAMs will be a promising therapeutic target and prognostic biomarker.
Title: SOX10 and folate receptor alpha are frequently expressed in triple negative and progesterone receptor negative breast cancers

Laura L Hoang¹, Weimin Qi¹, Charlie Yu¹ and David Tacha¹. Biocare Medical, Concord, CA.

Body: Background:
Specific biomarkers can be essential for developing effective treatments for aggressive breast cancers, especially triple negative subtypes, for which treatment options are limited. Folate receptor alpha (FRα), a critical membrane protein for DNA synthesis and cell metabolism, has been suggested to participate in the transformation of breast cancer into aggressive subtypes. It has been shown to be strongly associated with poor prognosis in triple negative breast cancers (TNBC) as well as estrogen receptor (ER) positive and progesterone receptor (PR) negative subtypes.

SOX10 is a nuclear transcription factor that participates in neural crest development and in the differentiation of cells of melanocytic lineage. Data suggests that SOX10 may contribute in stem cell or progenitor cell maintenance. Recently, SOX10 expression has also been documented in benign breast myoepithelial cells and in aggressive breast cancers. The correlation of FRα and SOX10 in breast cancer is not fully known. This is the first study to compare FRα and SOX10 immunohistochemical profiles in breast cancers with emphasis in TNBC.

Design:
166 cases of whole breast cancer tissues were classified according to their ER, PR, and HER2 immunohistochemical (IHC) status. These same cases were then IHC stained for mouse monoclonal SOX10 and FRα. Cut-off values of 1% and 5% for SOX10 and FRα, respectively, were used to determine positivity.

Results:
SOX10 achieved a sensitivity of 42.1% (8/19) in ER+/PR-/HER2- cases and was negative in all ER+/PR+/HER2+ cases (p<0.05). FRα was positive in 7.6% (7/92) of ER+/PR+/HER2- cases and was negative in all ER+/PR+/HER2+ cases. SOX10 identified more ER+/PR-/HER2- cases (42.1%, 8/19) than ER+/PR+/HER2+ cases (7.7%, 1/13) (p<0.05). Similarly, FRα stained 52.6% (10/19) of ER+/PR-/HER2- cases and was negative in all ER+/PR+/HER2+ cases (p<0.005). SOX10 and FRα were observed in 3.3% (1/30) and 20% (6/30) of HER2+ cases, respectively.

In ER-/PR-/HER2- (triple negative) cases, both markers were highly expressed with 40.0% (10/25) and 52.0% (13/25) positive cases with SOX10 and FRα, respectively, with 24.0% (6/25) of cases positive with both markers. Approximately one half of TNBC cases expressed SOX10 and FRα; however, most SOX10 positive TNBC cases did not overlap with FRα positive TNBC cases.

Table 1: SOX10 and FRα expression in breast cancer subtypes

<table>
<thead>
<tr>
<th>ER/PR/HER2 Classification</th>
<th>SOX10+ (%)</th>
<th>FRα+ (%)</th>
<th>Co-expression of SOX10 and FRα (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER+/PR+/HER2+ (n=13)</td>
<td>1 (7.7%)</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>ER+/PR+/HER2- (n=92)</td>
<td>6 (6.5%)</td>
<td>7 (7.6%)</td>
<td>2 (2.2%)</td>
</tr>
<tr>
<td>ER+/PR-/HER2+ (n=10)</td>
<td>0 (0%)</td>
<td>5 (50.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>ER+/PR-/HER2- (n=19)</td>
<td>8 (42.1%)</td>
<td>10 (52.6%)</td>
<td>5 (26.3%)</td>
</tr>
<tr>
<td>HER2+ (n=30)</td>
<td>1 (3.3%)</td>
<td>6 (20.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>ER-/PR-/HER2- (n=25)</td>
<td>10 (40.0%)</td>
<td>13 (52.0%)</td>
<td>6 (24.0%)</td>
</tr>
</tbody>
</table>

Conclusion:
SOX10 and FRα were frequently expressed in triple negative breast cancers and in progesterone receptor negative breast cancers. Our data suggests that there may be different mechanisms by which SOX10 and FRα are implicated in aggressive breast cancers. These findings may help achieve a better understanding of the two different pathways involving stem cells.
(SOX10) and growth factors (FRα), their potential prognosis and their therapeutic management in the future.
Expression of SOX10 in invasive ductal carcinoma of the breast

Laura L Hoang¹, Jianmin Wang², David Tacha¹, Huijiao Chan³, Bing Wei³, Zhang Zhang³, Hong Bu³, David G Hicks⁴ and Ping Tang⁴. ¹Biocare, Inc, Concord, CA; ²RTI Health Solutions, Research Triangle Park, NC; ³Sichuan University, West China Hospital, Chengdu, China and ⁴University of Rochester Medical Center, Rochester, NY.

Body: Background: The transcription factor SOX10 mediates the differentiation of neural crest-derived cells, and expression of SOX10 detected by IHC analysis is primarily used to support the diagnosis of melanoma. Expression of SOX10 has been recently reported in benign breast myoepithelial cells and triple negative breast cancer. The aim of the current study is to investigate the expression pattern of SOX10 in a cohort of invasive ductal carcinoma (IDC) tumors, and analyze its relationship with different clinicopathological features and clinical outcome. Methods: Four hundred-twenty eight cases of IDC of the breast diagnosed in our institution between 1997 and 2008 and having follow-up information were included in this study. Immunohistochemical studies for SOX10 (≥1% of tumor cells having nuclear staining designated as positive expression), ER, PR, HER2 and Ki-67 were performed on 25 pre-constructed tissue microassay blocks. The relationship between SOX10 expression and the clinicopathologic features, expression of ER, PR, HER2 and Ki-67, and clinical outcome were evaluated. Results: Among this cohort of patients, the majority of them were greater than 50 years old, with grade 1 or 2 tumors that were less than 2 cm and node negative. 81% of the tumors were ER positive, 75% were PR positive and 8% HER2 positive. The overall expression rate of SOX10 was 18%; however, its expression rates were significantly higher in high grade tumors (31%), and tumors that were ER (53%) and PR (41%) negative, and tumors with high Ki-67 expression (designed as >15% nuclear labeling, 47%). 51% of ER-/PR- tumors, 64% of triple negative tumors and 68% of Basal-like tumors expressed SOX10. SOX10 expression was not associated with patient age, tumor size, nodal status and over-expression of HER2. When we looked at the 4 different levels of SOX10 expression (1-25%, 26-50%, 51-75% and 76-100% of cells with positive nuclear staining) in different subgroups of breast cancer, we noted that the majority of all cases had high levels of SOX10 expression (76-100% cells with nuclear staining) regardless of their ER, PR, HER2 and Ki-67 status. No significant difference in overall survival (p value=0.8553) and disease-free survival (0.1810) between SOX10 positive and negative tumors was noted. Conclusion: Our data demonstrates a significant association between SOX10 expression and high grade, ER/PR negative, triple negative or basal-like IDC, suggesting their myoepithelial differentiation. Given that SOX10 has been recently described as a principle driver for melanoma, better understanding of SOX10 expression and signalling would provide important insight in development of novel diagnostic and therapeutic tools for SOX10 expressing tumors.
Expression of high affinity folate receptor in breast cancer brain metastasis

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Body: Background: Folic acid is required by proliferating cells for the synthesis of nucleotide bases among other tasks. The high affinity folate receptor (HFR) is a membrane protein that is upregulated in several cancers of epithelial origin and rarely present in most normal cells. High expression of HFR has been documented in ovarian, breast, kidney, uterine and lung cancers. In breast cancer patients, HFR is overexpressed in 33% of primary tumors (PT) and this is associated with poor prognosis. The HFR expression in breast cancer brain metastases (BCBM) is unknown. The aim of this study was to analyze the incidence of HFR expression in BCBM and its role in the prognosis of this high-risk patient subgroup.

Methods: We analyzed a database of 42 patients who underwent craniotomy with resection of breast cancer brain metastasis (BCBM). Of those, we collected 19 brain metastasis (BM) and 13 PT. HFR status was assessed by immunohistochemistry. Log-Rank test analyzed differences in overall survival (OS) between groups.

Results: Median age was 51 years (range 24-74). Median follow-up was 4.2 years (range 0.6-18.5). HFR was positive in 4/19 BM (21.1%) and in 1/13 PT (7.7%). Positive samples had low H-scores (range 1-50). Staining with H&E revealed that 11/19 BM (57.9%) and 7/13 PT (53.8%) had apocrine differentiation. Analysis of OS showed no difference between patients with positive HFR (median OS 48 months) and negative HFR (median OS 69 months) (P=0.25). Similarly, there was no difference in OS between patients with apocrine differentiation (median OS 63 months) and those without apocrine differentiation (median OS 69 months) (P=0.49).

Conclusions: To the best of our knowledge, this is the first analysis of HFR expression in BCBM. While previous studies associated the presence of HFR with worse prognosis; in this cohort of high-risk patients, HFR was positive in only 21.1% of BM with low levels of positivity. Around half of our patients had apocrine differentiation. Neither the status of HFR nor the presence of apocrine features had impact in OS in our cohort.
Title: Overexpression of mucin 4 and tumor necrosis alpha are molecular features of invasive micropapillary carcinoma of the breast

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Body: Background: Invasive Micropapillary Carcinoma (IMPC) of the breast is an infrequent tumor associated with an aggressive biology. Indeed, IMPC has a high incidence of lymph node metastases. Pure cases of IMPC are rarely seen, since it is usually associated with invasive ductal carcinoma (IDC). Interestingly, the relative amount of micropapillary features does not seem to have any significant bearing on the unfavorable prognosis. Therefore, lesions comprising an IMPC component, even if subtle, must be carefully explored and informed by pathologists and should be considered by oncologists, for treatment purpose. Lymphovascular invasion is a common feature in these tumors. In fact, it has already been described that tumor necrosis factor alpha (TNF) is related to microvessel density in IMPC. On the other hand, aberrant mucin 4 (MUC4) expression directly correlates with metastatic phenotype of many cancer cell types. Nonetheless, there are no markers that highlight IMPC at the moment and its diagnosis still relies only on histological features identified by pathologists.

Aims: To assess differentially expressed markers in IMPC, we studied the expression of TNF and MUC4 in order to enhance the diagnostic accuracy of this tumor subtype.

Methods: We studied 15 primary cases of IMPC, which represented 2.9% of our total cohort. To explore the difference between IMPC and IDC, we matched 15 IDC patients to the IMPC samples and we performed immunohistochemistry (IHC) for MUC4 (1G8, Santa Cruz) and TNF (ab9739, Abcam). Both groups of patients had a median age of 56 years (38-81), their tumors expressed estrogen (ER) and progesterone receptors (PR), and had comparable histological grades (2-3) and stages (I-III). Twenty percent of the tumors were HER2 positive. MUC4 and TNF staining intensity were scored as 0, 1, 2 and 3 (negative, weak, moderate and strong staining intensity, respectively). ER, PR, and HER2 were scored according to ASCO/CAP guidelines.

Results: We observed positive cytoplasmic staining in tumor cells for MUC4 (scores 2 or 3) and TNF (scores 2 or 3) in all the 15 cases of the IMPC samples. In the IDC group, positive staining was detected for MUC4 and TNF in 7/15 and 11/15 cases, respectively. Quantitative assessment of MUC4 expression revealed a more intense staining in IMPC than in IDC (median [CI 95%]: 2 [2.2-2.7]. vs 1 [0.8-1.8], P<0.0002). Similar results were obtained for TNF expression: 3 (2.3-2.9) for IMPC and 2 (1.4-2.5) for IDC (P<0.004). MUC4 was always co-expressed with TNF in all the samples tested. Interestingly, MUC4 staining was strikingly stronger in the IMPC component compared to the associated IDC (MUC4: 3 [1.9-3.0] vs. 2 [0.2-2.7] P<0.04). High intensity of MUC4 and TNF expression was evident in IMPC despite its percentage in the IDC, and was not related with of nodal metastasis at the time of diagnosis.

Conclusion: Our results demonstrated that MUC4 is expressed in IMPC and suggest that simultaneous MUC4 and TNF overexpression is a distinctive feature of IMPC. This co-expression should be considered as a molecular signature that is present at early stages of this tumor subtype.
Title: AQP3 expression predicts survival in patients with HER2-positive early breast cancer

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Body: Background: Recent studies have revealed aquaporins (AQPs) as targets for novel anti-tumor therapy, since they likely play a role in carcinogenesis, tumor progression, and invasion. Accordingly, we analyzed the prognostic impact of AQP3 expression and polymorphisms in a number of patients with early breast cancer (EBC).

Patients and Methods: AQP3 expression was investigated on the basis of the immunohistochemistry of tissue microarray specimens from 447 EBC patients who underwent surgery between 2003 and 2008. We scored the staining intensity (0 through 3) and percentage of positive tumor cells (0 through 4), and the staining score was defined as sum of these scores used to categorize the AQP3 expression as negative (0 through 2), weak (3 through 5), or strong (6 or more). For AQP3 polymorphisms, seven SNPs (rs10813981, rs34391490, rs591810, rs2227285, rs2228332, rs17553719, and rs3860987) were selected using in silico analysis and genotyped using the Sequenom MassARRAY.

Results: A total of 180 (40.3%) of the patients were identified as AQP5-positive (staining score >2), including 86 (19.2%) cases of strong expression (stating score >5). In a univariate analysis, AQP3 expression was significantly associated with survival for the patients with HER2-overexpressing EBC. Moreover, a multivariate survival analysis revealed that AQP3 expression was an independent prognostic marker of disease-free survival (DFS: HR=3.137, 95%CI=1.079-9.125, p=0.036; Distant DFS: HR=2.784, 95%CI=0.921-8.414, p=0.070) for the HER2-overexpressing EBC patients. Meanwhile, none of selected AQP3 polymorphisms were related with AQP3 expression in tumor tissue nor survival in the current study.

Conclusion: AQP3 expression in tumor tissue may be considered as a potential prognostic marker in patients with HER2-overexpressing EBC after curative surgery.
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Publication Number: P6-01-18
Average Grade: 0

Title: 2-Hydroxyestrone is associated with breast density measured by mammography and fat:water ratio magnetic resonance imaging in women taking tamoxifen

Cynthia A Thomson¹, Patricia A Thompson¹, Betsy C Wertheim¹, Denise Roe¹, Marilyn T Marron¹, John-Phillipe Galons², Matthew A Kupinski¹, Maria I Altbach¹, Gertraud Maskarinec³ and Alison Stopeck¹. ¹University of Arizona Cancer Center, Tucson, AZ; ²University of Arizona Bio5 Institute, Tucson, AZ and ³University of Hawaii Cancer Center, Manoa, Honolulu, HI.

Body: Research Objectives and Rationale. Tamoxifen (TAM) use has been shown to reduce breast cancer recurrence with the benefit greater in patients who experience a TAM-associated decrease in percent mammographic density(PMD); findings that support PMD as a biomarker of response to TAM. PMD is a radiographic phenomenon of breast fibroglandular tissue that is associated with breast cancer risk. PMD is inversely associated with body mass index (BMI) and sparse data have shown a weak positive association with the sex hormone levels. Limited data exist evaluating the relationship between TAM, 2OHE1:16 a-OHE1 ratio (concentrations previously hypothesized to be associated with a reduced risk of breast cancer) and PBD.

Methods. Using cross-sectional baseline breast density (BD) from an ongoing prevention trial of diindolylmethane (DIM) in 121 women receiving TAM, we evaluated BD in relation to circulating TAM metabolites [TAM, endoxifen, 4-OH TAM, ND TAM], estradiol (E2), sex hormone-binding globulin (SHBG) and urinary 2-OHE1 and 16α-OHE1. PMD was assessed by mammography (n=65; 54%) and also a novel, non-radiative, non-contrast magnetic resonance imaging-derived fat-water ratio (FWR-MRI) as the fat fraction (Fra)50 and 80 (n=53; 44%) developed for repeat BD assessment in short intervals. This is our first report that BD using digitized mammograms is correlated with FWR-MRI-derived measures designated Fra50 and Fra80; Spearman ρ = 0.90 and 0.86, respectively, p < 0.001.

Results. As previously demonstrated, BMI was inversely correlated with all measures of BD. No association was shown between TAM and TAM metabolites and BD or urinary 2OHE1. Further, we found no relationship between circulating E2 or SHBG concentrations and BD. In contrast, urinary 2OHE1 levels were positively correlated with BD across all measures of density; 2OHE1 levels were most strongly correlated with BD measured by FW-MRI using Fra80 (Spearman ρFra80=0.483; p=0.001 compared to ρFra50 =0.431; p = 0.004 and ρPD=0.400; p=0.003). A significant, but weaker, correlation was observed for the 2OHE1:16OHE1 ratio and BD (ρ values 0.34-0.38). The magnitude of the relationship between 2OHE1 and BD was similar in pre and post-menopausal women despite lower PBD after menopause.

Conclusions. Our results replicate earlier work from Maskarinec et al. wherein excreted 2OHE1 was an independent determinant of BD. These data challenge the hypothesis proposed by Yager and Liehr that higher urinary 2OHE1 to 16OHE1 ratio would be indicative of reduced hormone tumorigenesis. These results suggest a possible comparable binding affinity for the estrogen receptor that may modify endogenous steroid hormones and their effects on BD. Our findings strengthen the arguments favoring a better mechanistic understanding of BD, the biological determinants and their relationship to breast cancer. This is particularly timely given new mandates to provide BD measures to all women undergoing mammography and recent findings that while BD is associated with breast cancer risk high BD is not associated with greater breast cancer mortality.
Title: A novel anti-hormone receptors (estrogen receptor and progesterone receptor) targeted water-soluble QDs for breast cancer cell immunofluorescent labeling

Zheli Xu1, Xing Ren1, Hailong Huang2, Jingyuan Wang2 and Guang Sun1. 1Breast Surgery of the 3rd Clinical Medical College of Norman Bethune Health Science Center of Jilin University, Changchun, Jilin, China and 2Alan G. MacDiarmid Institute of Jilin University, Changchun, Jilin, China.

Body: Background:
Hormone receptors as estrogen receptor (ER) and progesterone receptor (PR), play important roles in appraise the molecular properties and prognostic evaluation of breast cancer. Semiconductor QDs as the new class of fluorescent labeling agents had recently been used for a broad range of biological applications, especially early cancer diagnosis. The probes were synthesized by this novel water-soluble quantum dots to the ER and PR monoclonal antibody via chemical reaction. This research aimed to develop a novel kind of biomarker based on water-soluble QDs conjugated with ER and PR antibodies for the detection of breast cancer.

Method:
The water-soluble QDs were incubated with SP1 clone rabbit anti-human ER or PR monoclonal antibodies to obtain the ER targeted QDs (QDs-ER) and PR targeted QDs (QDs-PR) probes. Human breast cancer cells MCF7(ER+PR+) and MD-MBA-231(ER-PR-) cells were chosen for the detection. QD-based immunofluorescent staining of ER, PR were adapted from previously described protocols. Coverslips were mounted on glass slides and observed by Confocal Laser Scanning Microscope. Image analysis was performed using FV10-ASW 3.1 Viewer software. We also designed an experiment to monitor the fluorescence intensity changes of the traditional organic fluorescent dye-Alexa Fluor 488 and QDs-ER probes. MTT assays were performed to evaluate the cytotoxicity of both QDs-ER probes and QD-PR probes.

Result:
The PL and UV-vis absorption spectrum of the QDs-ER showed that the conjugation process did not affect the structure of the QD nanocrystal. QD-based immunofluorescence staining results demonstrated either QDs-ER probes or QDs-PR probes provided homogeneous labeling of the cell nuclei with nonspecific binding to the cells in MCF7 cells. On the contrary, no staining can be observed in MDA-MB-231 cells (ER-negative and PR-nagative). Besides it required only one step antigen-antibody reaction to achieve the specific detection which meant simple, time-saving and efficiently. The fluorescence intensity changes indicated that our QD probes were much more photostable than the traditional fluorescent dye Alexa Fluor 488. The cytotoxicity of QDs-ER probes and QDs-PR implied that biocompatible and no obvious toxic side effects to living breast cancer cells in vitro.

Conclusion:
In this study, we conjugated the novel anti-estrogen receptor targeted water-soluble QDs probes and analyzed their effectiveness for the detection of ER and PR in different breast cancer cell lines. Both QDs probes can be specifically, time-saving and efficiently. QD-based multi-spectral immunofluorescence labeling demonstrated it can help us gain new insight into the complex process of tumor invasion, and formulate new anti-cancer strategies. In vitro neither QDs-ER probes nor QDs-PR probes had obvious toxic side effects on living MCF7 cells. According to the above analysis, these new kinds of probes have a potential for the diagnosis of breast tumor in vitro in future clinical application and in vivo long time observation of tumors.
Title: A role of MACC1 expression and its regulation of the HGF/c-Met pathway in breast cancer

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Body: Background
The newly identified gene, metastasis-associated in colon cancer 1 (MACC1), is suggested to be a transcriptional regulator of the receptor tyrosine kinase gene c-Met, leading to cancer progression and metastasis in colorectal cancer. Also in breast cancer, aberrant hepatocyte growth factor (HGF) / c-Met signaling has been shown to contribute to worse prognosis and confer resistance to endocrine therapy or trastuzumab treatment, however, little is known of the role of MACC1. Here, we report its impact on the survival for breast cancer patients and the biological function in the cell lines.

Methods
A total of 300 breast cancer patients who received both surgery and adjuvant treatment at Kumamoto University Hospital between 2001 and 2009 were selected. We analyzed expressions of MACC1 by reverse transcription polymerase chain reaction (RT-PCR) and immunohistochemistry (IHC) to evaluate the associations of its expression with breast cancer survival. In an in vitro study, the expressions of MACC1 were examined by Western blotting in breast and colorectal cancer cell lines. After transfection with a MACC1-harboing plasmid, we evaluated the activities of cMet protein and cell motility and proliferation. Further, the binding ability of MACC1 to the cMet promoter was evaluated using chromatin immunoprecipitation (ChIP) assay.

Results
In survival analyses, reduced MACC1 expressions were associated with patient mortality. Cox proportional hazards model showed that MACC1 mRNA (HR = 0.25, P = 0.001), MACC1 protein (HR = 0.37, P = 0.016), as well as axillary nodal status and estrogen receptor status, were independent predictors of mortality. No significant correlations between MACC1 expression and other clinicopathological factors were found. We found no strong positive correlation between MACC1 protein and c-Met mRNA expression with a Spearman’s coefficient of 0.16 (P = 0.0067). In the cell lines tested, MACC1 expression was much higher in colorectal cancer cells (DLD-1) than breast cancer cells (MCF7 and MDA-MB-231). To investigate the impact of MACC1 on the biological function of the cells, we transfected with MACC1 in breast cancer and colorectal cancer cells (SW480). MACC1 overexpression did not induce cMet expression in MCF7, whereas the corresponding cMet expression was upregulated in SW480 cells. Further, SW480 cells transfected with MACC1 showed enhanced migratory ability, whereas in MDA-MB-231 cells, transfection of MACC1 had no impact on this ability. In ChIP assay, the binding of MACC1 to the cMet promoter region was suggested in SW480 cells, but not in MCF7 cells.

Conclusions
Our findings provide some novel insights into the role of MACC1 for breast cancer, indicating that it plays different roles in breast cancer and in several other cancers. The biological mechanism of MACC1 which underlies improvement of breast cancer prognosis remains unelucidated. There is possibility that MACC1 does not act as the exclusive master regulator of the HGF/c-Met signaling involved in disease progression in breast cancer. Further studies to validate our results are needed.
Title: C-myc and bcl-2 overexpression in HER2-positive breast carcinomas

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Body: Background: Gene amplification plays a significant role in the transcriptional regulation of the genome; in breast cancer, amplification of the HER2 gene occurs in 20-30% of patients and confers a poor prognosis. Trastuzumab is now widely used in HER2-positive breast carcinomas and has significantly changed outcomes in this aggressive subtype of breast cancer. C-myc, another oncogene, is reported to be amplified in breast cancer; c-myc regulates cell proliferation. Bcl-2, the prototype anti-apoptotic gene, is overexpressed in human breast carcinomas. The anti-apoptotic action of bcl-2 is located downstream from HER2 and may serve as a potential drug target. This study evaluated the rate of c-myc and bcl-2 overexpression in a cohort of HER2-amplified breast carcinomas and correlated the dysregulation of these genes with patient demographics and histologic characteristics.

Design: The study population consisted of 96 patients with HER2-positive invasive breast carcinoma treated with surgical excision or mastectomy at Northwestern Memorial Hospital (2009-12) (mean age 53, range 18-80). Electronic medical records were reviewed for patient demographics. Pathologic tumor characteristics (histologic type, size, grade, lymph node status) and tumor marker profile at the time of diagnosis (ER, PR, p53 and ki-67) were evaluated. Tissue microarrays were constructed (3 cores from each case to account for tumor heterogeneity) for immunohistochemical evaluation of c-myc (Epitomics, clone V69) and bcl-2 (DAKO, clone 124).

Results: Overall, these HER2-amplified breast carcinomas were almost exclusively infiltrating ductal carcinomas (92/96, 96%) of high histologic grade: 68/96 (71%) were grade 3, while the other 28 were grade 2 tumors. C-myc was overexpressed in 49/96 (51%) of the cases and bcl-2 was expressed in 60/96 (62%). There was no association of c-myc or bcl-2 expression with age, patient race, tumor grade, tumor size, lymph node status, p53 expression or ki-67 proliferation index. Expression of both c-myc and bcl-2 was seen in 30/96 (31%) of the cases. Of interest, c-myc-positive/bcl-2-positive tumors more often had positive lymph nodes and higher ki-67 proliferation index compared to tumors with c-myc-negative/bcl-2-negative phenotype (46.7% v 29.4% and 75.8% v 56.2% respectively).

Conclusions: (1) C-myc is expressed in over 50% and bcl-2 in over 60% of HER2-amplified carcinomas. (2) C-myc and bcl-2 expression do not appear to have differential expression between age groups or racial groups and do not correlate with histologic tumor characteristics. (3) HER2-amplified carcinomas expressing both c-myc and bcl-2 are highly proliferative tumors with a high rate of lymph node positivity. These findings suggest that coupled c-myc and bcl-2 overexpression may contribute to the biologic aggressiveness of HER2-amplified breast carcinomas. Additional studies into the molecular mechanisms that drive HER2-amplified tumors are currently underway in this aggressive subtype of breast cancer.
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Title: PDE5 as a novel biomarker and a potential therapeutic target for breast cancer

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Body: Background: Phosphodiesterases are enzymes responsible for regulating second messenger signaling by hydrolyzing 3′-5′ cyclic guanosine monophosphate (cGMP), that activates specific pathways resulting in protein phosphorylation, ion fluxes, or cyclic nucleotide hydrolysis to affect gene expression or other aspects of cellular activity. Previous studies have reported increased PDE5 expression in multiple human carcinomas, including bladder, colon, lung and breast cancers, suggesting a role for PDE5 in tumorigenesis. In addition, several in vitro observations have shown antiproliferative and proapoptotic effects of sildenafil and other PDE5 inhibitors in cancer cell lines. However, very little is known about PDE5 expression in human breast tumours and its potential role in breast cancer initiation and progression. We therefore propose to determine whether PDE5 expression may be predictor of outcome in breast cancer patients, and examine PDE5 impact on breast cancer phenotype in vitro.

Methods: We employed MCF-10A normal breast epithelial cells, estrogen receptor (ER) α-positive (MCF-7/ZR-75/T-47D) and ERα-negative (BT-20/MDA-MB-468/SKBR-3/MDA-MB-435) breast cancer cells. We used RT-PCR, immunoblotting and immunofluorescence analyses for evaluating PDE5 expression. To examine PDE5 impact on breast cancer phenotype, MCF-7 cells were engineered to stably express PDE5 and four clones were selected. Cell proliferation was assessed by MTT and anchorage-independent assays, motility and invasion by wound-healing, transmigration and matrigel-based invasion assays. Retrospective analysis using 1959 breast cancer patients of the Metabric Project was performed to evaluate relationship between PDE5 expression and overall survival by Cox proportional hazard regression.

Results: PDE5 mRNA and protein were constitutively expressed at high levels in all the examined tumor cell lines compared to normal breast cells, except for the less motile and non-invasive MCF-7 cells. Interestingly, higher PDE5 expression was found in more aggressive ER-negative cells. Stable overexpression of PDE5 did not affect proliferation of MCF-7 cells, while it significantly increased motility and invasion of all the stable PDE5-transfected clones tested. Patients having high PDE5 expression had a statistically significant poorer survival compared to patients with low PDE5 expression (p=0.014, HR= 1.2). A more relevant discrimination was achieved in lymph node-negative patients (p=0.0015, HR= 1.6), suggesting that assessing PDE5 levels may be helpful to identify a subgroup of early-stage breast cancer patients who are most likely at the highest risk of progression.

Conclusions: PDE5 expression may enhance cancer cell invasive potential, thereby representing prospectively a potential molecular candidate as prognostic marker and target for breast cancer therapy.
Identification of subgroups of triple negative breast cancer cells with selective responses to mTOR, CDK, mitotic and proteasome inhibitors

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Body: Triple negative breast cancer (TNBC) is characterized by the lack of estrogen, progesterone and HER2/ErbB2 receptors. It is a highly heterogeneous class of breast cancer and transcriptomics has recently been used to define 6 major subtypes of TNBC. We studied a panel of 15 TNBC cell lines using a chemical biology approach where we measure the responses to 306 approved and investigational oncology drugs. Clustering of cell lines based on their overall drug responses resulted in a strikingly different grouping compared to the gene expression derived one, highlighting that the current TNBC subtyping is not easily converted to differential sensitivities to drugs.

To further evaluate the nature of the drug responses and to differentiate between their cell growth and cytotoxic effects, we multiplexed the standard cell viability readout in the cell line screening with detection of cytotoxicity. This simple multiplexed readout identified several drug classes that previously had been assumed to be cytotoxic based on strong effects on cell viability (cell numbers) while they in fact showed no or a very heterogeneous effect on cytotoxicity. Drug classes exhibiting this type of response included mTOR inhibitors, cyclin-dependent kinase inhibitors (eg. alvociclib), mitotic inhibitors (eg. paclitaxel) as well as proteasome inhibitors (eg. bortezomib) and RNA synthesis inhibitors (eg. dactinomycin).

Further investigation of these drug classes showed that their static effects were reversible and in some cases the cells even overcame the inhibitory effect in the presence of the drug in a matter of a few days. Given the non-toxic responses to major classes of anticancer compounds such as mTOR inhibitors, we performed combination screens with these compounds to identify other drugs with which they may synergize to promote cancer cell specific killing. Surprisingly, we instead found that mTOR inhibitors had an antagonistic effect on the activity of many other cancer drugs such as different cytotoxic and antimitotic drugs, tyrosine kinase inhibitors, HDAC inhibitors and PARP inhibitors, suggesting that combining these classes of drugs may be counterproductive also in the clinic. We also found out that accessing a cytotoxic readout allowed us to identify effective synergistic drug combination concentrations that were not seen in cell viability readouts. For example, these synergistic toxic combination responses were seen in DU4475 cells when the MEK inhibitor trametinib was combined either with the PARP inhibitor iniparib or with the broad spectrum tyrosine kinase inhibitor ponatinib.

In conclusion, multiplexed cell viability cell death readouts in drug sensitivity testing yields novel critical information on single drug and drug combination activities and liabilities. With this we were able to conclude that antimitotic, mTOR, CDK, proteasome and metabolic inhibitors have a heterogeneous cytotoxic effect across the panel of TNBC cell lines in contrast to their homogenous effect on metabolic inactivation.
Exploiting Isobutyl-deoxyynboquinone-induced DNA damage responses and metabolic changes for breast cancer therapy

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Body: During oncogenic or cellular stress new genes are frequently (over)-expressed that could be exploited for targeted cancer therapy. The enzyme, NAD(P)H:quinone oxidoreductase-1 (NQO1) is over-expressed in most solid cancers, including 60% of primary and metastatic breast cancers regardless of subtype. Normally, NQO1 detoxifies quinones resulting in the formation of stable hydroquinones that are subsequently excreted from the cell. However, NQO1 bioreduction can turn certain rare quinones, such as β-lapachone and deoxynboquinone (DNQ), into potent cell death-inducing compounds. These agents cause severe DNA damage, poly(ADP-ribose)polymerase-1 (PARP1) hyperactivation, NAD+/ATP loss, and programmed necrosis of NQO1-expressing cancer cells. Although β-lapachone (ARQ761) is in current clinical trials at UTSW, more potent tumor-selective NQO1 compounds are needed. Based on its structure and mode of action, isobutyl-DNQ (IB-DNQ) was recently added to the spectrum of NQO1 substrates as a more selective and potent anti-cancer agent whose mechanism of action remains to be elucidated.

Although NQO1 expression is a major determinant of IB-DNQ-induced lethality, previously published results from our group showed that increased catalase expression could cause marked cytoprotection. We conducted a screen for NQO1:Catalase ratios in 266 breast tumor samples, and 143 normal breast samples, for a total of 409 specimens. We found that NQO1 expression was significantly elevated in breast tumors compared to normal tissue. In contrast, catalase expression was suppressed in breast tumors versus adjacent normal tissue. These results predict that normal tissue, which typically has higher catalase levels than cancer cells, could be selectively spared from IB-DNQ-induced toxicity. Thus, NQO1:Catalase ratios favor use of IB-DNQ in breast cancers to exploit this large therapeutic window.

Since NQO1 bioactivatable drugs synergize with agents that damage DNA, we hypothesized that certain cancer vulnerabilities (e.g., BRCA1-deficient breast cancers) that have elevated endogenous DNA damage would synergize with IB-DNQ. Exposure of breast cancer cells with IB-DNQ induced DNA damage, PARP1 hyperactivation, dramatic loss of essential nucleotides (NAD+/ATP), and µ-calpain-mediated programmed necrosis with 10X greater potency than β-lapachone.

IB-DNQ-induced DNA double-strand breaks (DSBs) that occurred in cells in S/G2 phases were mainly repaired by error-free homologous recombination (HR), and therefore BRCA1-deficient cancers, being HR defective, would be particularly vulnerable to IB-DNQ treatment. Indeed, HCC1937 breast cancer cells, deficient in BRCA1, were extremely sensitive to low dose IB-DNQ due to the overwhelming levels of IB-DNQ-induced DNA damage and their inability to repair it due to their compromised HR. In fact, IB-DNQ was far superior to PARP inhibitors in targeting BRCA1-deficient cells. Studies in vivo showed equivalent antitumor efficacy of IB-DNQ to β-lapachone and DNQ, but with much greater potency at lower doses. These findings offer preclinical ‘proof-of-concept’ for IB-DNQ as a potent chemotherapeutic agent for the treatment of breast cancers, especially those deficient in BRCA1. This research was supported by grant CA102972 to DAB.
Title: SUMO Inhibitors affect tumorigenesis of novel breast cancer xenograft model

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Body: A novel basal breast cancer cell line IOWA-1T was derived from chemotherapy resistant locally advanced breast cancer tumor. The cells rapidly form large, skin-eroding xenografts in nude mice. The SUMO inhibitor anacardic acid (AA) effectively cleared CD44⁺/hi/CD24⁻/low cancer stem cell (CSC) population in IOWA-1T and BT-20 basal cancer cell lines and delayed tumor outgrowth of basal cancer xenografts. The effect of SUMO inhibitors to clear the CSC population was dependent upon the SUMO unconjugated form of TFAP2A (Bogachek MV et al, Cancer Cell, 2014). Herein we show that tumors that eventually form from IOWA-1T xenografts in mice treated with AA are not capable of developing secondary xenografts, confirming eradication of the CSC population by SUMO inhibitors. As further mechanistic evidence for the SUMO pathway, transient knockdown of UBC9 and PIAS1 SUMOylation enzymes repressed CD44 expression and increased tumor free and overall survival in mice inoculated with IOWA-1T xenografts. Furthermore, CD44 downregulation was demonstrated in IOWA-1T cells after treatment in vitro with UBC9 inhibitor PYR-41 and PIAS1 inhibitor NSC-207895. Overall survival of mice with IOWA-1T xenografts was increased to 43±0.5 and 39±2 days with PYR-41 and NSC-207895 i.p. injections, respectively, compared to a vehicle treated control group 33±1 days (p<0.05). By contrast, doxorubicin treatment was not able to extend survival of mice with IOWA-1T xenografts. These findings establish the class of SUMO inhibitors as potential therapeutic drugs that eliminate the breast CSC population and may be effective in basal breast cancer cases resistant to conventional chemotherapy.
Title: Eribulin mesylate promotes a mesenchymal-epithelial transition (MET) and effectively radio-sensitizes triple negative breast cancer cells

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Body: Purpose: Eribulin mesylate is a novel non-taxane microtubule dynamics inhibitor that irreversibly induces mitotic arrest, and has been shown to promote a shift from mesenchymal to epithelial phenotypes in breast cancer. It has demonstrated therapeutic activity in heavily pretreated patients with metastatic breast cancer, with a trend toward greater efficacy in patients with triple negative breast cancer (TNBC). TNBC is an aggressive tumor with a mesenchymal phenotype and higher locoregional recurrence (LRR) compared to estrogen receptor (ER) positive breast cancer. One postulated cause for higher LRR in TNBC is that these tumors have a great proportion of cancer stem cells (CSC). CSCs are radio- and chemo-resistant when compared to the bulk of the tumor, and are thought to be the source of both LRR and distant metastasis after treatment, thus an important target for therapy. Microtubule inhibitors are known radiation sensitizers given that they arrest cells at G2/M, the most radiation sensitive phase of the cell cycle. We hypothesize that because eribulin induces an irreversible mitotic arrest and promotes a mesenchymal-to-epithelial transition (MET) it will enhance the radiation sensitivity of TNBC through reduction in CSCs and inhibition of DNA damage repair (DDR) pathways.

Methods: Experiments were conducted in 3 subtypes of TNBC cells, all with p53 mutations and PTEN loss: SUM149 (basal B), BT-549 (mesenchymal-like), and MDA-MB-468 (basal A). Cells were treated with 0.5nM-3nM Eribulin, alone or in combination with increasing doses of radiation from 0-8 Gy. In vitro studies performed included; cell survival assays, immunoblot analysis for markers of MET, FACS analysis for cell cycle, cancer stem cell population, and apoptosis, and finally P-γH2AX staining to measure DDR.

Results: We found that combining ionizing radiation (IR) and eribulin is synergistic. The IC50 of eribulin combined with 6Gy IR, was 0.5-1 nM, 10 fold less than drug alone, and a 40-60% decrease in cell viability was observed with the combination compared to IR alone. A significant decrease in clonogenic survival was seen after 24 hours pretreatment with 1nM eribulin, with the greatest efficacy seen in BT-549 cells. No significant increase in apoptosis was seen with the combination treatment by FACS analysis though a significant delay in DDR was observed. Cell cycle analysis demonstrated eribulin increases the proportion of cells in G2/M arrest following IR, as well as suppressed cell proliferation. 24-hour eribulin treatment also caused morphological changes in culture consistent with transition from a mesenchymal to an epithelial phenotype, and IHC showed a decrease in mesenchymal markers and increase in epithelial markers. Finally eribulin treatment decreased the proportion of CSCs by FACS analysis by 80% in SUM149 cells and prevented an increase in CSC population following IR. Studies are ongoing to further characterize the effect of combined eribulin and IR on DDR and the CSC population in TNBC cells.

Conclusions: These studies demonstrate that eribulin effectively radiosensitizes TNBC cells. We believe this occurs through inhibition of DDR as well as promotion of MET which reduces the radiation resistant CSC population.
Title: Systemic statin treatment and intra-tumoral lipid metabolism - report from a window-of-opportunity breast cancer trial

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Body: Background:
In recent years, attention has been drawn to the plasma cholesterol lowering drugs statins (HMGCR-inhibitors) due to their exertion of pleiotropic intratumoral effects such as induction of apoptosis and inhibition of proliferation, generating a possible utility in cancer prevention and treatment. However, the molecular mechanisms are complex and remain largely undefined. The aim of this study was to assess the statin induced changes of the intra-tumoral levels of cholesterol, to gain enhanced understanding of the role of the mevalonate pathway in cancer lipid metabolism.

Methods:
The study was designed as a window-of-opportunity trial, where 50 patients with primary invasive breast cancer were included, and treated with 80 mg of atorvastatin/day for two weeks, between the time of diagnosis and surgery. From frozen tumor tissue samples pre- and post atorvastatin treatment, lipids have been extracted, and the cholesterol levels have been measured using a cholesterol quantification assay. In vitro experiments on MCF7-cells treated with atorvastatin was used for optimization and comparison on the cellular level.

Results:
42 patients completed all study parts, and assessment of the cholesterol levels in the paired frozen tumor tissue was achievable in 14 pairs. Following atorvastatin treatment, the intra-tumoral levels of total cholesterol was significantly increased (P=0.035), with an increase in 11 of the pairs, and decrease in 3 pairs. Similar findings were observed in the in vitro experiments, demonstrating increased levels of total cholesterol and storage in lipid droplets in the cells treated with atorvastatin.

Conclusions:
We have earlier reported statin-induced anti-proliferative effects in breast cancer. This study shows a statin-induced increase in the intra-tumoral cholesterol levels, which may however rely on the increased cholesterol storage in lipid droplets, a phenomenon observed in the in vitro experiments with MCF7 cells treated with atorvastatin. Our results suggest that the anti-proliferative effects exerted by statins reluctantly reduce cell metabolism and consequently increase the storage of e.g. lipids.
Title: Safety and feasibility of neoadjuvant combined chemotherapy of breast cancer with paclitaxel carried in a lipid nanoemulsion (LDE) associated with adriamycin and cyclophosphamide

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Body: Background
The introduction of neoadjuvant chemotherapy considerably changed the natural history of breast cancer by permitting a big tumors to be operable, permitting less aggressive surgical procedures and when achieving complete pathological response prolonging the disease-free period. However, the chemotherapy schemes bear high toxicity rates and are even life-threatening. Neoadjuvant chemotherapy aims at reducing mortality and improving surgical options and offers an in vivo chemosensitivity testing at the same time. It is the ideal setting for clinical and translational research. Compared to the classical adjuvant treatment, it offers several advantages.

Methods
We performed a pilot study to evaluate the safety and feasibility of neoadjuvant combined chemotherapy of breast cancer with paclitaxel carried in a lipid nanoemulsion (LDE) associated with adriamycin and cyclophosphamide in comparison to the classical neoadjuvant chemotherapy paclitaxel, adriamycin and cyclofosfamide (TAC). All the patients were women with recently diagnosed breast cancer who needed primary chemotherapy as the first treatment selected from Perola Byington Hospital in São Paulo - Brazil.

Results
From April 2006 to June 2008, 39 patients from a center for breast disorders (Pérola Byington Hospital) were included in the study. These patients were randomized to the standard chemotherapy scheme (control arm – n=16) or LDE-paclitaxel (LDE arm – N=17). Six patients were excluded from the study because they did not complete at least 5 cycles of chemotherapy. We also excluded three patients because of lack of follow up. The majority of patients had stage III (37% IIIA and 27% IIIB). There was no significant difference among the groups, considering the clinical variables, so the sample was considered homogeneous. The patients were grouped in four different groups regarding the expression of ER, PR and CerbB2: Luminal subtype, Triple Negative, Her2-positive and Hybrid Luminal. We observed 37,5% of complete response in the control arm and 23,5% in the LDE arm. The partial response were 43,7% in the control group and 58,8% in the LDE group. The toxicity particularly in grade 3 and 4 events were substantially lower in LDE group: Nausea 11% vs 2,2%; Vomiting 7,3% vs 0.7% and neutropenia 8,8% vs 2,2%.

Conclusion
LDE scheme was effective and had lower grad 3 and 4 events than the TAC regiment. More studies are necessary to evaluate this approach.
Body: Background: FGFR2 is amplified in 4% of triple-negative breast cancers (TNBC). In addition, the mRNA expression levels of FGFR2 were significantly increased in amplified vs nonamplified tumor samples, suggesting a potential role for FGFR2 in TNBC (Turner et al. 2010). We evaluated efficacy of allosteric inhibition of FGFR2 in high- and low-expressing TNBC xenografts. Methods: Immunocompromised mice were used for xenotransplantation of FGFR2 high-expressing TNBC cells (SUM52PE) or FGFR2 low-expressing TNBC cells (HS578T). Forty animals with measurable tumors were selected on day 10 and randomized into treatment groups (low-molecular weight allosteric inhibitor RPT835, 30 mg/kg; gavage, daily) or vehicle (water; gavage, daily). Measurements of tumor volume (mm3) were performed by digital calipers every 3 days during 40 days after tumor inoculation.

Results: RPT835 significantly inhibited aggressive growth of SUM52PE tumor xenograft (P<0.0001). At study day 31, mean tumor volumes (± SE) were 2712.2±37 mm3 in the vehicle group, and 1080.7±49 mm3 in the study group. In addition, animals of the study group received RPT835 from day 31 to day 40 with disease stabilization (no differences in tumor volume between days 31 and 40, P=0.167). The tumor growth curve shows a nearly exponential increase in median tumor volume up to day 31 in the vehicle group and a high rate of slow growing tumors up to day 40 in the study group. In the HS578T xenograft study differences in tumor volume between study and control groups were weak at day 31 (mean tumor volumes ± SE, 703±89.1 mm3 vs. 1053±179.8 mm3, respectively; P<0.001) and at day 40 (1104±162.2 mm3 vs. 1592±335 mm3; P=0.01).

Conclusions: The allosteric FGFR2 inhibitor RPT835 significantly impacts on growth of FGFR2-expressing TNBC.
Title: Novel flavonoid Anto-028 shows promising antitumor activity in preclinical models of breast cancer

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Body: New drugs are required to treat both endocrine resistant and triple negative breast cancers (TNBC). Among new structures being investigated are flavonoids, which are natural polyphenolic compounds with antitumor properties. Clinical trials are underway to assess their application as chemopreventatives and as sensitisers to chemotherapy. Among flavonoids, myricetin has shown particular promise, inducing cell cycle arrest and mitochondrial-dependent apoptosis in cancer cell line models. In this study we evaluated a novel series of myricetin-derived flavonoids with improved antioxidant and mitochondrial targeting properties. We hypothesised that these compounds might have potential in breast cancer management and in particular to treat estrogen receptor positive (ER+) tumors, in which estrogen controls tumor growth at least partially through oxidative stress-related mitochondrial signalling. We first assessed the effect of 8 flavonoids on 4 breast cancer cell lines (MDA-MB-231, BT-549, MCF-7 and HBL-100) and 3 MCF-7-derived cell lines with reduced sensitivity to endocrine therapy (LCC-1/LCC-2/LCC-9). The novel flavonoids were designed to assess the involvement of redox potential and mitochondrial targeting through selective structure-activity changes. Anto-028 was the most potent compound identified with 4 to 140-fold lower IC₅₀ values than myricetin, as shown by sulforhodamine B (SRB) assays applied to assess antiproliferative effects. Although endocrine-resistant cell lines were less sensitive, Anto-028 exerted a strong, ER-independent antiproliferative effect on both ER+ and TNBC cell lines, with IC₅₀ values in the low micromolar range. Treatment for 8 hours exerted dose-dependent reduction in cell viability and induction of cytotoxicity and apoptosis, with a 2 to 5-fold increase in caspase activation, as detected by luminescence-based plate assays. The involvement of reactive oxygen species (ROS) regulation in these effects was demonstrated by plate-based assays and microscopic detection of fluorescent probes for mitochondrial hydrogen peroxide and superoxide. Results indicated that different species of ROS are sequentially generated by treatment with a range of concentrations of Anto-028, suggesting a possible biphasic effect of the drug at different concentrations. Experiments in mice implanted with subcutaneous MDA-MB-231 xenografts showed that Anto-028 can significantly inhibit tumour growth in vivo with associated changes in percentage of viable areas within the xenografts (from 56% in controls to 37% in mice treated with 25mg/kg/day of Anto-028) and reduced Ki67 proliferation index (from 60% in controls to 36% in treated xenografts). Current studies are assessing combinatorial effects with TRAIL and chemotherapy which have produced synergistic effects in ovarian cancer models, with nanomolar concentrations of Anto-028 sensitising resistant cells to induce strong, significant antiproliferative effects. In conclusion, Anto-028 is a novel flavonoid with promising antitumor activity in preclinical models of breast cancer.
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Title: Structure-activity relationship of ruthenium (Ru) complexes to inhibit breast cancer growth and metastasis

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Body: Previous studies demonstrated that transition metal complexes inhibit cancer cell growth. We have examined a series of ruthenium (Ru (II)) complexes for their ability to inhibit growth of breast cancer cells. Among three complexes with the general formula [Ru(η6-p-cym)(N-N)Cl]+ tested for their abilities to inhibit MDA-MB-231 cell growth, the complex with o-phenalenediamine (o-pda) as the bidentate nitrogen donor (N-N) ligand, [Ru(η6-p-cym)(o-pda)Cl]+, showed significant inhibitory activity in both a concentration- and a time-dependent manners. Although [Ru(η6-p-cym)(o-pda)Cl]+ inhibited growth of various human cancer types such as MCF-7 (breast), SK-Br3 (breast), B-cell lymphoma (Raji), osteosarcoma (HT1080), and melanoma (Bowes) cells, two TN breast cancer cells, HCC1806 and HCC38, were resistant to this treatment, suggesting cell-type specific functions of [Ru(η6-p-cym)(o-pda)Cl]+ for inhibiting cell growth. When MDA-MB-231 cells were treated with [Ru(η6-p-cym)(o-pda)Cl]+, both cleavage of caspase-3 and release of HMGB-1 into conditioned medium increased in a concentration dependent manner, suggesting [Ru(η6-p-cym)(o-pda)Cl]+ induced both apoptosis and necrosis processes. Importantly, [Ru(η6-p-cym)(o-pda)Cl]+ synergistically inhibited MDA-MB-231 cell growth with cyclophosphamide but not doxorubicin and paclitaxel. These results suggest that [Ru(η6-p-cym)(o-pda)Cl]+ is a potent tumor growth inhibitor per se and enhances tumoricidal activity of chemotherapeutic agents such as cyclophosphamide. Thus, Ru (II) complexes are promising anti-cancer drugs which could be used alone and/or in combination with chemotherapeutic agents for breast cancer patients. The opinion and assertions contained herein are the private views of the authors and are not to be construed as official or as representing the views of the Department of the Army or the Department of Defense.
Title: Targeting protein-protein interactions in the proteasome assemblies as a novel strategy to treat triple negative breast cancers

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Body: The ubiquitin-proteasome pathway is responsible for the most of regulated intracellular protein degradation in human cells. The proteasome, a multifunctional, multisubunit, modular proteolytic enzyme is an essential part of the pathway. The proteasome, which comprises several proteolytic assemblies sharing a catalytic core, is a recognized anti-cancer drug target. Competitive inhibitors such as bortezomib and carfilzomib bind to the active centers of the core and are successfully used to treat aggressive blood cancers, most notably refractory/relapsed multiple myeloma and lymphomas. Unfortunately, so far breast cancers performed disappointingly in clinical trials with the proteasome inhibitors, even if a recent genome-wide screen identified proteasome addiction as vulnerability of triple negative breast cancers. Triple negative breast cancers are among the most deadly and difficult to treat and such vulnerability is more than worth to explore. Here we aimed at searching for new concepts in proteasome targeting, better suited for breast cancers than active-centers blocking drugs. The sophisticated structure of the proteasome offers ample opportunities to design noncompetitive inhibitors. The most physiologically relevant 26S proteasome is an assembly of a 20S catalytic core and a 19S regulatory particle "cap". The active sites bearing core is the canonical target of proteasome targeting anti-cancer drugs. On the other hand, the 19S protein complex is responsible for recognizing and processing the majority of intracellular protein substrates tagged for degradation by polyubiquitin. We designed small molecules that instead of binding to the active sites of the core, target protein-protein interactions between the core and the cap. The lead compound B1 in vitro prevents assembly of 20S and 19S components, destabilizes 26S proteasome and noncompetitively, most likely allosterically, inhibits the 20S core at low-nanomolar concentrations. We tested the performance of B1 in the culture of triple negative breast cancer MDA-MB-231 cells. Apparently, B1 at nanomolar concentrations suppresses the cell culture growth, lowers the content of 26S proteasome and compromises activity of the ubiquitin-proteasome system, as manifested by the accumulation of polyubiquitinated protein substrates in the cytosol of B1 treated cells. Importantly, B1 strongly synergizes with bortezomib, even if these cells are relatively resistant to the treatment with bortezomib alone. The cytotoxic effect of the combined treatment on the MDA-MB-231 cell culture is apparent with single-digit nanomolar concentrations of both drugs. Summarizing, targeting protein-protein interactions in the proteasome assemblies with small molecules, alone or in combination with competitive inhibitors, seems to provide a promising strategy to treat proteasome addicted triple negative breast cancers.
Title: A phase Ib study of the CXCR1/2 inhibitor reparixin in combination with weekly paclitaxel in metastatic HER2 negative breast cancer – First analysis

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Body: Background. Experimental models and retrospective clinical observations point to cancer stem cells (CSCs) as the culprits for tumor recurrence and metastasis. CSCs account for a small proportion of tumor cells, suggesting that their elimination through pharmacological targeting would not necessarily translate in any sizeable tumor regression. Thus, an ideal CSCs targeting agent should be a nontoxic molecule that can be safely administered in combination with chemotherapy to reduce tumor burden and improve disease control. CXCR1, one of the receptors for CXCL8, has been identified on breast cancer (BC) CSCs (Ginestier C et al., JCI 2010). Reparixin, an allosteric inhibitor of CXCR1 with a large safety database in non-cancer patients, effectively targeted CSC in BC xenografts (Ginestier C et al., JCI 2010).

Methods. Patients were female aged > 18 years with HER-2 neg metastatic breast cancer (MBC), eligible for treatment with paclitaxel (not taxane-refractory), had received up to 3 prior CT lines for advanced BC (not including neo/adjuvant chemotherapy), had measurable disease according to RECIST 1.1, had ECOG PS of 0-1, had adequate organ function, and had no brain metastases. Patients received a 3-day run-in with reparixin oral tablets 3 times daily (tid) followed by paclitaxel 80 mg/m2/week (Days 1, 8, and 15 for 28-day cycle) + reparixin oral tablets tid for 21 days. Three dose levels of 3-6 subjects were explored: 400 mg, 800 mg and 1200 mg oral reparixin tid. A further 17 subjects were enrolled at the highest tolerated dose (total 20 patients). Safety was assessed following one cycle. Treatment continued until disease progression, unacceptable toxicity or withdrawal of consent. Primary endpoints were safety and tolerability, and pharmacokinetic (PK) profile of the combination treatment. Among secondary endpoints, assessment of disease response every 2 cycles for indication of efficacy and correlative evaluations on peripheral blood samples were conducted.

Results. From 02/12 to 04/14, 33 patients entered the study. PK of reparixin at 400 mg tid, $t_{\text{max}} = 0.5-1.5 \text{ hr}$, $t_{\text{1/2}} = 1.7 \text{ hr}$; at 800 mg tid, $t_{\text{max}} = 0.5-3 \text{ hr}$, $t_{\text{1/2}} = 4.6 \text{ hr}$; at 1200 mg tid, $t_{\text{max}} = 0.5-2 \text{ hr}$, $t_{\text{1/2}} = 1.6 \text{ h}$. Co-administration of reparixin on days 1 and 8 had no effect on paclitaxel kinetics. Fifteen SAEs were recorded, none of which was related to Reparixin. Grade 3-4 adverse reactions were recorded in 30% (10/33) patients including haematological toxicity (5/10). Only one patient discontinued treatment for a reversible GI adverse reaction due to reparixin at the 1200 mg dose level. To date, 5 confirmed responses (2 CR, 3 PR) were recorded among 18 patients who underwent at least 1 tumor assessment (at 8 weeks). Response duration was 20m+ and 3m+ (for CR) and 9m+, 6m+, 2m+ (for PR). Final data will be presented at the meeting.

Conclusions. Combination treatment was safe and well tolerated at all dose levels without evidence of pharmacologic interactions and the recommended dose for subsequent studies is 1200 mg tid. Efficacy was demonstrated both in hormone receptor positive and triple receptor negative disease. A randomized phase II study of the combination versus single agent weekly paclitaxel in patients with MBC is warranted.
Body: The estrogen receptor (ER) is the principal driver of growth and differentiation in breast cells and de-regulated receptor function is a key feature of almost 75% of breast cancers. Here, we investigated the role of de-ubiquitinating enzymes (DUBs), which act to remove ubiquitin moieties from proteins, in regulating the transcriptional activity of ER in breast cancer. To identify DUBs involved in the regulation of ER transcriptional activity, we performed an RNAi loss-of-function screen using a library of shRNA vectors targeting all known human DUB genes (108 genes/432 shRNAs in total). We found that suppression of a number of DUBs markedly repressed or enhanced the activity of an estrogen-response-element (ERE) luciferase reporter to estradiol (E2). In particular, suppression of the BRCA2-associated DUB, USP11, was found to down-regulate ERα transcriptional activity (both in the presence and absence of E2), as demonstrated by a pronounced decrease in estrogen-response element (ERE) luciferase reporter activity. Subsequent validation of the screen using multiple individual hairpins and ZR-75-1 stable USP11 knockdown cells confirmed the suppression of ERE-reporter activity and further revealed a notable reduction in expression of the endogenous ER target genes TFF1 and PgR following USP11 knockdown. In vitro phenotypic analysis also revealed a global decrease in cell survival, decreased ERK and AKT phosphorylation and increased sensitivity to DNA-damaging agents in USP11 knockdown cell lines compared to non-targeting controls.

In silico analysis of publically available breast cancer gene expression datasets revealed a highly significant association between high USP11 mRNA levels and poor prognosis. We observed a highly significant correlation between high expression of USP11 mRNA in ER positive patients and poor overall survival (OS)(HR 1.51, CI 1.07-2.14, p=0.018) and distant metastasis-free survival (DMFS)(HR 1.35, CI 1.04-1.73, p=0.023). This correlation was also significant in ER positive patients who had received endocrine therapy (OS, HR 3.4, CI 1.2-9.81, p=0.014), DMFS, HR 2.16, CI 1.23-3.8, p=0.0083). These results suggest a role for USP11 in driving cellular growth and identify USP11 as a rationale and novel therapeutic target in breast cancer.
Title: Enrichment of janus kinase-2 (JAK2)-amplified tumor cell populations in triple-negative breast cancers (TNBC) during chemotherapy treatment

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Body: Neoadjuvant chemotherapy (NAC) is frequently used in the treatment of triple-negative breast cancer (TNBC). Approximately 30% of TNBC patients achieve pathological complete response with excellent prognosis. However, the remaining 70% are at increased risk of recurrence with no molecularly targeted therapeutic options in the adjuvant setting. We performed targeted next-generation sequencing in residual TNBC tumors after NAC to identify potentially actionable genomic alterations. As previously reported, we found amplifications in JAK2 (JAK2AMP) in 7/68 (10.2%) post-NAC tumors compared with 1/30 (3%) in the pre-NAC cohort; all cases were confirmed by JAK2–fluorescence in situ hybridization (FISH). In contrast, JAK2AMP was detected in 1% or less of primary untreated breast tumors in other independent cohorts.

Patients with JAK2AMP after NAC compared with JAK2NORMAL patients were younger (39.9yo vs 47.6yo), more frequently pre-menopausal (71.4% vs 57.9%), and had little or no anti-tumor response to NAC (Miller & Payne I: 42.9% vs 15.8%). RNA expression of the JAK2-activating ligand interleukin-6 (IL6) was also higher in these patients (p=0.008).

In matched untreated and post-NAC specimens, including 2 patient derived xenograft models generated from a single patient’s pre- and post-NAC specimens, FISH analysis identified a subpopulation of tumor cells with JAK2AMP that was enriched after NAC treatment. Preliminary data from RNA in situ hybridization (RNAScope) showed that tumor cells expressing high levels of JAK2 are distinct from their counterparts, with higher IL6 expression, suggesting a paracrine signaling event.

We also explored the intra-tumor heterogeneity of JAK2AMP in cell lines and patient tumors, performing chemical and genetic loss of function studies in 4 TNBC cell lines: HCC-1143 (JAK2DELETED/LOW), SUM-159PT (JAK2HIGH), HCC-38 (JAK2GAIN, HIGH) and HCC-70 (JAK2AMP, HIGH).

In vitro, IL6 expression after adriamycin and/or docetaxel treatment was higher in the JAK2HIGH cell lines compared with the changes registered in HCC-1143 (around 2, 4 and 100 fold increase from their respective IL-6 basal levels in HCC-38, HCC-70 and SUM159PT respectively, compared with 0.5 fold increase in HCC-1143). Treatment with chemotherapy also abrogated the IL-6 downregulation produced by the JAK1/2 inhibitor ruxolitinib. Ruxolitinib decreased mammosphere formation by 50% in SUM-159PT cells, with a 10% reduction of the CD24low/CD44high stem cell compartment. Interestingly, we observed no change with ruxolitinib treatment in the JAK2GAIN/AMP cell lines. However, in HCC-38, siRNA knockdown of JAK2 reduced the CD24low/CD44high compartment (around 15%) and mammosphere formation (around 80%).

These findings suggest that a JAK2AMP cell population may escape from chemotherapy-induced apoptosis resulting in lack of response to treatment and eventual disease recurrence. Furthermore, chemotherapy may induce a wound-healing response in the tumor, upregulating and/or selecting JAK2AMP cells via a paracrine or autocrine IL-6/JAK1/pSTAT3 signal. In vivo models confirming these observations and further exploring the therapeutic potential of ruxolitinib to treat JAK2AMP TNBCs are underway.
Title: TGF-β receptor type III is a tumor promoter in mesenchymal-stem like triple negative breast cancer

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Body: Introduction: There is a major need to better understand the molecular basis of triple negative breast cancer (TNBC) in order to develop effective therapeutic strategies. Using gene expression data from 587 TNBC patients we previously identified six subtypes of the disease, among which a Mesenchymal-Stem Like (MSL) subtype. The MSL subtype has significantly higher expression of the transforming growth factor beta (TGF-β) pathway-associated genes relative to other subtypes, including the TGF-β receptor type III (TβRIII). We hypothesize that TβRIII is tumor promoter in mesenchymal-stem like TNBC cells.

Methods: Representative MSL cell lines SUM159, MDA-MB-231 and MDA-MB-157 were used to study the roles of TβRIII in the MSL subtype. We stably expressed short hairpin RNAs specific to TβRIII (TβRIII-KD). These cells were then used for xenograft tumor studies in vivo; and migration, invasion, proliferation and three dimensional culture studies in vitro. Furthermore, we utilized human gene expression datasets to examine TβRIII expression patterns across all TNBC subtypes.

Results: TβRIII was the most differentially expressed TGF-β signaling gene in the MSL subtype. Silencing TβRIII expression in MSL cell lines significantly decreased cell motility and invasion. In addition, when TβRIII-KD cells were grown in a three dimensional (3D) culture system or nude mice, there was a loss of invasive protrusions and a significant decrease in xenograft tumor growth, respectively. In pursuit of the mechanistic underpinnings for the observed TβRIII-dependent phenotypes, we discovered that integrin-α2 was expressed at higher level in MSL cells after TβRIII-KD. Stable knockdown of integrin-α2 in TβRIII-KD MSL cells rescued the ability of the MSL cells to migrate and invade at the same level as MSL control cells.

Conclusions: We have found that TβRIII is required for migration and invasion in vitro and xenograft growth in vivo. We also show that TβRIII-KD elevates expression of integrin-α2, which is required for the reduced migration and invasion, as determined by siRNA knockdown studies of both TβRIII and integrin-α2. Overall, our results indicate a potential mechanism in which TβRIII modulates integrin-α2 expression to effect MSL cell migration, invasion, and tumorigenicity.
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Title: Dual HER2 blockade of an activating driver HER3 mutation: A proof of principle study in a metastatic breast cancer patient

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Body: Background: HER3 is the only member of the EGFR family with an impaired kinase domain. Pre-clinical work has suggested that activating hotspot point mutations in HER3/ERBB3 may constitute drivers of tumor progression. Mutant HER3 oncogenic activity was shown to be dependent on HER2 signaling. In vitro and in vivo experiments have suggested that HER2-targeting therapies could be effective to block mutant HER3 oncogenic signaling. Through high-depth whole exome sequencing analysis of the primary breast cancer and liver metastasis of a patient with stage IV disease, we identified a clonal, activating HER3 mutation in both the primary tumor and metastasis, and sought to define whether this mutation would constitute a driver of the disease and be a therapeutic target.

Patient and method: A 46 yo women was diagnosed in 2012 with de novo synchronous metastatic breast cancer. Biopsies of the primary breast cancer and of the liver metastasis were performed before any treatment within the ESOPE prospective study (NCT01956552). Pathologic examination revealed a high-grade invasive ductal carcinoma with low expression of estrogen and progesterone receptors (both 20%) and intermediate HER2 (++) expression by immunohistochemical analysis without HER2 gene amplification by fluorescence in situ hybridization. While high-depth (250x) whole exome massively parallel sequencing (HiSeq) of the primary tumor and liver metastasis was ongoing, the patient received two lines of chemotherapy (anthracycline- and taxane-based). Data analysis revealed that both the primary tumor and the liver metastasis displayed a clonal G284R HER3 mutation at a high allelic frequency compatible with the distribution of a driver genetic alteration. Single nucleotide polymorphism (SNP6) arrays and sequencing confirmed the lack of HER2 gene amplification. At time of progression, after two lines of chemotherapy (05/2014), the patient had a baseline blood draw and PET-CT and was administered trastuzumab (6 mg/kg q3w) in combination with lapatinib (1250mg qd), with no chemotherapy.

Results: Treatment was well tolerated with no dose reduction. Protein-ligation assay (PLA) by rolling-circle amplification performed at baseline showed HER2-HER3 heterodimers in circulating tumor cells. After only 15 days of treatment, PET-CT displayed a complete metabolic response and a slight decrease of the diameter of liver metastasis (-20%). At day 21, no CTCs were detected in 7.5ml of peripheral blood and CA15.3 dropped from 45 (baseline) to 32 (upper limit = 30). ctDNA was also collected and is currently being analyzed. Dual blockade has been pursued; updated follow-up will be presented at the meeting.

Conclusion: HER3 mutations are particularly rare in breast cancer (<1% of breast cancers) and no trial is currently assessing the relevance of HER2-blockade in this subgroup. On the basis of an extreme response to dual HER2 blockade with no chemotherapy, we confirm the published preclinical data and suggest that these patients may benefit from anti-HER2 therapies. A prospective global registry for HER3 mutated breast cancers cases and their response to specific systemic therapies may facilitate the accrual of data to support the use of anti-HER2 agents in this patient population.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P6-03-06  
**Average Grade:** 6.00

**Title:** Therapeutic targeting of ER$\beta$ in triple negative breast cancer

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**Body: Background:** While the biological functions and clinical importance of ER$\alpha$ are well understood in breast cancer, much less is known about its most closely related family member, ER$\beta$, particularly in the setting of triple negative disease. Additionally, the ability to therapeutically target ER$\beta$ in triple negative breast cancer (TNBC) has not been fully explored.

**Methods:** Expression of ER$\beta$ protein was determined using a well characterized and validated ER$\beta$ specific monoclonal antibody that only recognizes the full-length form of this receptor (PPG5/10) in a cohort of 71 TNBCs. To further define the biological functions of ER$\beta$ in TNBC, novel ER$\beta$ expressing triple negative cell lines (MDA-MB-231 and Hs578T) were developed and comprehensively characterized at the level of global gene expression profiling, modulation of important biological pathways, cellular proliferation and response to targeted therapies.

**Results:** In TNBCs from 71 patients, nuclear and cytoplasmic ER$\beta$ was detected at moderate to high levels in 24% and 32% of cases respectively. This moderate to high expression of both nuclear and cytoplasmic ER$\beta$ was associated with higher levels of Ki67. Of the 17 tumors expressing ER$\beta$, 13 (76%) were negative for androgen receptor expression. In the triple negative MDA-MB-231-ER$\beta$ and Hs578T-ER$\beta$ cell lines, expression of ER$\beta$ led to inhibition of proliferation in response to both estrogen and multiple ER$\beta$ specific agonists. Microarray analysis and RT-PCR profiling of these cells revealed that estrogen and ER$\beta$ agonists highly induced the expression of multiple cystatins, a family of small secreted cysteine protease inhibitors, while suppressing the expression of many interleukins. Conditioned media isolated from estrogen or ER$\beta$ agonist treated MDA-MB-231-ER$\beta$ cells inhibited the proliferation rates and blocked TGF$\beta$ signaling in non-ER$\beta$ expressing TNBC cells, effects that were completely reversed following depletion of cystatins from the conditioned media.

**Conclusions:** ER$\beta$ is expressed in a substantial proportion of TNBCs, and most do not express the androgen receptor. In TNBC, where targeted therapies are lacking, our data suggest that estrogen or ER$\beta$ specific agonists would be expected to elicit anti-tumor effects when ER$\beta$ is expressed. These tumor suppressive effects of ER$\beta$ appear to be mediated in part through the actions of cystatins, and their inhibition of TGF$\beta$ signaling, suggesting that this family of secreted proteins may represent novel biomarkers for monitoring ER$\beta$ specific drug responsiveness and/or patient outcomes.
Targeting multiple pathways in breast cancer: Androgen receptor, HER2, and mTOR

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Background: Androgen receptor (AR) is expressed in ~60% of breast cancers (BC) and current evidence indicates that some BC become reliant on AR signaling for growth. AR activation affects numerous cellular functions and signaling cascades, including the HER2/PI3K/mTOR pathway. Conversely, the HER2/PI3K/mTOR pathway regulates steroid hormone receptors including AR. Therapeutics targeting this pathway such as the anti-HER2 trastuzumab (TRAS) and the anti-mTOR everolimus (EVE) have shown significant clinical benefit; however the impact that AR inhibition may have on enhancing the efficacy of these agents and a potential mechanism of synergy have not been explored in BC. The anti-androgen enzalutamide (ENZ) inhibits nuclear entry of AR, is clinically effective in castrate-resistant prostate cancer, and is currently being tested in clinical trials for BC.

Hypothesis: Given the overlap in these pathways, simultaneous inhibition of AR pathway with agents targeting HER2/PI3K/mTOR may be more effective in BC therapy.

Methods: HER2+ and triple-negative BC (TNBC) cell lines were treated with ENZ, TRAS, and EVE, either alone or in combination, at three clinically relevant doses per drug. Cell proliferation was measured either by crystal violet (MDAMB453, SUM225) or on an IncuCyte live cell imager (Essen Bioscience) with nuclear-red labeled cells (BT474, SKBR3, BT549). Drug synergy was calculated using Calcusyn (Biosoft, Inc.) from IncuCyte proliferation data for selected cell lines (BT474, SKBR3, BT549) by comparing combinations of multiple drug concentrations where a combination index (CI)<1 indicated synergy. Protein expression of pathway components was measured by western blot, and gene expression was measured by RT-qPCR.

Results: ENZ inhibited proliferation of HER2+ BC cells, and a combination of ENZ plus TRAS inhibited proliferation more effectively than either agent alone. The effect was synergistic in SKBR3 cells (CI<0.1), and additive in BT474 cells. Treatment of a HER2+/ER+ cell line (BT474) as well as an ER-, HER2-non-amplified but overexpressing cell line (MDAMB453) with dihydrotestosterone (DHT) caused increased expression of pHER3 and total HER3 protein, which was attenuated by addition of ENZ. However, in HER2+/ER- cell lines (SKBR3 and SUM225), pHER3 and total HER3 levels did not change, but rather pHER2 levels increased in response to DHT and were attenuated with ENZ treatment. Additionally, ENZ inhibited proliferation of TRAS-resistant SKBR3 cells. However, unlike the parental SKBR3 cells, ENZ did not affect pHER2 levels in the TRAS-resistant cells. ENZ plus EVE treatment resulted in a significant decrease in proliferation compared to single agent; this effect was synergistic in two HER2+ cell lines (SKBR3 CI<0.1, BT474 CI<0.1) as well as one TNBC cell line (BT549 CI<0.5). Treatment with EVE alone caused a compensatory increase in AR protein and downstream AR gene expression in all cell lines. This increase was attenuated with ENZ plus EVE combination treatment.

Conclusion: ENZ synergizes in vitro with FDA-approved BC therapies TRAS and EVE through distinct mechanisms. Combination therapies containing ENZ may provide benefit for multiple BC subtypes, including HER2+ and triple negative BC.

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Title: Androgen receptor (AR): A novel target for radiosensitization and treatment in triple-negative breast cancers (TNBC)

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Body: Background: Increased rates of locoregional recurrence have been observed in TNBC despite the use of chemotherapy and radiation (RT). Thus, approaches that result in radiosensitization of TNBC are critically needed. We have previously characterized the radiation response of 21 breast cancer cell (BCC) lines using clonogenic survival assays. We now pair this data with high-throughput drug screen data available through cancer cell line encyclopedia studies to identify AR as a top target for radiosensitization and assess AR inhibition as a radiosensitization strategy for TNBC.

Methods: Clonogenic survival assays were performed to determine the intrinsic RT sensitivity of 21 BCC lines (0-8 Gy RT). IC50 values were determined for 130 clinically available compounds and correlation coefficients were calculated using IC50 values (for drug sensitivity) and SF-2Gy (for radiation sensitivity). Gene expression was measured using Affymetrix microarrays and protein expression was measured using reverse-phase protein lysate arrays (RPPA) of human tumor samples (n=2,061) and BCC lines (n=51). AR function was assessed using siRNA knockdown or inhibition with MDV3100 (enzalutamide). Kaplan-Meier analysis was performed to determine the clinical impact of AR expression on local control and survival. A Cox proportional hazards model was constructed to identify potential factors of survival, and multivariate analysis was used to determine variables most significantly associated with LRF survival.

Results: Our radiosensitizer screen nominated bicalutamide as one of the most effective drugs in treating radioresistant BCC lines (R²= 0.46, p-value <0.001). Recognizing that a subgroup of TNBC includes AR expressing tumors, we interrogated the expression of AR in >2000 human breast tumor samples and found significant heterogeneity in AR expression with an increase in TNBC (35% of tumors) compared to non-TNBC (28% of tumors). This same heterogeneity was also identified in human BCC lines. There was a strong correlation between AR RNA expression and protein expression (R²= 0.72, p <0.0001). Inhibition of AR using both siRNA and MDV3100 induced radiation sensitivity in vitro with an enhancement ratio (ER) of 1.35-1.42 in AR-positive TNBC lines. No such radiosensitization was seen in AR-negative TNBC or ER-positive, AR-negative BCC lines. Radiosensitization was at least partially dependent on impaired dsDNA break repair mediated by DNAPKcs. In vivo assessment of tumor growth inhibition with RT and anti-AR strategies are currently underway. Clinically, analyses of patients with TNBC showed that patients whose tumors had high expression of AR had markedly higher rates of LR after RT than patients with low expression of AR (HR for LR 2.9-3.2, p-value <0.01, 2 independent datasets). There was no difference in LR in TNBC patients not treated with RT when stratified by AR expression status. In multivariate analysis, AR expression was the variable most significantly associated with worse LRF survival after RT with a HR of 3.58 (p-value < 0.01).

Conclusion: Our results implicate AR as a mediator of radioresistance in breast cancer and support the rationale for developing clinical strategies to inhibit AR as a novel radiosensitizing target in TNBC.
Targeting the c-myc/E2F1 pathway in TNBC promotes a DNA damage dependent synthetic lethality

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The c-myc/E2F1 pathway is highly upregulated in TNBC and dictates increased genomic instability, a higher frequency of relapse and poor overall survival. C-myc is a potent oncogene with overexpression activity that influences several pathways including metabolism, DNA replication, DNA damage, cell cycle and apoptosis. The essential role of c-myc in normal cells has made therapeutic intervention elusive. However the role of c-myc influencing cell cycle progression has demonstrated that CDK inhibition downregulates c-myc expression in TNBC breast cancer cells. We hypothesize that CDK inhibition downregulates c-myc expression resulting in an accumulation of DNA damage thus increasing the susceptibility of TNBC cells to PARP inhibition. A panel of TNBC cell lines was assessed for c-myc/E2F1 pathway expression and downregulation in the presence of CDK inhibitor Dinaciclib. An MTT-based High Throughput Survival Assay (HTSA) was utilized to assess growth inhibition response to drug treatment in TNBC cells. FACS analysis was performed on Dinaciclib treated TNBC cell lines to assess cell cycle response to CDK inhibition. DNA damage and Homologous Recombination DNA repair was assessed by γH2AX, BRCA1, RAD51 foci formation by immunofluorescence. Lentiviral knockdown of c-myc, E2F1, BRCA1 and RAD51 were performed to assess susceptibility to PARP inhibition. Combination treatment of Dinaciclib + PARP inhibitor MK4827 was assessed via combination index values calculated by CalcuSyn. CDK inhibition resulted in downregulation of both c-myc and E2F1 in all TNBC cells. The pan-CDK inhibitor Dinaciclib universally inhibited proliferation in all TNBC cell lines in the presence of the drug however drug removal allowed TNBC cells to recover a normal proliferative profile. Dinaciclib induction increased γH2AX foci formation while decreasing BRCA1 and RAD51 foci formation resulting in an increase in DNA damage induced cell death that correlated with c-myc downregulation. CDK inhibition correlated with downregulation of c-myc/E2F1 target genes. Lentiviral knockdown of c-myc and not E2F1 induced susceptibility to PARP inhibitor MK4827. Combination CDK + PARP inhibitor resulted in a dose dependent synthetic lethal increase in PARP inhibitor efficacy in both BRCA1 mutant and wild-type cells. Together these results suggest that c-myc downregulation via CDK inhibition in combination with PARP inhibition may pose a novel combination therapy for TNBC patients.
Title: Targeting voltage-gated sodium channels with the antiepileptic drug phenytoin inhibits breast tumor growth and metastasis

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Body: Voltage-gated Na⁺ channels (VGSCs) are heteromeric protein complexes containing pore-forming α subunits and smaller, non-pore-forming β subunits. The β subunits are multifunctional channel modulators and are members of the immunoglobulin superfamily of cell adhesion molecules (CAMs). VGSCs are classically expressed in electrically excitable cells, e.g. neurons, where they mediate action potential firing, neurite outgrowth and migration during development. An increasing body of evidence indicates that VGSCs are also expressed in metastatic cells from a number of cancers, including breast cancer. The Na⁺v1.5 α subunit (encoded by SCN5A) is expressed in cancer cell lines, where it enhances migration and invasion. SCN5A is up-regulated in tumor samples in several published datasets, associating with recurrence, metastasis, and reduced overall survival. We have previously shown that the VGSC-blocking antiepileptic drug phenytoin inhibits the migration and invasion of metastatic MDA-MB-231 cells in vitro. In addition, we have recently shown that the VGSC β1 subunit enhances breast tumor growth and metastasis in a xenograft model of breast cancer. The purpose of the present study was to establish whether or not VGSCs might be a viable therapeutic target by testing the effect of phenytoin on tumor growth and metastasis in vivo. We found that Na⁺v1.5 expression was retained on MDA-MB-231 cells in orthotopic xenografts in immune-deficient mice. Mice were treated with phenytoin (60 mg/kg) or vehicle by daily intraperitoneal injection for 3 weeks, starting 1 week following implantation of tumor cells. Plasma phenytoin concentration was measured at the end of the experiment by liquid chromatography-mass spectrometry with single reaction monitoring (LC-SRM-MS) using metaxalone as an internal standard. Phenytoin significantly reduced tumor growth, detected by in vivo bioluminescent imaging and caliper measurements, without affecting animal weight. Phenytoin reduced the density of Ki67-positive cycling cells in the primary tumors, but did not affect the density of apoptotic cells expressing activated caspase-3, or the density of CD31-positive vessel structures. Phenytoin also reduced local invasion and the density of MMP9-positive cells within primary tumors. Finally, phenytoin significantly reduced metastasis to the liver, lungs and spleen, detected by bioluminescent imaging and GFP immunohistochemistry. This is the first study showing that phenytoin reduces tumor growth and metastasis in vivo. Together, our data support the hypothesis that VGSCs are up-regulated in breast cancer, favoring an invasive phenotype, and may thus be promising targets for therapeutic intervention. We propose that pharmacologically targeting VGSCs should be further studied as a potentially novel, cost-effective, anti-cancer therapy. Repurposing FDA-approved oral VGSC-blocking antiepileptic or antiarrhythmic drugs, e.g. phenytoin, may improve patient outcomes in the adjuvant setting.
**Title:** Erk5 as a therapeutic target in triple negative breast cancer (TNBC)

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**Body:** TNBC is a subtype of breast cancer known for its aggressive behavior and poor prognosis. TNBC accounts for 17-24% of all invasive breast cancers and is a subtype that lacks the expression of hormone receptors; oestrogen [ER], progesterone [PR]) and epidermal growth factor receptor 2 (HER2) receptors. Currently there has been a shift from using empirically derived agents that inhibit tumor cell growth and/or survival, to molecular therapies that target specific molecules that regulate these and other important biological processes. Despite the success of some new-targeted therapies to treat breast cancer, the outlook for the majority of patients with TNBC remains poor. Therefore we have utilized the KinexTM antibody array to resolve potential new targetable "nodes". We have screened 43 primary breast cancer biopsies (16 TNBC, 16 ER/PR positive and 11 HER2-positive) and 16 breast cancer cell lines for protein/phosphoprotein levels. Extracellular-signal-regulated kinase 5 (Erk5) is a member of the MAPK family. Erk5 has a large, unique C-terminal-half not found in other MAPK family members and because of this unique 400-amino acid extension, ERK5 is also called big MAP kinase 1 (BMK1). By extensive in silico analyses, we identified that the Erk5 metagene is prognostic and identifies TNBC/BLBC with poor prognosis. Our kinome study of primary breast cancer has further confirmed that the MEK5-Erk5 pathway is upregulated in the TNBC subgroup compared to the other TNBC subgroups and that pharmacological inhibition of Erk5 is therapeutic against primary MDA-MB-231 TNBC xenografts (1).

Our preliminary data has shown that Erk5 is overexpressed in the TNBC subtype at the protein level. We have also shown that silencing Erk5 by siRNA or shRNA inhibits migration of TNBC cell lines in vitro. Expression of Erk5 (total and phosphoproteins) will be investigated in primary breast tumours and metastases samples from patient by immunohistochemistry. We will also overexpress Erk5 in non-metastatic breast cancer cell lines to study the effect of Erk5 overexpression on metastases. In an elegant study, site directed mutagenesis was used to modify Erk5 and show that phosphorylations of the C-terminal domain of kinase activated Erk5 is required for the transcriptional activity of Erk5. Using in vitro and in vivo models we will determine if the transcriptional activity of activated Erk5 is a major player to drive metastatic phenotype. Moreover, whether kinase-dependent or transcription-dependent activity of Erk5 on several pathways is sufficient to drive metastasis will also be determined. Our results strongly suggest the MEK5-Erk5 pathway as a prognostic and therapeutic pathway in a poor-prognosis subset of TNBC/BLCL. Characterization of this pathway in clinical cohorts particularly in TNBC tumors, mechanistic understanding of its contribution to metastasis and therapeutic targeting in vivo would help develop a personalised approach to identify and treat TNBC tumors and metastases with activated Erk5 pathway.

Title: High MELK expression levels correlate with shorter overall survival in breast cancer

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Body: Background

Triple-negative breast cancer (TNBC) is thought to relapse and metastasize because cancer stem cells (CSCs) resist conventional chemotherapy and radiotherapy and later give rise to secondary tumors. MELK (maternal embryonic leucine zipper kinase), a protein kinase of the Snf1/AMPK kinase family, is known to play a critical role in promoting cell proliferation, CSC maintenance, apoptosis, and transformation. MELK is frequently upregulated in basal-like breast cancers but is not expressed in normal vital organs (Lin et al, Breast Cancer Res 2007;9:R17; Wang et al, eLife 2014;10.7554/eLife.01763). We hypothesized that MELK is upregulated in TNBC and that high expression correlates with poor progression-free survival (PFS), distant metastasis-free survival (DMFS), and overall survival (OS) in breast cancer.

Methods

This retrospective study involved the World Inflammatory Breast Cancer Consortium dataset comprising 389 patients from 3 institutions (The University of Texas MD Anderson Cancer Center, General Hospital Sint-Augustinus, and Institut Paoli-Calmettes) with clinical and tumor data and gene expression (GE) profiles. We studied the 314 patients with stage I-III breast cancer (84 TNBC and 230 non-TNBC) within this dataset. Hormone receptor (HR) and HER2 status was defined using the GE data [ESR1 (probe set 205225_at), PGR (208305_at), ERBB2 (216836_s_at)]. We defined high MELK mRNA GE (probe set 204825_at) as ≥7.0 using a Martingale residual plot. Time-to-event endpoints were summarized using the Kaplan-Meier method and compared between or among groups using the log-rank test. Multicovariate Cox proportional hazard models were applied to assess the effect of covariates on survival endpoints.

Results

Median age of patients was 54 yrs (range, 24-89). Disease stage was III in 48.4% of patients, II in 30.9%, and I in 20.7%. Most tumors had ductal histology (82.5%) and almost half had nuclear grade III (49.7%). MELK expression was significantly higher in TNBC than in the HR+HER2-, HR+HER2+, and HR-HER2+ subtypes (p<0.0001, Kruskal-Wallis test). Median follow-up was 5.7 years. In univariate analysis, patients with MELK-overexpressing tumors had significantly shorter PFS, DMFS, and OS times than patients with low MELK expression (table).

In multicovariate Cox regression model, high MELK expression did not have independent prognostic value for PFS (p=0.37) or DMFS (p=0.29); however, compared with low expression, was associated with a higher risk of death (hazard ratio=1.79; 95% CI=1.11-2.89; p=0.02), adjusted for tumor stage, IBC status and TNBC status and stratified by the study center.

Conclusion

High MELK expression was an independent prognostic factor of OS in breast cancer. Our preliminary results and those of others suggest that MELK may be an important therapeutic target for breast cancer, particularly TNBC, where the MELK expression level was significantly higher than in other subtypes; this warrants further investigation with a large dataset. This study justifies developing MELK inhibitors in TNBC.

5-year DFS, DMFS and OS rates

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**Title:** Inhibition of Src increases radioiodide uptake in breast cancer cells by inhibiting phosphorylation of pituitary tumor transforming gene binding factor (PBF)

Vikki L Poole\(^1\), Martin L Read\(^1\), Rachel J Watkins\(^1\), Bhavika Modasia\(^1\), Waraporn Imruetaicharoenchoke\(^1\), Kristien Boelaert\(^1\), Vicki E Smith\(^1\) and Christopher J McCabe\(^1\). \(^1\)University of Birmingham, Birmingham, West Midlands, United Kingdom.

**Body:** Although not detectable in normal breast tissue, the sodium iodide symporter (NIS) has been found to be expressed in 70-80% of breast cancers. However, the majority of NIS is intracellular, leaving only 20-30% functional at the plasma membrane. Whilst radiodine therapy has been proposed as a potential treatment for breast cancer, effective therapy would require increased levels of membranous NIS localisation in tumours. Previous work revealed that overexpression of pituitary tumor transforming gene binding factor (PBF) in thyroid cells leads to the redistribution of NIS from the plasma membrane into intracellular vesicles, thereby reducing radioiodide uptake, a process modulated by Src phosphorylation of PBF. Here we show that PBF and NIS have a consistent relationship in breast cancer, with phosphorylation of PBF at residue Y174 being critical for the association. Immunofluorescent microscopy revealed co-localisation between NIS and PBF in co-transfected MDA-MB-231, MCF-7 and T47D cells, with increased intracellular staining for NIS compared to cells transfected with NIS alone. Phosphorylated PBF was also observed to co-localise with NIS in T47D cells. Treatment with PP1, a Src inhibitor which modulates the phosphorylation of PBF, led to increased NIS plasma membrane staining and less intracellular co-localisation with PBF. Functional studies in MCF-7 and MDA-MB-231 cells demonstrated that PBF significantly repressed radioiodide uptake in cells expressing exogenous NIS (25% and 30% reduction respectively; \(n=3\), \(p<0.05\)). Treatment with PP1 restored the ability of MCF-7 and MDA-MB-231 cells to uptake I-125 (1.24 and 1.69 fold increase respectively; \(n=3\), \(p<0.05\)). Combined all-trans retinoic acid (ATRA) and dexamethasone treatment has previously been shown to enhance NIS expression and radioiodide uptake in MCF-7 cells. Importantly, in the face of NIS-induction via ATRA and dexamethasone, PBF retained its ability to repress iodide uptake (31% reduction; \(n=3\), \(p<0.05\)). This repression was overcome by PP1 treatment, which restored radioiodide uptake to vector only transfected levels (1.22-fold increase; \(p=ns\), \(n=3\)). In keeping with PP1 data, the Src inhibitors saracatinib and dasatinib inhibited the phosphorylation of PBF in MCF-7 and MDA-MB-231 cells. Radioiodide uptake studies revealed that 1nM dasatinib was capable of restoring the ability of MDA-MB-231 cells to uptake I-125 (2 fold increase; \(n=3\), \(p<0.05\)) when expressing exogenous NIS and PBF compared to vehicle only treated cells. 10nM saracatinib was also capable of restoring I-125 uptake but with less potency than dasatinib. Inhibition of focal adhesion kinase (FAK) with PF573228 failed to ameliorate PBF’s repression on I-125 uptake, suggesting that FAK is not responsible for PBF phosphorylation. Taken together, these data suggest that PBF alters the subcellular location of NIS and potently represses its activity in breast cancer cells. Further, tyrosine phosphorylation of PBF by Src modulates the ability of breast carcinoma cell-lines to take up radioiodide, with important implications for adapting NIS as a potential therapy in breast cancer.
Title: Activation of myristoylated alanine-rich C kinase substrate (MARCKS) potentiates cancer malignancy and paclitaxel resistance in triple-negative breast cancer

Ching-Hsien Chen¹, Muhammad Arif¹, Wen-Hsin Chang¹, Yuan Yuan², Jing Zhai², David K Ann² and Reen Wu¹. ¹University of California, Davis, CA and ²City of Hope National Medical Center, Duarte, CA.

Body: Increasing evidence has suggested the importance of protein kinase C (PKC)-mediated phosphorylation of myristoylated alanine-rich C kinase substrate (MARCKS) in modulating various cellular processes, including cancer cell growth and metastasis. However, there is a lack of information regarding to the roles of MARCKS and its pSer 159/163 phosphorylated product in breast cancer. To tackle this concern, we recently initiated both immunohistochemical and western blot analyses on a series of clinical specimens and several breast cancer cell lines. We have observed a significant elevation of phospho-MARCKS associated with advanced-stage breast cancer tissues as compared with benign ones, particularly in triple-negative breast cancer (TNBC) tissues and cell lines. Knockdown of MARCKS expression resulted in suppressions of lamellipodia/filopodia formation and migration/invasion as well as down-regulation of Src activity in these TNBC cell lines. Of note, MARCKS-silenced TNBC cells grew much more slowly and more sensitive to paclitaxel than the control and parental cells. Treatment with paclitaxel was shown to induce MARCKS phosphorylation in a dose-dependent manner, which may be related to an enhanced paclitaxel resistance in some of breast cancer cells. Interestingly, blockade of paclitaxel-induced MARCKS phosphorylation was able to abrogate Src activation. Consistent with this potential, treatments of TNBC cells with a MARCKS N-terminus sequence peptide, namely MANS, to down regulate MARCKS activity not only attenuated the associated aggressive phenotype but also synergistically enhanced anticancer efficacy of paclitaxel treatment through regulation of angiogenesis. These results demonstrate an association of PKC-mediated MARCKS phosphorylation with breast cancer malignancy potential and MARCKS phosphorylation as a predictor of paclitaxel resistance in TNBC cells. It is suggestive that an inhibition of PKC/MARCKS pathway may serve as an alternative therapeutic strategy for enhancing efficacy of chemotherapy.
Title: Melatonin on angiogenesis in breast cancer

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Body: The rapid tumor growth results in hypoxia on tumor microenvironment, leading to a cascade of events that induce angiogenesis and subsequent cancer progression. Thus, the identification of therapeutic agents that can inhibit angiogenesis is essential for the control of tumor progression. Exogenous administration of melatonin, a hormone secreted by the pineal gland, has been shown several oncostatics effects on different types of cancers. The aim of this study was to evaluate the effectiveness of melatonin treatment on angiogenesis in breast cancer, in the in vitro and in vivo studies. In the in vitro study, breast cancer cell lines (MCF-7 and MDA-MB-231) were treated with melatonin under cobalt chloride (CoCl2)-induced hypoxic conditions. Cell viability was measured by MTT assay, the expression of hypoxia-inducible factor 1-alpha (HIF-1α) and vascular endothelial growth factor (VEGF-A) was assessed by real-time PCR and immunocytochemistry. Additionally, other proteins involved in angiogenesis were evaluated by the Protein Array. In the in vivo study, the MDA-MB-231 cells were implanted in athymic nude mice, which were treated with melatonin (40 mg/kg) for 21 days. The tumor was measured weekly and evaluation of angiogenesis was performed by single-photon emission computed tomography (SPECT) with Tc-99m-HYNIC-VEGF-c, which is specific for VEGF receptors (VEGFR2/VEGFR3). Moreover, VEGFR2, VEGFR3, von Willebrand factor (vWF) and cell proliferation marker (Ki-67) were evaluated in tumor tissue by immunohistochemistry, and other angiogenic proteins by Protein Array. Results from the in vitro study showed that 1 mM of melatonin under hypoxic conditions (200 µM CoCl2) led to decreased cell viability, protein levels of HIF-1α and gene and protein expression of VEGF-A in both cell lines (p < 0.05). Among other proteins evaluated, melatonin treatment under hypoxia resulted in a decrease of VEGF-C, VEGFR2, VEGFR3, matrix metalloproteinase 9 (MMP-9) and angiogenin in MCF-7 cell line (p < 0.05). For MDA-MB-231, a significant reduction was observed for VEGFR2 protein, epidermal growth receptor (EGFR), and angiogenin (p <0.05). In vivo study, mice treated with melatonin showed reduced tumor growth compared to control animals (144.90 ± 38.38 mm³ vs 282.00 ± 88.53 mm³, p < 0.05). Furthermore, one animal showed tumor regression during melatonin treatment (Day 7 = 27.38 mm³, 8.79 mm³ Day = 14, mm³ Day 21 = 4.8). SPECT detect less radioactivity of Tc-99m-HYNIC-VEGF-c and consequent reduced expression of VEGFR2/3 in tumors treated with melatonin (150.46 ± 17.06 % vs 183.55 ± 20.92 %) but statistical significance was not achieved (p > 0.05). The reduction of VEGFR2 in tumors treated with melatonin was confirmed by immunohistochemistry (p <0.05), as well as the reduction of micro-vessel density (vWF) and cell proliferation (ki-67) (p < 0.05). There was no change in the expression of the other proteins evaluated, however a significant increase in EGFR and Insulin-like growth factor (IGF-I) was observed in tumors treated with melatonin (p< 0.05). Taken together, our results showed that melatonin has an important anti-angiogenic effect, suggesting its potential therapeutic action in breast cancer.

Support: FAPESP.
Title: TAMs assisted tumor neo-angiogenesis in hypoxic milieu: Role of IL-16

Chakrapani Tripathi¹, Khemraj S Baghel¹, Brij N Tewari², Mehraj U-Din Lone¹, Richa Shrivastava¹ and Smrati Bhadauria¹. ¹Central Drug Research Institute, Lucknow, Uttar Pradesh, India and ²KGMU, Lucknow, Uttar Pradesh, India.

Body: TAMs, a unique and distinct M2-skewed myeloid population of tumor stroma, exhibiting pro-tumor functions is fast emerging as a potential target for anti-cancer immunotherapy. Macrophage-recruitment and M2-polarization represent key TAMs-related phenomenon that are amenable to therapeutic intervention. However successful translation of these approaches into effective therapeutic regimen requires better characterization of tumor-microenvironment derived signals that regulate macrophage recruitment and their polarization. Owing to hypoxic milieu being a persistent feature of tumor-microenvironment and a major contributor to malignancy and treatment resistance, the current study was planned with an aim to decipher tumor cell responses to hypoxia vis-a-vis macrophage homing and phenotype switching. We recently demonstrated that hypoxia-primed cancer cells chemoattract and polarize macrophages to M2-polarized subtype via Eotaxin and Oncostatin M. The current study was planned to evaluate whether these M2 polarized macrophages may potentiate tumor angiogenesis. Using in vivo CAM assay we demonstrated that these macrophages exhibited enhanced pro-angiogenic potential. Furthermore, apart from potentiating tumor neo –angiogenesis on their own these M2 polarized macrophages also impart proangiogenic properties to normoxic cells that have never encountered hypoxia directly. Cytokine profiling of the conditioned media indicated for involvement of IL-16. The findings may establish these cytokine as novel targets for devising effective anticancer therapy particularly for tumors that are refractory or develop resistance to anti-angiogenic therapeutics.
Title: Endoplasmic reticulum resident protein transmembrane protein 33 (TMEM33) induces apoptosis via UPR signaling and autophagy in breast cancer cells

Rong Hu¹, Xiyuan Zhang¹, Leena Hilakivi-Clarke¹, Usha Kasid¹ and Robert Clarke¹. ¹Georgetown University, Washington, DC.

Body: Breast cancer is the most common cancer type in women, with expected incidence of around 232,670 new cases in the US alone each year. Endoplasmic reticulum stress (EnR stress) and the related unfolded protein response (UPR) are activated in breast cancer cells to promote cell survival and endocrine resistance. TMEM33 is a novel transmembrane protein that resides in the endoplasmic reticulum (EnR) and has been shown to activate two of the UPR branches (PERK; IRE1α). However, the cellular functions and underlying mechanism of this EnR resident protein remains largely unknown. In this study, we show that overexpression of TMEM33 induces robust cell death in breast cancer cells. TMEM33 overexpression strongly activates the pro-death JNK-p53 pathway, possibly through the activation of IRE1α. We also observe a significant inhibition of the downstream survivin (also called BIRC5), which is known to inhibit cell death by binding to caspases and blocking their activation. We further show that either the blockage of JNK activation with a small molecule inhibitor, or the overexpression of survivin, protects cells against TMEM33-induced apoptosis. In addition, we demonstrate that TMEM33 overexpression induces autophagy in breast cancer cells (changes in LC3 and p62 expression). Inhibition of autophagy with either the autophagy inhibitor chloroquine, or by knockdown of the autophagy gene Atg5, further sensitizes breast cancer cells to TMEM33 overexpression. Conversely, cell death induced by TMEM33 is decreased by overexpression of the autophagy gene Beclin 1. The findings in this study demonstrate that the novel EnR resident protein TMEM33 induces cell death by activating the IRE1α-JNK-p53-survivin pathway in breast cancer cells. Paradoxically, autophagy is also activated by TMEM33 but the prosurvival action is generally not sufficient to block all of the cell death signaling.
2014 San Antonio Breast Cancer Symposium

**Publication Number:** P6-05-02  
**Average Grade:** 0

**Title:** Expression of ING1b-derived peptide inhibits viability of breast cancer cells and promotes apoptosis

Oleksandr Boyko¹ and Karl Riabowol¹. ¹University of Calgary, Calgary, AB, Canada.

**Body:** The ING1b protein is a type II tumor suppressor and a stoichiometric member of HDAC-containing protein complexes. Primarily by altering chromatin structure, ING1b contributes to regulation of gene expression, senescence and apoptosis. Mislocalization and decreased levels of ING1b are commonly observed in human tumors and cancer cell lines. Multiple independent studies in varied cell types report that ING1b overexpression promotes apoptosis. Since the inactivation of apoptosis pathways is frequent in cancer cells, modulating ING1b expression in tumors may serve as a viable approach for cancer therapy.

We have defined the minimal ING1b region that confers apoptotic function as estimated by the levels of PARP cleavage, FACS analysis and using an Annexin V binding assay. We have established that ING1b-derived peptides containing its third alpha helix (A3H) and NLS/NTS domains are able to induce apoptosis at levels comparable to those of full length ING1b. The A3H-NLS/NTS peptide exhibited strong nucleolar localization characteristic of full length ING1b. Cells overexpressing the full length ING1b and A3H-NLS/NTS peptide showed similar changes in cell morphology characteristic of apoptosis and exhibited increased levels of PARP cleavage. While the A3H region that includes the N-terminal part of the highly conserved Lamin Interacting Domain (LID) was necessary but not sufficient, the NLS/NTS domain that mediates nuclear and nucleolar localization of ING1b was required and partially sufficient for induction of apoptosis. Adenoviral-mediated expression of A3H-NLS/NTS peptide in cells of osteosarcoma, glioblastoma and breast cancer origins resulted in strong reduction of cell viability. Depending on the cell line, treating cells with 45 MOI of virus expressing A3H-NLS/NTS peptide resulted in a 60 - 85% decrease in cancer cell survival compared to cells treated with the control construct (Ad-GFP), and the highly transformed triple negative tumorigenic MDA-MB-468 breast cancer line was sensitive to the peptide when infected with 5 MOI of A3H-NLS/NTS adenovirus. These pro-apoptotic effects were found to be dose and time dependent. Furthermore, using p53 wild-type, p53 mutant and p53-null cancer cell lines we demonstrated that the effects of A3H-NLS/NTS expression on cell survival and apoptosis were independent of p53-status. The evaluation of the synergy between the A3H-NLS/NTS peptide and common chemotherapeutic agents is currently ongoing. Our long-term goal is to develop ING1b-based therapeutics that can be used as an adjuvant therapy in combination with the existing breast cancer treatments.
2014 San Antonio Breast Cancer Symposium

**Publication Number:** P6-05-03  
**Average Grade:** 5.20

**Title:** CD4 Th1 cytokines and HER-2/HER-3 blockade induces tumor senescence in breast cancer

Cinthia Rosemblit¹ and Brian J Czerniecki¹. ¹University of Pennsylvania, Philadelphia, PA.

**Body:** Background: HER-2 a molecular oncodriver in breast tumorigenesis is over expressed in 25% of human breast cancers, and its expression correlates with enhanced tumor aggressiveness. While targeted therapies have improved outcomes many patients become resistant or recur. We have recently established a progressive loss in anti-HER-2 CD4 Th1 responses during disease progression and is associated with outcomes. The pleiotropic Th1 cytokines IFN-γ and TNF-α have diverse effects on tumor epithelial cells. In this study we sought to determine whether these Th1 cytokines induce senescence of HER-2 expressing breast cancer cells and assess the impact of IFN-γ and TNF-α with simultaneous HER-2 and HER-3 blockade.

**Results:** All breast cancer cell lines tested express IFN-γ and TNF-α receptors measured by western blot analysis. The high and intermediate HER-2 expressing cells are sensitive to tumor senescence induction when treated with combinations of IFN-γ and TNF-α in a dose dependent manner. Low HER-2 expressing cells were less sensitive to senescence induction as measured by positive β-galactosidase activity and the expression of p15INK4b and p16INK4a by western blot. Addition of IFN-γ and TNF-α treatment to HER-2-depleted cells by RNAi resulted in an increase senescent phenotype and was increased further when the cells were double HER-2- and HER-3-depleted. To determine whether CD4 Th1 mediated effects on high HER-2 human breast cancer cell lines, we co-cultured increasing number of HER-2 antigen-primed CD4+ T cells with high HER-2 human breast cancer cells in a transwell cell culture system. This resulted in a dose-dependent senescence of breast cancer cells compared with CD4+ T cells primed either with immature dendritic cells (DC) or mature DC plus irrelevant (Class II BRAF) peptide. In addition, SK-BR-3 breast cancer cells incubated with the supernatant of the CD4+ T cells-immature DC or mature DC co-culture demonstrated similar results. CD4+ Th1-elaborated cytokines IFN-γ and TNF-α in the supernatants were confirmed using ELISA. Blocking antibodies against IFN-γ and TNF-α demonstrated reduced senescence induction.

**Conclusions:** Our results establish a role for IFN-γ and TNF-α in inducing tumor senescence in breast cancer. An effective CD4 Th1 responses combined with HER-2 and HER-3 blockade can significantly drive tumor senescence in breast cancer that can be explored as treatment to effectively eliminate residual breast cancer cells and prevent recurrence.
Title: Studies on tumor heterogeneity using a preclinical model of breast cancer caused by genetic alteration of the ATX-LPA axis

Lorenzo Federico¹, Kang Jin Jeong¹, Dong Zhang¹, Zhenlin Ju¹, Zechen Chong¹, Jennifer B Dennison¹ and Gordon B Mills¹.
¹University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Targeted alterations of the ATX/LPA signaling axis in mice creates a tumorigenic environment from which mammary neoplasias can develop. Using mammary tumors derived from this model, we have established a transplantation procedure in which fragments of different tumors are orthotopically transplanted in syngeneic mice. Under these conditions, primary murine tumors displayed a wide spectrum of growth rates suggesting that alteration of ATX/LPA signaling axis engenders a variety of different breast cancer subtypes with distinctive propensity to progress, which is similarly observed in human neoplastic disease. Comprehensive analysis of high-throughput RNAseq and Reverse Phase Protein Array (RPPA) data revealed that despite being passaged multiple times, tumors retained their distinct growth profiles and also maintained surprisingly stable molecular characteristics. The remarkable consistency in terms of growth, transcriptional landscape, and protein expression demonstrates that in this model a small fragment randomly taken from any region of the tumor can accurately and reproducibly regenerate an entire developmental program when grown in vivo. It follows that each consistent molecular change detected in the tumor likely represents either a marker of the tumor or its mechanistic oncodriver. Using unbiased multidimensional scaling analysis of RNAseq data we have identified tumors that were strictly related to normal mammary tissue in terms of gene expression as well as individual gene signatures that differentiate slow- vs fast-growing tumors. Furthermore, we have identified several stable alterations at a protein level that are commonly found in human breast cancers including cMYC, BRCA2, PARP1, Claudin-7, Her3, and E-Cadherin. These results demonstrate that an orthotopic transplantation procedure in immunocompetent hosts represents a relevant preclinical platform to probe human breast cancer heterogeneity and pinpoint molecular alterations that are mechanistically responsible for malignant behavior.
Title: Molecular characterization of a patient-derived xenograft (PDX) resource for triple negative breast cancer

Joel H Graber¹, James G Keck¹, Susan D Airhart¹, Carol J Bult¹ and Edison T Liu¹. ¹Jackson Laboratory, Bar Harbor, ME.

Body: The Jackson Laboratory (JAX) has developed a resource of human tumors implanted into immune deficient mice (patient derived xenografts; PDX) as a platform for testing standard of care and novel therapeutic options for Triple Negative Breast Cancer. PDX models provide an advantage over cell culture based models for testing therapeutic interventions because they retain properties such as tumor cell heterogeneity that are critical to the biological properties of a patient’s tumor and response to treatment.

Tumor material acquired from biopsy or surgical resection was implanted subcutaneously into the flank of immune deficient NOD-scid IL2r gamma-chain null (NSG) mice. The PDX resource currently contains 21 established breast cancer PDX models (12 TNBC) with 24 additional models currently in development. Two of the established TNBC PDX models have BRCA1 mutations. The median age of the patients from whom tumor material was obtained for all breast models is 53 (45-89). Tumors that successfully engrafted were characterized for somatic mutations using the new JAX Clinical Cancer Panel, Copy Number Variants using the Affymetrix human 6.0 SNP array, and gene expression using both Affymetrix U133 plus v2 and RNA-Seq. Normalized gene expression was analyzed for characteristic patterns in a pan-cancer approach across all PDX models and further compared with the previously identified TNBC molecular subtypes (Lehmann et al. 2011. JCI 121:2750-2767). The combination of principal components analysis and classification via expression pattern resulted in putative matches of models to most of the known molecularly defined subtypes of TNBC tumors.

Tumor bearing mice for the TNBC PDX models have been treated with docetaxel, cisplatin, cyclophosphamide and doxorubicin. Preliminary studies of tumor response to these treatment regimes revealed systematic differences that can be correlated with features of the genomic analysis, including expression subtype characterization.

The JAX collection of TNBC cancer PDX models is a well-annotated, publically available resource of models with deep genomic characterization and standard of care therapy response data for use in the development of advanced therapeutic options. Genomically defined subgroups within the collection suggest strategies to refine patient selection and treatment algorithms.

Information about the models along with summarized genomic data is publicly available at the Mouse Tumor Biology database PDX web portal (http://tumor.informatics.jax.org).
The development of malignant phyllodes tumor models

Yayoi Takamoto¹, Yoshimi Arima¹, and Hideyuki Saya¹. ¹Institute for Advanced Medical Research (IAMR), School of Medicine, Keio University, Tokyo, Japan.

Body:
Phyllodes tumors are rare fibroepithelial tumors in breast that are composed of epithelial and stromal components. Surgical resection is the standard treatment for patients with phyllodes tumors. With worsening histological grades, phyllodes tumors show more aggressive clinical behaviors such as a local recurrence, especially malignant phyllodes tumors may demonstrate distant metastasis. Although the pathological characterization of this tumor has been intensively studied, only a few molecular characteristics have been described, and none have been used for specific targeted therapy. To better understand these tumors, the mouse models, which recapitulate the tumor tissues biologically and pathologically similar to human phyllodes tumor, are extremely useful and required.

We constructed retrovirus vectors overexpressing oncogene, H-RasG12V or anaplastic lymphoma kinase (ALK)R1174Q. The normal mammary glands of C57BL/6 mice were digested and cultured in floating as mammospheres. The mammosphere-derived cells (MDCs) were infected with retroviruses having H-RasG12V or ALK1174Q and were transplanted into mice mammary glands. We investigated whether or not tumors developed. We also obtained normal mice mammary cells expressing CD24med/CD49high, which are defined as basal stem cells (BSCs), by fluorescence activated cell sorting (FACS). We retrovirally introduced H-RasG12V or ALK1174Q into BSCs, and these cells were transplanted into mice mammary glands.

FACS analysis revealed that both MSCs and BSCs predominantly expressed CD24low/CD49high which is defined as stromal components, after a passage of some time.

Both H-RasG12V/MSCs and H-RasG12V/BSCs formed tumors in mice, whose tumorigenic rate was 97.4% (37/38), and 100% (10/10), respectively. The tumorigenic rate of ALK1174Q/MSCs and ALK1174Q/BSCs was 35.7% (15/42), and 0% (0/9), respectively. Those mice tumors were pathologically similar to human malignant phyllodes tumors, which are characterized by an overgrowth of stromal cells like sarcoma with pericanalicular and/or intracanalicular patterns or leaf-like patterns, and have high recurrence rates with lung metastasis. The H-RasG12V-induced tumors tended to have more leaf-like patterns than the ALK1174Q-induced tumors, and the frequency of tumorigenesis and metastasis in H-RasG12V-induced tumors was higher than that of ALK1174Q induced tumors.

We established mice malignant phyllodes tumor models by introducing H-RasG12V or ALK1174Q into mice mammary stromal cells. These models would aid in revealing the pathophysiology of malignant phyllodes tumors.
Title: The role of sphingosine kinase 1 in breast carcinogenesis

Yoshiko Shimizu\(^1\), Hideki Furuya\(^1\), Paulette M Tamashiro\(^1\), Kayoko Iino\(^1\), Charles J Rosser\(^1\) and Toshihiko Kawamori\(^1\). \(^1\)University of Hawaii, Honolulu, HI.

Body: Introduction: The bioactive sphingolipids, ceramide/sphingosine and sphingosine 1-phosphate (S1P) possess opposite effects on cell fate. Sphingosine kinase 1 (SphK1) is an enzyme that principally regulates the balance or the "rheostat" of the sphingolipids by phosphorylating sphingosine to form S1P. In this study, we investigated the effect of SphK1 deficiency on HER2/neu-induced mammary carcinogenesis using a MMTV-neu transgenic (Tg) mice model. Methods: MMTV-neu Tg mice were crossbred with SphK1 knockout (KO) mice to generate MMTV-neu Tg mice with KO or wild-type for SphK1 gene. The number and size of the palpable tumors were recorded weekly until mice reached 35 weeks of age or the largest tumor diameter reached 20 mm in size. At necropsy, tumors and blood were collected for analysis. The sphingolipid profiles in blood were analyzed using tandem mass spectrometry. Results: The incidences of mammary tumor development in the homozygous SphK1 KO (1/13; 8%) and heterozygous SphK1 KO (11/44; 26%) were significantly reduced (P=0.0112 and 0.0208, respectively) when compared to wild-type mice (8/13; 62%). The mammary tumor multiplicity was significantly reduced in homozygous SphK1 KO (0.08±0.08; P=0.0036) when compared to wild-type mice (0.69±0.17). The S1P levels in blood were significantly decreased in homozygous SphK1 KO mice (P<0.0001), while sphingosine levels were significantly increased in the blood of heterozygous SphK1 KO mice (P<0.001). In both homozygous and heterozygous SphK1 KO mice, the blood level of C16:0-Ceramide was significantly increased (P<0.001). Conclusion: The results provide novel evidence that SphK1 mediates HER2/neu-induced mammary carcinogenesis by regulating the ceramide/sphingosine-S1P "rheostat". Thus, we propose that SphK1 inhibition may be a novel therapeutic option in the treatment of HER2 breast tumors.
Title: Anti-tumor efficacy of PI3K-mTOR pathway inhibitors in combination with PARP inhibitor plus carboplatin in BRCA1-competent TNBC, beyond PTEN: A proof of concept study

Nandini Dey¹, Yuliang Sun¹, Jennifer Carlson¹, Lori Friedman², Pradip De¹ and Brian Leyland-Jones¹. ¹Avera Research Institute, Sioux Falls, SD and ²Genentech, San Francisco, CA.

Body: Background: Recently we reported that PI3K-mTOR inhibition potentiated anti-tumor effects in BRCA-competent TNBC cells when combined with ABT888 (A) and carboplatin (C) (De et al., Neoplasia, 2014). Pro-proliferative and anti-apoptotic actions of this pathway constitute one of the main effector signals of RAS. Here, we tested the anti-tumor effect of either single or dual node blockade of PI3K-mTOR pathway when combined with A and C on BRCA-competent TNBC cells with WT-PTEN background and activated RAS-RAF pathway.

Materials & Methods: Athymic mice bearing TNBC xenograft tumors were treated with pan PI3K inhibitor, GDC-0941 or PI3K-mTOR dual inhibitor, GDC-0980 alone or in combination with A and C. Mechanism-based in vitro studies were performed using a panel of BRCA-wt/mutants TNBC cells with varying genetic backgrounds.

Results: Blocking a single nodal point of PI3K by GDC-0941 failed to significantly inhibit growth of pre-established tumors (> 20%) even in combination with A and C in MDA-MB231 xenografts, while GDC-0980 potentiated an anti-tumor effect by inhibiting tumor growth by 90%. In vitro treatment (1) decreased proliferation signals (pAKT, pP70S6K, p4EBP1, pS6RP), cell cycle progression, vascular mimicry & 2D clonogenic growth, and (2) increased apoptosis markers (cl-caspase3, 9, BIM, cl-PARP, & annexinV positivity). GDC-0980 in combination with A plus C concomitantly decreased Ki67, cl-caspase3, pVEGFR, CD31, p4EBP1, and pS6RP in vivo (IHC). In contrast, combination of GDC-0941 with A plus C failed to affect the cell cycle, apoptosis or 2D clonogenic growth in MDA-MB231 cells. Alternatively, Treatment with RAD001 increased pAKT while it perturbed the activation of S6RP/4EBP1.

Conclusion: Our data indicate that following dual inhibition of PI3K-mTOR, S6RP/4EBP1 de-phosphorylation tracks more consistently with the drug’s tumor-growth inhibitory response rather than the upstream state of AKT-activation. Unlike mTORC1 inhibitor RAD001, GDC-0980 potently eliminates (in vitro & in vivo) feed-back re-activation of the pathway as, (1) it targets PI3K, reactivation of AKT³⁰⁸ is blocked and (2) inhibition of the mTORC2 complex blocks the reactivation of AKT⁴⁷³. TNBC tumors with PTEN independent RAS/RAF mutation-mediated activation of PI3K-mTOR pathway can be controlled by dual node blockade of the pathway when combined with a PARP inhibitor and carboplatin.
Title: Amplified DERE-mediated epigenetic repression in ERα-mediated breast tumorigenesis

Pei-Yin Hsu¹, Hang-Kai Hsu¹, Yi-dong Chen¹, Victor X Jin¹, Zelton D Sharp¹ and Tim H-M Huang¹. ¹UTHSCSA, San Antonio, TX.

Body: Epidemiological studies reveal that maternal exposure to estrogenic chemicals during pregnancy results in higher risk of breast cancer in offspring. One of possible causal mechanisms is epigenetic reprogramming, but how estrogen/ERα signaling contributes to tumorigenesis through epigenetic machinery remains unclear. In this study, we demonstrate that the epigenetic modulation mediated by estrogen-driven amplification of distant-acting regulatory elements, or enhancers, may be an explanation addressing to this question. Enhancers contain transcription factor binding sites known to remotely regulate transcription through chromatin looping or transvection. Using our in vitro model to mimic maternal estrogen exposure and a "Seq-to-Seek" strategy integrating three next-generation sequencing approaches, we comprehensively mapped distant estrogen response elements (DEREs) that remotely control transcription of target genes through chromatin proximity. Surprisingly, a densely mapped DERE region located on chromosome 20q13 frequently amplifies in ERα-positive breast cancer with poor prognosis. Progressive accumulation of DERE copies was observed in normal breast progenitor and cancer cells chronically exposed to estrogen. Upon estrogen stimulation, these aberrantly amplified DEREs are clustered as a potential transcriptional depot for suppressing target gene expression through altering chromatin interactions, leading to accumulation of repressive histone marks (e.g. trimethylated H3K27 and H3K9), polycomb repressive complex 2, the presence of heterochromatin protein 1 (HP1) and DNA methylation, following prevention of RNA polymerase II and active histone mark- H3K4me3 binding. Furthermore, neutralization of HP1 function can significantly attenuate estrogen-driven DERE clustering and DERE-mediated repressive chromatin interactions, resulting in inhibition of cell proliferation. Deletion of the interested DERE regions using CRISPR/Cas9 genomic editing system further demonstrated that DERE-driven remote transcriptional control play a crucial role in tumor cell growth. Our data support a model in which amplified DEREs preferentially induce repressive epigenetic modulation of target genes. These findings indicate that 20q13 DERE region is a potential transcriptionally repressive domain whose aberrant amplification can result in suppressing expression of tumor suppressor genes, leading to tumorigenesis. In summary, our findings suggest that amplification of DNA regulatory elements can profoundly alter target transcriptome during tumorigenesis and amplified DEREs can be used as potential prognostic markers for endocrine resistance and predicative for progeny at risk of breast cancer.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-07-03
Average Grade: 7.00

Title: An exhaustive algorithm for detecting copy number aberrations and large structural variants in whole-genome mate-pair sequencing data

Yan W Asmann¹, Chen Wang², Brian M Necela¹, Xianfeng Chen², Jean-Pierre A Kocher², Matthew J Maurer², Thomas M Habermann², Susan L Slager², Andrew L Feldman², Anne J Novak², James R Cerhan², Edith A Perez¹ and E Aubrey Thompson¹. ¹Mayo Clinic, Jacksonville, FL and ²Mayo Clinic, Rochester, MN.

Body: Objectives and Rationale: Structural variants (SV) including large copy number aberrations (CNV), translocations, inversions, and large insertions and deletions (INDEL) play a critical role in tumorigenesis and progression. In fact, we now know that tumors can be categorized according to the size of mutations harbored. In several cancers, including ovarian and breast cancer, the large structural mutations, rather than single site mutations, play a dominate role in tumor etiology. Therefore, it's critical to implement reliable algorithm for the detection of structural variants in DNA sequencing data. Mate-pair sequencing is a protocol specifically implemented for detection of the whole-genome level structural variants. It requires less sequencing depth therefore is cost effective, and enables the detection of CNVs, translocations, and inversions simultaneously. However, so far there has been no reliable bioinformatics pipeline for the analyses of the mate-pair sequencing data.

Methods: Our novel algorithm, the SnowShoes-SV, is an exhaustive search algorithm designed specifically for mate-pair DNA sequencing data analyses. It calls the SVs based on disconcordant read pairs. The false SVs are filtered according to the following criteria: (i) the number of the supporting read pairs; (ii) the lack of reads from control data that implicate SV at the same region; (iii) the mappability and uniqueness of the region based on data from the ENCODE project; (iv) consistencies of the mapping orientations of the supporting read pairs; (v) the similar sizes between sequencing library and the two end read clusters.

Results: Using a set of samples previously genotyped by aCGH, the SnowShoes-SV successfully detected all known CNVs and other SVs. It also identified copy number neutral translocations and inversions previously not identified by aCGH. In addition, the algorithm nominated novel SVs which are to be validated by PCR.

Conclusions: SnowShoes-SV is a highly sensitive and specific algorithm for SV detection from the mate-pair DNA sequencing data.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-07-04
Average Grade: 5.33

Title: Targeting ErbB2 with human PEPD

Lu Yang¹, Yun Li¹, Arup Bhattacharya¹ and Yuesheng Zhang¹. ¹Roswell Park Cancer Institute, Buffalo, NY.

Body: ErbB2, also known as Her2 or Neu, belongs to the ErbB family of plasma membrane-bound receptor tyrosine kinases, which also include ErbB1, ErbB3 and ErbB4. ErbB2 is best known for its involvement in human breast cancer. ErbB2 gene amplification occurs in ~20% of breast cancer, and ErbB2 amplification or overexpression is a strong predictor of poor disease prognosis. ErbB2-targeted therapies, particularly humanized monoclonal antibody trastuzumab (Ttzm) in combination with chemotherapy, have shown considerable clinical efficacy. However, primary and secondary resistance remains a clinical challenge, and Ttzm, produced in mammalian cells, is very expensive.

We have found that human prolidase, also known as peptidase D (PEPD) among several other names, binds to ErbB2 with high affinity (Kd = ~7 nM) and binds as a homodimer (493 amino acids per subunit) to subdomain 3 in the extracellular domain of ErbB2. Each monomer of PEPD binds to one copy of ErbB2. However, PEPD is a weak ErbB1 binder (Kd = ~5 µM) and does not bind to ErbB3 or ErbB4. PEPD is the first-ever natural ligand of ErbB2, and unlike the other ligands of ErbB receptors, it is devoid of an EGF motif. PEPD has been long known to hydrolyze dipeptides with proline or hydroxylproline at the carboxy terminus, but the dipeptidase activity of PEPD is not involved in ErbB2/ErbB1 modulation. In cells overexpressing ErbB2, where both activated dimers and inactive monomers of ErbB2 exist, as ErbB2 overexpression causes spontaneous dimerization, auto-tyrosine phosphorylation and recruitment and activation of downstream signals, PEPD rapidly binds to ErbB2 homodimers (<~10 min) and silences the existing ErbB2-SRC signaling, a key oncogenic pathway of ErbB2, by disrupting SRC association with ErbB2. In contrast, PEPD binds to ErbB2 monomers relatively slowly (>~30 min), but this binding causes ErbB2 dimerization, ErbB2 phosphorylation and downstream signaling. PEPD binding to ErbB2 subsequently causes pronounced ErbB2 depletion, resulting from its internalization and degradation. PEPD also strongly inhibits the DNA synthesis, anchorage-independent growth and invasion of cells that overexpress ErbB2, but has no effect on cells without overexpression of ErbB2. In fact, cells become sensitized to inhibition by PEPD upon achieving stable ErbB2 overexpression. Thus, the overall impact of PEPD on ErbB2 is inhibitory, and PEPD targets cells addicted to ErbB2. In ErbB2-overexpressing cells, at equimolar concentrations, PEPD was more effective than Ttzm in driving ErbB2 depletion, but is weaker than Ttzm in stimulating ErbB2 phosphorylation. In mouse tumor models, PEPD administered by intraperitoneal injection (Monday, Wednesday, Friday) at 0.2-2 mg/kg body weight strongly inhibited the growth of ErbB2-overexpressing tumors, but had no impact on tumors without ErbB2 overexpression, and the PEPD-treated mice showed no adverse effects.

Given that the findings described above were made using human PEPD generated in bacteria, there is a distinct possibility that recombinant human PEPD may be a low cost alternative to Ttzm. Further investigation of the antitumor activity of PEPD and its modulation of ErbB2 signaling is warranted.
Title: Single-cell TOF-SIMS reveals that human breast cancer stem cells have significantly lower content of palmitoleic acid compared to their counterpart non-stem cancer cells

Yoshimi Ide\textsuperscript{1,2}, Michihiko Waki\textsuperscript{2}, Itsuko Ishizaki\textsuperscript{3}, Yasuyuki Nagata\textsuperscript{2,4}, Yumiko Taki\textsuperscript{1}, Yuko Hosokawa\textsuperscript{1}, Ryoichi Matsunuma\textsuperscript{1}, Hiroyuki Ogura\textsuperscript{1}, Norihiko Shiiya\textsuperscript{1}, Noriaki Sanada\textsuperscript{3} and Mitsutoshi Setou\textsuperscript{2}. \textsuperscript{1}Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan; \textsuperscript{2}Hamamatsu University School of Medicine, Hamamatsu, Shizuoka, Japan and \textsuperscript{3}ULVAC-PHI, Chigasaki, Kanagawa, Japan.

Body:

Background

Distinguishing individual cancers according to their biochemical heterogeneity have provided much useful information to clinical site. Recently, the cancer stem cell (CSC) theory has been accepted as a concept that explains the mechanism of cancer recurrence and resistance to treatment. To characterize such particular cell populations in heterogeneous tissues, we conducted combination of fluorescence activated cell sorting (FACS) and time-of-flight secondary-ion mass spectrometry (TOF-SIMS) and applied the method to analyses of breast CSCs. TOF-SIMS, which enables to visualize the composition of molecules with mass over 100 Da that were obtained in specimen, has been employed to analyze surface of industrial materials and biomaterials. This method is thus suitable for performing single cell analysis of membranous lipids.

Methods

Breast cancer specimens surgically resected from two patients were enzymatically dispersed into cells. They were labeled with fluorescence-conjugated antibodies of CD45, CD44, and CD24. The cells of CD45-CD44+ CD24- were sorted as CSCs with FACS as well as CD45-CD44- CD24+ cells as non-stem cancer cells (NSCCs). TOF-SIMS analysis and fatty acid analysis was performed according to our previous study published in Surface and Interface Analysis (1). The surface of the sorted cells was analyzed by a PHI TRIFT V (ULVAC-PHI Inc., Kanagawa, Japan) TOF-SIMS instrument. Primary ion beam is irradiated to the surface of the samples and, secondary ions derived from samples are calculated by time-of-flight with the information of the place where the molecular ions were ejected. Negative secondary ions were obtained with a mass range of m/z 0–1850. Mass spectra were analyzed by WinCadenceN software (ULVAC-PHI Inc.) to obtain ion counts and ion images. Integrated ion intensities of FA were normalized using phosphoric acid intensity. The Welch’s t-test was used to compare the normalized ion counts with P-value < 0.05 taken as statistically significant.

Results

FACS analyses successfully collected CD45-/CD44+/CD24- CSCs and CD45-/CD44-/CD24+ non-stem cancer cells (NSCCs) in both two cases, which were corresponding to 0.33% and 0.74% of all cells in case 1, and 0.14% and 1.14% in case 2. TOF-SIMS analyses visualized phosphoric acids and four fatty acid (FA) species in the sorted CSCs and NSCCs. These ions probably came from membranous phosphopolipids and they were uniformly detected from the locus where the cell attached. Integrated ion intensity of palmitoleic acids [FA(16:1)] of CSCs normalized by phosphoric acids signals were significantly decreased than that of CD45-/CD44-/CD24+ NSCCs as a counterpart. Therefore, our novel method successfully provided lipid composition analysis of individual cells classified with complicated combination of marker expressions in clinical specimens composed of heterogeneous cellular populations, and characterized lipid composition of CSCs.

Reference

Title: Post-transcriptional coordination of gene expression during breast cancer tumorigenesis

Laura Simone Bisogno¹ and Jack D Keene¹. ¹Duke University, Durham, NC.

Body: We investigate mechanisms of RNA regulation central to human breast cancer progression. Aberrant gene expression is known to be an important factor in cancer, yet little is known about the role RNA-binding proteins (RBPs) play in disease onset and progression. Sequence specific RBPs coordinately regulate subsets of functionally related mRNAs within ribonucleoprotein complexes (RNPs), which are remodeled in response to cellular perturbations, allowing for the rapid and coordinated translation of proteins that have common functions. These post-transcriptional events robustly influence expression patterns of proto-oncogenes, growth factors, cytokines, and cell cycle regulators by influencing both mRNA stability and translation. Therefore, understanding the post-transcriptional layer of gene regulation is critical to understanding cancer development and progression. We have identified significant transcriptomic changes during the stepwise transition from primary mammary epithelial cells to a fully malignant state, as well as coordinated RNA dynamics of transient cellular RNP complexes. For this analysis, we generated a model of human breast cancer formation in which normal mammary epithelial cells were first immortalized through the expression of hTERT, p53DD, Cyclin D1, CDK4R24C and C-MYC{T58A}, and then subsequently transformed by the addition of H-RAS{G12V}. RNA-sequencing analysis demonstrated that the genes most significantly changed in this model are those involved in cell adhesion. In fact, while primary cells have a gene expression pattern typical of normal epithelial cells, both immortalized and transformed cells exhibit an mRNA expression pattern typical of mesenchymal cells. Interestingly, we found that N-cadherin protein, as well as other prototypical mesenchymal proteins, are only robustly expressed in the fully malignant cells, but not in immortalized cells. This suggests coordinated translational regulation of mRNAs that are essential for activating the epithelial-to-mesenchymal transition (EMT). Many of these EMT-related mRNAs are targets of both the HuR protein and microRNAs. HuR is a translational activator that competes with microRNAs and is mislocalized in many cancers. To integrate the quantitative RNA dynamics of HuR and microRNAs on a global scale, we used RNP-Immunoprecipitation followed by high-throughput sequencing to identify and quantify the remodeling of mRNA subsets associated with HuR and the Ago2/RISC complex in our breast cancer progression model. This integrative work provides global information about the underlying biology of carcinogenesis at the level of post-transcriptional coordination of gene expression, resulting in a more comprehensive understanding of the many layers of complex gene regulation. Therefore, the results from this study may ultimately direct our ability to counter the RNA regulatory changes that underlie malignancy.
Title: Low dose of CDB-2914 and CDB-4124 efficiently inhibit the growth of T47D spheroid induced by pre-menopausal and post-menopausal concentrations of estrogen and progesterone

Oukseub Lee¹, Mi Ran Choi¹, Susan E Clare¹ and Seema A Khan¹. ¹Northwestern University Feinberg School of Medicine, Chicago, IL.

Body: Background: The anti-progestins (RU-486, CDB-2914 and CDB-4124) may have potential to prevent estrogen receptor (ER) and progesterone receptor (PR) positive breast cancer. Physiological estradiol (E2), and progesterone (P4) levels are different in pre- and post-menopausal women. The purpose of this study was to determine: 1) Whether the physiological female hormones at pre- and postmenopausal concentrations affect the growth of an in vitro model of breast cancer, i.e., T47D spheroids, and 2) Whether anti-progestins at pharmacological concentrations work as growth inhibitors in high and low hormonal environments.

Methods: T47D cells were grown as spheroids in the presence of serum concentrations of E2 and P4 consistent with pre- and postmenopause. Premenopausal luteal phase concentrations were 262 pM (71.3 pg/mL) of E2 + 18 nM (5.66 ng/mL) of P4; postmenopausal concentrations were 122 pM (33.2 pg/mL) of E2 + 3 nM (1.08 ng/mL) of P4. T47D cells (5000 per well) were seeded in 1.5% agarose coated 96 well plates, and grown in phenol red-free mammary epithelial cell growth basal medium (Lonza) supplemented with 10% double charcoal-stripped FBS. Hormones and anti-progestin treatments started 24 hrs after cell seeding. Three concentrations (50, 250, 1000 nM) of the anti-progestins were tested. Images of each spheroid were taken daily for 14 days, and the sizes of spheroids were analyzed by area (Pixel) using ImageJ software.

Results: Results are presented in Table 1.

Table 1. Relative spheroid growth compared to the hormone treatment alone

<table>
<thead>
<tr>
<th>Hormone treatments Drug treatments (nM)</th>
<th>RU-486</th>
<th>CDB-2914</th>
<th>CDB-4124</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premenopausal condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>+</td>
<td>50</td>
<td>0.8</td>
<td>0.56</td>
</tr>
<tr>
<td>+</td>
<td>250</td>
<td>0.72</td>
<td>0.63</td>
</tr>
<tr>
<td>+</td>
<td>1000</td>
<td>0.84</td>
<td>0.64</td>
</tr>
<tr>
<td>Postmenopausal condition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>+</td>
<td>50</td>
<td>0.95</td>
<td>0.76</td>
</tr>
<tr>
<td>+</td>
<td>250</td>
<td>0.9</td>
<td>0.83</td>
</tr>
<tr>
<td>+</td>
<td>1000</td>
<td>0.96</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Premenopausal and postmenopausal hormone concentrations stimulated spheroid growth by two- fold and 1.7 fold higher, respectively, when compared to the vehicle control (0.1% DMSO) at14 days.

In the premenopausal condition, RU-486 showed moderate inhibition of spheroid growth at all three concentrations; CDB-2914 was more efficient at inhibition than CDB-4124 at 50 nM. At 250 nM and 1000 nM, CDB-2914 and CDB-4124 showed similar efficacy.

In the postmenopausal condition, RU-486 showed very minor inhibition on spheroid growth at all three concentrations; CDB-2914 showed significantly higher inhibition than CDB-4124 at 50 nM. At 250 nM and 1000 nM, CDB-4124 was more efficient than CDB-2914.
Conclusions: Our results indicate that T47D spheroids will grow in postmenopausal hormone concentrations, but growth is enhanced in premenopausal hormone conditions. RU-486 did not produce effective inhibition at postmenopausal hormone levels; however pharmacological concentrations (50, 250 and 1000 nM) of both CDB-2914 and CDB-4124 efficiently decrease the spheroid growth induced by both premenopausal and postmenopausal hormone levels. These data suggest that low doses of CDB-2914 and CDB-4124 should be further investigated for ER/PR positive breast cancer prevention and therapy.
Title: Contemporary risk of local, regional and contralateral breast cancer recurrence

Kim C Aalders¹, Annelotte CM van Bommel², Thijs van Dalen¹, Gabe S Sonke³, Paul J van Diest⁴, Liesbeth J Boersma⁵ and Margriet van der Heiden-van der Loo⁶. ¹Diakonessenhuis, Utrecht, Netherlands; ²Leiden University Medical Center, Leiden, Zuid-Holland, Netherlands; ³Netherlands Cancer Institute, Amsterdam, Noord-Holland, Netherlands; ⁴University Medical Center, Utrecht, Netherlands; ⁵University Hospital Maastricht, (GROW Maaastro Clinic), Maastricht, Limburg, Netherlands and ⁶Comprehensive Cancer Centre the Netherlands (IKNL), Utrecht, Netherlands.

Body: Background
Long-term follow-up of breast cancer patients aims to detect curable recurrence, and focuses on ipsilateral in-breast recurrence (LR), regional lymph node recurrence (RR) and contralateral breast cancer (CBC). In recent years there is mounting evidence of a decrease in locoregional recurrence rates. Non-surgical-treatment modalities have evolved extensively, while surgery has become less invasive over the last fifteen years. The present study aimed to address contemporary loco-regional recurrence rates evaluating time trends and the role of contributing factors.

Material and methods
The Netherlands Cancer Registry was searched for all female patients diagnosed and operated for a unilateral primary breast cancer (pT1-2, anyN, M0) between 1-1-2003 and 31-12-2006. Exclusion criteria were previous cancer, neo-adjuvant chemotherapy or incurable disease. Data on 5-year follow-up were available from hospital records and included the first site of recurrence and contralateral breast cancer (CBC). The 5-year risk of developing LR, RR and CBC were estimated using Kaplan Meier curves. Patients were censored at time of death, lost to follow-up or the development of distant metastases. Prognostic influence of various patient- and disease characteristics was assessed.

Results
A total of 35,006 eligible patients were identified. The 5-year rates of LR, RR, and CBC are presented in Table 1. The risk of CBC was higher than LR and RR. Over time, the rates decreased significantly for all three endpoints.

Table 1. Overall 5-year risk of local, regional and contralateral recurrence and distant metastases over time (period 2003-2006)

<table>
<thead>
<tr>
<th></th>
<th>Local recurrence(a)</th>
<th>Regional recurrence</th>
<th>Contralateral breast cancer</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>no. of events</td>
<td>rate (%)</td>
<td>no. of events</td>
</tr>
<tr>
<td>2003 (n=8933)</td>
<td>185</td>
<td>2.40%</td>
<td>86</td>
</tr>
<tr>
<td>2004 (n=9048)</td>
<td>181</td>
<td>2.35%</td>
<td>83</td>
</tr>
<tr>
<td>2005 (n=9055)</td>
<td>144</td>
<td>1.84%</td>
<td>75</td>
</tr>
<tr>
<td>2006 (n=7970)</td>
<td>131</td>
<td>1.87%</td>
<td>50</td>
</tr>
<tr>
<td>Overall (n=35,006)</td>
<td>641</td>
<td>2.12%</td>
<td>294</td>
</tr>
</tbody>
</table>

(a) Local recurrence (ipsilateral in-breast recurrence + new primary). Rates represent Kaplan Meier estimates

The LR-rate was lower with breast conserving surgery (BCS) vs. amputation (1.8% vs. 2.5%), T1a-b vs. T1c-T2 tumors (2.0% vs. 2.5%), ER+ vs. ER- tumors (1.8% vs. 3.5%) and inversely related with age (highest in pts. <35 yrs: 2.9%). LR rate seemed independent of HER2 status.

The 5-year RR-rate was 0.9% for N0 patients, and decreased from 1.0% to 0.7% over time. The risk of RR after amputation decreased from 1.8% to 0.9% over time, but was higher than after BCS (1.6% vs. 0.6%). Overall, the RR-rate was highest in the N>1 group (1.4%) and the triple negative group (2.0%).

The CBC-rate was lower for patients who received chemotherapy (CT) than for patients who did not (1.6% vs. 3.1%). The CBC-rate only decreased over the years in the CT-group (3.7% to 2.5%).

Conclusions
Loco-regional recurrence rates have decreased substantially in recent years and have become very low. For the vast majority of patients the risk of LR is substantially lower than the risk of CBC and the risk of RR is rarely larger than 1.0%. These low rates might reflect improvements in systemic treatment.
Body: Background
Obesity (body mass index (BMI) >30) increases breast cancer (BC) risk and promotes BC metastases probably through high estrogen levels. A high BMI as a risk and prognostic factor is consistently reported in postmenopausal women. Obese women develop particularly hormone receptor positive, HER2 negative BC. In this study, we investigate the influence of BMI on the prognostic effect of the progesterone receptor (PR) in postmenopausal patients with estrogen receptor (ER) positive, HER2 negative BC.

Patients and methods
Women over 50 years of age diagnosed with primary operable BC between 2000 and 2012 at University Hospitals Leuven were retrieved from the database. Patients were subdivided into normal weight (<25 kg/m²), overweight (>25 and ≤30) and obese (>30). We investigated for each BMI category the distant metastasis free interval (DMFI) and BC specific survival (BCSS) by PR status. Apart from the total cohort, subgroup analysis was performed for luminal A (grade 1-2) and luminal B HER2 negative BC (grade 3). We used Fine and Gray’s competing risk regression for the analyses. Covariates were age at diagnosis, tumor size, lymph node status, and therapy.

Results
In total, 3227 patients were analyzed for DMFI. For BCSS, 3192 patients were analyzed as patients with unknown cause of death were excluded from the analysis. 2395 of all patients had luminal A and 832 had luminal B HER2 negative BC (table 1). Median time of follow-up was 6.5 years.

Table 1: Percentage of luminal A and luminal B patients per BMI category

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>Luminal A</th>
<th>Luminal B</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &lt;25</td>
<td>1105 (76%)</td>
<td>355 (24%)</td>
</tr>
<tr>
<td>BMI &gt;25 and ≤30</td>
<td>820 (75%)</td>
<td>278 (25%)</td>
</tr>
<tr>
<td>BMI &gt;30</td>
<td>470 (70%)</td>
<td>199 (30%)</td>
</tr>
</tbody>
</table>

BMI: body mass index

BMI interacts with the prognostic effect of PR for DMFI and BCSS in luminal HER2 negative BCs. Only patients with BMI <25 had a reduction in the risk of distant metastasis and BC-related death if the tumor was PR positive as compared to patients with PR negative BC. This was observed in the total cohort and only seen in the luminal B subgroup (table 2). A similar effect was observed in obese patients but this did not reach statistical significance, and was mainly present during the first 5 years following diagnosis. For the overweight patients, no difference in DMFI and BCSS was observed by PR status.

Table 2: Multivariate analysis for DMFI and BCSS for PR positive versus PR negative in the 3 BMI categories in the total and the luminal B cohort.

<table>
<thead>
<tr>
<th>BMI Category</th>
<th>DMFI (HR, 95% CI)</th>
<th>BCSS (HR, 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cohort</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI &lt;25</td>
<td>0.25, 0.16 to 0.40</td>
<td>0.22, 0.12 to 0.40</td>
</tr>
</tbody>
</table>
Conclusion
Normal weight patients have a reduced risk of developing distant metastases and of BC-related death if the tumor is PR positive compared to PR negative BC. No difference between PR positive and PR negative cases was observed in overweight BC patients. This BMI-dependent prognostic effect of PR was limited to luminal B BC patients.

<table>
<thead>
<tr>
<th>BMI &gt;25 and ≤30</th>
<th>HR: 1.24, 0.59 to 2.60</th>
<th>CI: 0.95, 0.40 to 2.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &gt;30</td>
<td>0.70, 0.33 to 1.49</td>
<td>0.53, 0.21 to 1.33</td>
</tr>
<tr>
<td>luminal B</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI &lt;25</td>
<td>0.19, 0.11 to 0.33</td>
<td>0.14, 0.06 to 0.31</td>
</tr>
<tr>
<td>BMI &gt;25 and ≤30</td>
<td>1.23, 0.44 to 3.48</td>
<td>1.02, 0.36 to 2.85</td>
</tr>
<tr>
<td>BMI &gt;30</td>
<td>0.82, 0.31 to 2.20</td>
<td>0.61, 0.18 to 2.00</td>
</tr>
</tbody>
</table>

BCSS: breast cancer specific survival, BMI: body mass index, CI: confidence interval, DMFI: distant metastasis free interval, HR: hazard ratio
Title: Transcriptional profiling of breast cancer metastases identifies liver metastasis-selective genes associated with adverse outcome in luminal A primary breast cancer

Siker Kimbung¹, Ida Johansson¹, Anna Danielsson³, Srinivas Veerla¹, Suzanne Egyhazi⁴, Jonas Bergh⁴, Zakaria Einbeigi³, Barbro Linderholm³⁴, Elisabet Lidbrink⁴, Niklas Loman¹², Per Malmström¹², Martin Söderberg², Thomas M Walz², Mårten Fernö¹, Thomas Hatschek¹, Ingrid Hedenfalk¹ and the TEX Study Group⁴. ¹Lund University, Lund, Sweden; ²Skåne University Hospital, Lund, Sweden; ³Sahlgrenska University Hospital, Gothenburg, Sweden; ⁴Karolinska Institutet and Karolinska University Hospital, Solna, Sweden and ⁵Linköping University Hospital, Linköping, Sweden.

Body: Background: The site of relapse is associated with the prognosis of metastatic breast cancer, but our understanding of the molecular determinants of organ-specificity of metastasis is incomplete. This study aimed to provide additional insight into the biology of breast cancer liver metastases and to identify liver metastasis-selective genes associated with outcome in primary breast cancer.

Methods: A cohort of 304 women with locally advanced and metastatic breast cancer was studied. Estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and Ki67 expression were quantified in primary tumors (N=217) by immunohistochemistry and in situ hybridization on tissue microarrays, and molecular subtypes were assigned according to the 2013 St Gallen guidelines. In addition, fine-needle aspirates of metastases (N=91) were subjected to whole genome transcriptional profiling.

Results: Liver relapse was significantly associated with ER positivity (P<0.002) and the luminal B-like subtype (P<0.01) and was prognostic of an inferior post-relapse survival (P<0.001). Transcriptional profiling revealed that the major variation in the transcriptional landscape of breast cancer metastases was associated with the expression of hormone receptors and the tumor molecular subtype. However, liver metastases displayed unique transcriptional fingerprints, characterized by down-regulation of extracellular matrix (i.e. stromal) genes involved in adhesion and skeletal development. Importantly, we identified a subset of 17 liver metastasis-selective genes that displayed significantly decreased expression in primary tumors of high histological grade (grade 3) and of the luminal B and basal subtypes (P<0.001). This 17-gene signature was significantly and independently prognostic of shorter relapse-free (P =0.001) and overall (P=0.03) survival among patients with ER positive primary tumors. Remarkably, the 17-gene signature remained an independent predictor of shorter relapse-free survival (P=0.004) among patients with luminal A primary tumors.

Conclusion: These results highlight a possible role of stroma-related genes in breast cancer liver metastasis biology and validate the prognostic relevance of extracellular matrix/stromal genes in hormone receptor positive primary breast cancer, specifically of the luminal A subtype.
Title: Creation of a robust algorithm utilizing minimal gene sets normalized against a reference gene set to identify triple-negative breast cancer (TNBC) subtypes

Rob S Seitz¹, David R Hout¹, Stephan W Morris¹, Rebecca B Smith¹, Brian D Lehmann², Xi Chen², Jennifer A Pietenpol² and Brian Z Ring¹. ¹Insight Genetics, Inc, Nashville, TN and ²Vanderbilt-Ingram Cancer Center, Vanderbilt University School of Medicine, Nashville, TN.

Body: Introduction: Treatment of TNBC has been challenging due to the absence of well-characterized molecular targets and the heterogeneity of the disease. Using TNBC gene expression (GE) microarray profiles, the Pietenpol group molecularly binned the malignancy into six distinct subtypes: two basal-like [BL1, BL2], two mesenchymal-like [M, MSL], an immunomodulatory [IM], and a luminal subtype expressing androgen receptor [LAR]). Importantly, initial evidence suggests specific TNBC subtypes exhibit different sensitivities to various targeted and conventional chemotherapies (1). For example, BL1 and BL2 showed sensitivity and resistance, respectively, to taxanes (2).

Background: The original TNBCtype algorithm was generated from a meta-analysis of existing GE data from tumors and clustering the data into the six subtypes listed above and a seventh "unclassified" subtype (1). To transition the test into the clinic, we have modified the method of classification by reducing the number of signature genes. We then downloaded a publicly available GE dataset to validate the optimized assay on an independent cohort.

Methods: Gene set enrichment followed by shrunken centroid analysis were used for feature reduction, resulting in 258 genes used for model building. Linear regression, targeted minimum loss based estimation, random forest, and elastic-net regularized linear models were employed, with the latter giving the best fit with the least number of required genes. Models were created to identify each class individually or together using a centroid model. Coefficient and cutoffs were established on a normalized TNBC training data set consisting of 14 cohorts (N=386) and then applied to a seven cohort validation data set (N=201). Finally, both the original 2188 gene and the optimized 101 gene classifier models were applied to an independent cohort (278 TNBC patients treated neoadjuvantly with mitotic inhibitors: GSE41998) to identify the TNBC subtypes.

Results: In the validation cohorts used by Lehmann et al. (1), there was strong agreement between the 2188 and 101 gene models (Fisher exact test, P<0.0001). Specificity for the individual class models ranged from 90% (M) to 100% (LAR), while the 101 gene centroid resulted in a 4% rate of cases discordantly classified compared to the original 2188 gene classifier. As was seen in the initial clustering in the training data set, there was notable overlap between the subtyping of BL1 and M. On the discovery cohorts normalized with the reference gene set, the specificity for the individual class models ranged from 79% (M) to 97% (BL1). For the 101 gene centroid model the misclassification error was 5%. When the additional independent TNBC cohort was examined, the agreement between the 2188 and 101 gene models was 89%. Furthermore, patients defined as BL1 or BL2 by the minimal gene set matched previous observations of sensitivity and resistance, respectively, to mitotic inhibitors.

Conclusions: These initial results show that the minimal gene set shows agreement with the larger original TNBC algorithm and recapitulates the initial clinical observations. Future work will determine the clinical utility of this assay for patient management.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P6-08-05  
**Average Grade:** 5.50

**Title:** Prognostic value of the progesterone receptor by proliferation rate in patients with luminal HER2 negative breast cancer

Kathleen Van Asten¹, Ben Van Calster², Anneleen Lintermans¹, Olivier Brouckaert³, Giuseppe Floris⁴, Hans Wildiers⁵ and Patrick Neven³. ¹KU Leuven, Oncology, Leuven, Belgium; ²KU Leuven, Development and Regeneration, Leuven, Belgium; ³University Hospitals Leuven, Leuven, Belgium; ⁴University Hospitals Leuven, Leuven, Belgium and ⁵University Hospitals Leuven, Leuven, Belgium.

**Body:**

**Background**

Estrogen receptor (ER) positive, HER2 negative breast cancer (BC) can be classified into luminal A and luminal B-like tumors according to tumor grade. Several evidences point to the fact that IHC expression of the progesterone receptor (PR) has prognostic value. In this study, we assess to what extent a negative PR in luminal A BCs increases the risk of distant metastasis and whether or not luminal B BC might have a good prognosis if PR is positive.

**Patients and methods**

Women with primary operable ER positive, HER2 negative BC treated at University Hospitals Leuven between 2000 and 2009 were retrieved from our database. So called luminal A tumors were defined as grade 1-2 BC, whereas so called luminal B BC were defined as grade 3 BC. Distant metastasis free interval (DMFI) and breast cancer specific survival (BCSS) were investigated by their PR status. PR was considered negative if the semi-quantitative Allred score was 0-2. Before 2003 the semi-quantitative H-score was used and a score <50 was considered negative. Statistical analysis for DMFI was performed using the cox proportional hazards regression. For BCSS we used Fine & Gray’s competing risk regression. Covariates were age at diagnosis, tumor size, lymph node status, and therapy.

**Results**

In total, 3294 patients from Leuven were analyzed. From this cohort, 285 patients experienced metastases (8.7%) and 172 patients died of BC (5.2%). Details are shown in table 1. The median age at diagnosis was 58 years with ages ranging from 22 to 95 years, 2358 patients (71.6%) were aged above 50 at diagnosis. The median follow-up period was 8.1 years.

<table>
<thead>
<tr>
<th>Metastases</th>
<th>BC-related death</th>
</tr>
</thead>
</table>
| luminal A  | PR positive 110/2103 (5%) 61/2103 (3%)  
PR negative 16/267 (6%) 9/267 (3%) |
| luminal B  | PR positive 120/786 (15%) 75/786 (10%)  
PR negative 39/138 (28%) 27/138 (20%) |

**Table 1:** Number of patients that metastasized and died of BC by luminal subgroup and PR status.

In Leuven, the reduction in risk of metastasis in patients with PR positive luminal A and luminal B BC was respectively 14% (Hazard ratio (HR): 0.86, 95% confidence interval (CI) 0.52-1.51) and 47% (HR: 0.53, 95% CI 0.37-0.78) compared with PR negative tumors. PR positive luminal A and luminal B BC patients had a 16% (HR: 0.84, 95 % CI 0.42-1.69) and 53% (HR: 0.47, 95% CI 0.30-0.75) reduction in the risk of BC-related death compared with PR negative tumors respectively. The same analysis was also carried out for postmenopausal patients (older than 50 years) only. In this subcohort, PR positivity was associated with a 9% (HR 0.91, 95% CI 0.50 to 1.83) and 59% (HR 0.41, 95% CI 0.28 to 0.63) reduction in the risk of metastatic events in luminal A and luminal B lesions, respectively. For BCSS, a 31% (HR 0.69, 95% CI 0.30 to 1.57) and 66% (HR 0.34, 95% CI 0.20 to 0.57) reduction in the risk of BC-related death for respectively PR positive luminal A and luminal B BC patients was found.

**Conclusion**

These results suggest that the prognostic effect of PR in primary operable BC depends on the tumor grade. Compared with luminal PR negative BC, PR positivity improves outcome more in luminal B than in luminal A lesions.
Title: The multidisciplinary application of genomics in clinical practice (MAGIC) survey: Identification of early stage hormone receptor-positive (HR+), HER2– breast cancer (BC) patients in whom multigene assays may have their highest utility


Multidisciplinary Oncology Institute, Clinic of Genolier, Genolier, Switzerland; †Instituto Nacional de Cancerologia, Mexico City, Mexico; ‡National Cancer Institute G. Pascale Foundation, Naples, Italy; §Hospital Dr. I. Pirovano, Buenos Aires, Argentina; ¶Uzsoki Teaching Hospital, Budapest, Hungary; ¶Sahlgrenska Academy and University Hospital, Gothenburg, Sweden; ¶University of Florida Health Cancer Center at Orlando Health, Orlando, FL; ¶Athens University Medical School, Athens, Greece; ¶Medical Oncology Service, Hospital General Universitario Gregorio Marañón, Madrid, Spain; ¶Multidisciplinary Breast Centre and Gynaecological Oncology, UZ Leuven, Leuven, Belgium; ¶Russian Cancer Research Center, Moscow, Russian Federation; ¶School of Cancer Sciences, University of Birmingham, Birmingham, United Kingdom; ¶¶Institut Curie-Université Versailles-Saint-Quentin, Paris-Saint-Cloud, France; ¶¶Leiden University Medical Center, Leiden, Netherlands; ¶¶Medical Affairs, Genomic Health, Stockholm, Sweden and ¶¶¶Martin Luther University of Halle-Wittenberg, Halle (Saale), Germany.

Body: Background

Treatment recommendations for early stage HR+, HER2– BC patients depend on many factors. The MAGIC survey evaluated which criteria clinicians use regarding the need for adjuvant chemotherapy (AdjCT) and showed that there was substantial heterogeneity across clinicians and countries in treatment decisions (Aapro et al, EBCC 2014, abstract 24). Multigene assays (MGA) help to make more-informed decisions by providing prognostic and predictive information beyond traditional parameters, but are not always needed. The data presented here show for which BC patient profiles there is a high heterogeneity in treatment recommendations. We suggest that MGAs may be useful to guide treatment recommendations in these cases.

Methods

From August 2013 until January 2014, physicians with ≥5 years’ experience in BC treatment and participating in multidisciplinary teams were invited for the online MAGIC survey. The survey evaluated respondent characteristics and registered treatment recommendations for randomly generated early BC patient profiles (n=672). A conjoint analysis was used to assess which patient attributes were considered for treatment decisions.

Results

The survey was completed by 911 physicians from 52 countries, of whom 72% had >10 years’ experience. Their treatment recommendations showed that for BC patient profiles with only high-risk or only low-risk characteristics, there was a high consensus to recommend AdjCT or no AdjCT (endocrine treatment alone); 42% of the profiles had >75% probability of being recommended AdjCT and 6% had >75% chance of being recommended no AdjCT.

If interactions between patient characteristics were not considered, age was ranked as the most important patient characteristic for AdjCT decisions, followed by tumor grade, tumor size, nodal status, and expression of Ki67, estrogen receptor (ER), and progesterone receptor. The combination of patient attributes and their interactions were, as expected, of importance; some node-positive patients or patients with a Grade 3 tumor had >75% probability to be recommended no AdjCT (eg, older patients or patients with a small [<2 cm] tumor). Conversely, some patients with small, Grade 1 tumors had >75% probability to be recommended AdjCT (eg, young or node-positive patients).

In total, 104 patient profiles (15%) were identified for which treatment recommendations were highly heterogeneous, with a probability of <50% for both an AdjCT treatment recommendation and no AdjCT as a treatment recommendation. These patient profiles tended to have the following characteristics: >50 years old, tumor size <3 cm, Grade 1 or 2 tumor, high ER expression, and Ki67 expression <20%.

Conclusions

There was substantial heterogeneity in treatment recommendations and an overall tendency to give chemo-endocrine rather than endocrine treatment alone. The highest uncertainty in treatment decisions was seen in patients with intermediate risk by clinical and pathological parameters. This opens questions concerning treatment decisions and in such cases MGAs may be most useful.
Average Grade: 5.67

Title: Optimal method of detection and threshold for early intervention to prevent lymphoedema: A multi-centre prospective study

Nigel J Bundred¹, Charlotte Stockton¹, Katie Riches², Linda Ashcroft³, Abigail Evans⁶, Anthony Skene⁷, Maria Bramley⁴, Tracey Hodgkiss⁴, Arnie Purushotham⁵, Vaughan Keeley² and BEA Investigators¹. ¹University Hospital of South Manchester NHS Foundation Trust, Manchester, United Kingdom; ²Derby Hospitals NHS Foundation Trust, Derby, United Kingdom; ³Christie NHS Foundation Trust, Manchester, United Kingdom; ⁴Pennine Acute Hospitals NHS Trust, Manchester, United Kingdom; ⁵Guy's and St Thomas' NHS Foundation Trust, London, United Kingdom; ⁶Poole Hospital NHS Foundation Trust, Poole, United Kingdom and ⁷Royal Bournemouth & Christchurch Hospitals NHS Foundation Trust, Bournemouth, United Kingdom.

Body: Introduction

Women who undergo axillary surgery are at risk of developing lymphoedema. Early detection is recommended by measuring arm volume from a baseline before surgery to enable early intervention. The optimal measurement method to enable early detection and time to intervention are unclear. This prospective multi-centre study compares multi-frequency bioimpedance spectroscopy (BIS, ImpediMed) with the validated perometer method to determine which test is more sensitive for detecting the optimal threshold to prevent lymphoedema.

Methods

Participants (N = 960) undergoing axillary clearance at 9 UK centres have pre-operative and regular arm volume measurements post-surgery (1, 3, 6, 9 & 12 months, then 6 monthly), by the validated arm perometry compared with BIS (L-Dex) measurements as well as self-reported symptoms questionnaire. Change in arm volume was calculated using relative arm volume change (RAVC). The predictors of lymphoedema development and optimal method were assessed.

Results

Currently 612 patients, median age 55 (range 24 to 90) years, have 6 month follow-up data and 327 have 18 month follow-up data. Seventy six percent were ER positive and received endocrine therapy, 84% percent received radiotherapy and 67% received chemotherapy in addition to surgery. Lymphoedema by 18 months was detected in 19% (n=79) of women by perometry (≥10% RAVC) and a change in L-Dex of 10 was observed in 31% of women. A moderate correlation between perometer and BIS at 3 months (r=0.40) and 6 months (r=0.60), with a sensitivity of 73% and specificity of 84% was found.

Univariate analysis revealed a threshold for early intervention to prevent lymphoedema was RAVC ≥5%-<10% (p=0.03). Multivariate analysis indicated that Oestrogen Receptor (ER) negative breast cancer (p=0.01, hazard ratio (HR)=0.43, 95% confidence interval (CI)=0.24 to 0.84), number of positive nodes (p=0.01, HR=1.05, 95% CI=1.01 to 1.09) and a measurement of ≥5%-<10% (p=0.04, HR=1.67, 95% CI=1.03 to 3.54) at 6 months after surgery predicted development of lymphoedema. Further investigation of why ER negative patients are at increased risk of developing lymphoedema is planned.

Conclusions

The optimal threshold for early intervention to prevent progression to lymphoedema is ≥5%-<10% relative arm volume change by perometry. Further data on the sensitivity of BIS will be obtained in this study. Arm volume measurements remain necessary before and after ANC to allow early intervention.

(Funded by NIHR Programme Grant).
Title: Postpartum breast cancer demonstrates increased liver and brain metastasis with a proposed role for postpartum involution

Virginia F Borges¹, Erica Goddard¹, Ann H Partridge² and Pepper Schedin¹. ¹University of Colorado, Denver, CO and ²Dana-Farber Cancer Institute, Boston, MA.

Body: Background: Postpartum breast cancer, which we have defined as a breast cancer diagnosis within 5 years of giving birth (PPBC), has a 3-fold increased risk for metastasis and death when compared to nulliparous women that is independently significant when age at diagnosis, tumor stage, receptor status and year at diagnosis are accounted for. Models of postpartum breast cancer developed by our group have shown that the process of breast involution after lactation ceases or after pregnancy if lactation does not occurs is a key event that drives tumors toward increase metastasis. To further investigate the increased metastatic potential of postpartum involution, we performed a nested cohort study of metastatic young women’s breast cancer. We have also developed an immune-competent mouse model of postpartum breast cancer to fully characterize the metastatic capacity of the postpartum involution milieu. Hypothesis: The reduced survival of postpartum breast cancer is due, in part, to an altered pattern of metastatic spread to the liver driven by postpartum involution. Methods: Two independent cohorts from the University of Colorado (UC) and Dana Farber Cancer Institute (DFCI) were utilized to identify a nested cohort of women with first presentation of systemic metastasis. Location of metastasis was verified and frequency of end organ involvement recorded for the pre-determined targets of liver, bone, brain and lungs. The cohort was compared by parity status of nulliparous or PPBC. For determining the contribution of postpartum involution to metastatic spread and investigate mechanism, an intracardiac immune-competent mouse model of postpartum breast cancer metastasis was developed. Results: Cases of young women diagnosed ≤45 that had documented metastatic disease and for which site of metastasis could be identified were included (n=79). Cases with missing parity data or for whom site specific information was not available were excluded. Overall, the dominant organ for metastatic involvement at first presentation of metastatic disease was the bones. In PPBC, liver was the second most common site of metastasis (35%) and higher than in nulliparous women (24%) where lung metastases were the second most frequent. Individual organs were evaluated by parity status and a notable skewing of frequency of liver and brain metastasis to PPBC was seen. 63% versus 37% of the cases with liver mets and 73% versus 27% of cases with brain involvement were PPBC versus nullipara at first metastatic presentation. No differences were seen in lung or bone metastasis between the parity groups. To test whether the postpartum involution supports increased liver metastasis, mice were injected with mammary tumor cells into the left ventricle. Similar to the human data, significantly increased liver metastasis, but not bone or lung was observed in the postpartum group compared to age-matched nulliparous controls. Conclusion: PPBC demonstrates enrichment for liver and brain metastasis that is in part confirmed through an animal model and supports the role of postpartum involution in increased metastasis and death. Further studies into the mechanism by which increased liver metastasis occur in postpartum breast cancer patients are underway.
**Title:** Genetic polymorphisms (SNPs) as predictive markers for paclitaxel-induced peripheral neuropathy (PNP) and capecitabine-induced hand-foot syndrome (HFS) in HER-2 negative metastatic breast cancer patients

Siu W Lam, Charlotte N Frederiks, Tahar van der Straaten, Steffen M de Groot, Agnes Jager, Monique MEM Bos, Sabine C Linn, Joan van den Bosch, Hans J Braun, Ankie MT van der Velden, Maartje Los, Joanneke EA Portielje, Judith R Kroep, Aafke H Honkoop, Carolien H Smorenburg, Bea Tanis, Johanna MGH van Riel, Jetske M Meerum Terwogt, Marien O den Boer, Joep Douma, Frank Jeurissen, Johan Berends, Henk-Jan Guchelaar and Epie Boven. 1 Medical Oncology, VU University Medical Center, Amsterdam, Noord-Holland, Netherlands; 2 Comprehensive Cancer Centre the Netherlands, Amsterdam, Noord-Holland, Netherlands; 3 Erasmus MC Daniel den Hoed Cancer Center, Rotterdam, Netherlands; 4 Reinier de Graaf Hospital, Delft, Netherlands; 5 Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; 6 Albert Schweitzer Hospital, Dordrecht, Netherlands; 7 Vlietland Hospital, Schiedam, Netherlands; 8 Tergooi Hospitals, Hilversum, Netherlands; 10 St Antonius Hospital, Nieuwegein, Netherlands; 11 Haga Hospital, Hague, Netherlands; 12 Isala Clinics, Zwolle, Netherlands; 13 Medical Center Alkmaar, Alkmaar, Netherlands; 14 Groene Hart Hospital, Gouda, Netherlands; 15 St Elisabeth Hospital, Tilburg, Netherlands; 16 Onze Lieve Vrouwe Gasthuis, Amsterdam, Noord-Holland, Netherlands; 17 Laurentius Hospital, Roermond, Netherlands; 18 Rijnstate Hospital, Arnhem, Netherlands; 19 Medical Center Haaglanden, Hague, Netherlands and 20 Gemini Hospital, Den Helder, Netherlands.

**Body: Background**

Newly identified SNPs in genes encoding metabolizing enzymes (CYP2C8, CYP3A4) and drug target (TUBB2A) of paclitaxel have been associated with PNP. A recent GWAS has found novel SNPs in EP40A5 and FGD4 possibly predictive for PNP. Likewise, SNPs in genes (CDA, CES2) involved in capecitabine activation may play a role in HFS. Here, we attempted to confirm these SNPs as predictive markers for PNP and HFS in patients (pts) receiving first-line paclitaxel (T) and bevacizumab (A) without or with capecitabine (X).

**Patients and methods**

In the phase II ATX trial (NTR1348; BOOG2006-06), 312 pts were randomized 1:1 to AT (T 90 mg/m² d1, 8, 15 & A 10 mg/kg d1, 15 q4w x 6 cycles → A 15 mg/kg d1 q3w for next cycles) or ATX (T 90 mg/m² d1, 8, A 15 mg/kg d1 & X 825 mg/m² bid d1–14 q3w x 8 cycles → the same dose of A & X q3w for next cycles). Toxicity was graded by using NCI CTCAE v3 at each cycle. Germline DNA was isolated for genotyping CYP2C8*3 (1196A>G & 416G>A), CYP3A4*22 (522-191C>T), TUBB2A 943insC and CDA 451C>T. Results of CDA and CDA SNPs will be presented at the meeting.

The association between SNPs and toxicity was analyzed for 1) maximum grade of treatment-related toxicity per patient by Pearson’s X² or Fisher’s exact test; 2) cumulative dose level of drugs until first grade ≥1 toxicity or first dose reduction by Kaplan-Meier curve or Gehan-Breslow test.

**Results**

188 pts had SNPs genotyped; characteristics reflected the trial cohort. Table 1 shows the maximum grade of PNP. When grouped into grades 0–1 and 2–3, no differences in rates of PNP were noted among SNPs. Median cumulative dose of T until grade ≥1 PNP was 1,800 mg/m² (95% CI 1,534–2,065). Carriers of the CYP2C8 416 A-allele had a significantly lower cumulative dose of T until grade ≥1 PNP compared to those with a homozygous G/G genotype (Gehan-Breslow p=0.011). Carriers of the FGD4 2044-236 A-allele had a significantly higher cumulative dose until first dose reduction of T compared to those with a homozygous G/G genotype (Gehan-Breslow p=0.037).

<table>
<thead>
<tr>
<th></th>
<th>Grade 0</th>
<th>1</th>
<th>2</th>
<th>3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paclitaxel-treated</td>
<td>PNP, No. (%)</td>
<td>62 (33)</td>
<td>33 (36)</td>
<td>41 (22)</td>
</tr>
<tr>
<td>Capecitabine-treated</td>
<td>HFS, No. (%)</td>
<td>17 (18)</td>
<td>21 (23)</td>
<td>25 (27)</td>
</tr>
</tbody>
</table>

**Table 1.** toxicity by maximum grade per patient
For ATX-treated pts, rates of HFS are shown in Table 1. With regard to maximum grade of HFS, there was a trend of a lower rate of grade 2–3 HFS for CES 823C/G compared to C/C genotype (33% vs 64%, Fisher’s exact p = 0.059). The median cumulative dose of X until grade ≥1 HFS was 98.7 g/m² (95%CI 82.1–115). CES 823C>G was not associated with the cumulative dose of X until grade ≥1 HFS or dose reduction.

**Conclusions**

Our results indicate that CYP2C8 416G>A and FGD4 2044-236G>A might be predictive markers for paclitaxel-induced neurotoxicity, whereas reported associations of other SNPs with toxicity could not be confirmed.


Financial support from Roche Netherlands.
Title: Prospective study of the impact of the Prosigna™ assay on adjuvant clinical decision-making in women with estrogen receptor-positive, HER2-negative, node-negative breast cancer: A GEICAM study

Miguel Martín1,2, Milagros González-Rivera2, Serafín Morales3, Juan de la Haba4, Lucía González-Cortijo5, Luis Manso6, Joan Albanell7, Antonio González-Martín8, Sónia González9, Angels Arcusa10, Luis de la Cruz-Merino11, Federico Rojo12, María Vidal13,14, Uxue Goicoechea15, Patricia Galván14, Rosalía Caballero15, Eva Carrasco15, Steven Michalopoulos16, John Hornberger16,17 and Aleix Prat13,14. 1Hospital Gregorio Marañón, Madrid, Spain; 2Laboratory of Traslational Oncology. Hospital Gregorio Marañón, Madrid, Spain; 3Hospital Arnau de Vilanova, Lleida, Spain; 4Hospital Reina Sofía, Córdoba, Spain; 5Hospital Quirón, Madrid, Spain; 6Hospital Doce Octubre, Madrid, Spain; 7Hospital del Mar, Barcelona, Spain; 8MD Anderson Cancer Center, Madrid, Spain; 9Hospital Mutua de Terrassa, Barcelona, Spain; 10Consorci Sanitari de Terrassa, Barcelona, Spain; 11Hospital Virgen de la Macarena, Sevilla, Spain; 12Fundación Jiménez Díaz, Madrid, Spain; 13Hospital de la Vall d’Hebron, Barcelona, Spain; 14Translational Genomics Group, Vall d’Hebron Institute of Oncology (VHIO), Barcelona, Spain; 15Spanish Breast Cancer Research Group (GEICAM), San Sebastián de los Reyes, Madrid, Spain; 16CEDAR Associates, Menlo Park, CA and 17Stanford University School of Medicine, Palo Alto, CA.

Body: Background: Prosigna™ (PAM50) is a standardized test that measures the expression levels of 50 classifier genes in formalin-fixed paraffin-embedded (FFPE) breast tissue tumor samples. It provides subtype classification based on the fundamental biology of an individual patient’s tumor (referred to as "intrinsic subtyping"), as well as a prognostic score (referred to as "risk of recurrence [ROR] score") that predicts the probability of distant recurrence over 10 years. This decision impact study examines whether the Prosigna™ test influences both physician and patient adjuvant treatment selection, beyond standard immunohistochemistry (IHC) testing.

Methods: The analytic sample was comprised of postmenopausal women with node-negative, estrogen-receptor positive (ER+), HER2 negative (HER2-), early-stage breast cancer with tumors <5 cm (T1-T2). FFPE surgical specimens were analyzed using Prosigna™ in a central laboratory. Patients were classified according to the intrinsic tumor subtype (i.e., Luminal A, Luminal B, HER2-enriched, basal-like) and ROR score (low, intermediate or high risk groups). The primary endpoint was the effect of the Prosigna™ test on oncologists’ treatment recommendations, and the actual treatment received by patients (hormonal therapy [HT], chemotherapy [CT], chemotherapy and hormonal therapy [CHT]). All samples were analyzed in two independent laboratories to measure site-to-site concordance. Prosigna™ subtypes were compared with IHC intrinsic subtypes based on the St. Gallen 2013 criteria (cut-points: PgR≥20% and Ki67≥14%).

Results: A total of 200 patients met eligibility criteria and were enrolled in the study between June 2013 and January 2014. According to Prosigna™ results, intrinsic tumor subtypes of these patients were distributed as follows: 129 Luminal A (64.5%), 66 Luminal B (33.0%), 3 HER2-enriched (1.5%) and 2 basal-like (1.0%). Modifications to the adjuvant treatment recommendations by ROR score can be seen in the following table:

<table>
<thead>
<tr>
<th>Change in physician pre- to post- Prosigna recommendation</th>
<th>Low ROR (N=101), N (%)</th>
<th>Intermediate ROR (N=66), N (%)</th>
<th>High ROR (N=33), N (%)</th>
<th>TOTAL (N=200), N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT to CHT</td>
<td>0 (0.0)</td>
<td>8 (12.1)</td>
<td>10 (30.3)</td>
<td>18 (9.0)</td>
</tr>
<tr>
<td>CHT to HT</td>
<td>11 (10.9)</td>
<td>9 (13.6)</td>
<td>0 (0.0)</td>
<td>20 (10.0)</td>
</tr>
<tr>
<td>TOTAL</td>
<td>11 (10.9)</td>
<td>17 (25.7)</td>
<td>10 (30.3)</td>
<td>38 (19.0)</td>
</tr>
</tbody>
</table>

Treatment decisions changed for 19.0% of all patients: 10.0% and 9.0% of patients went from CHT to HT and HT to CHT, respectively. The percentage of patients who received chemotherapy in the low, intermediate and high risk groups was 5%, 36% and 88%, respectively. Both the central and each local laboratory analyzed the samples using IHC. We found 60% concordance between central IHC and Prosigna™ intrinsic subtypes (Kappa=0.2365). Prosigna™ results were consistent across labs (Kappa =
Conclusions: The Prosigna™ test can be reliably performed in hospital laboratories to provide useful information beyond standard clinical-pathological variables that oncologists can use to optimize adjuvant treatment decisions in clinical practice. Subtype determined using IHC is not an interchangeable proxy for subtype determined by Prosigna™.

*Two last authors have contributed equally to the study.
Title: UK OPTIMA-prelim study demonstrates economic value in more clinical evaluation of multi-parameter prognostic tests in early breast cancer

Peter S Hall¹, Alison F Smith¹, Armando Vargas-Palacios¹, Robert C Stein³, John Bartlett², Jane Bayani², Andrea Marshall¹ś, Janet A Dunn¹, Amy F Campbell⁴, Carrie Cunningham⁵, Leila Rooshenas⁶, Monika Sobol⁸, Adrienne Morgan⁷, Christopher Poole⁸, Sarah E Pinder⁶, David A Cameron⁶, Nigel Stallard⁸, Jenny Donovan⁶, Luke Hugh-Davies¹¹, Helena Earl¹², Andreas Makris¹², Claire Hulme¹ and Christopher McCabe¹⁰. ¹Leeds Institute of Health Sciences, University of Leeds, Leeds, United Kingdom; ²Ontario Institute for Cancer Research; ³National Institute for Health Research University College London Hospitals BioMedical Research Centre, London, United Kingdom; ⁴Warwick Clinical Trials Unit, University of Warwick; ⁵Cancer Research Centre, University of Edinburgh, Edinburgh, United Kingdom; ⁶University of Bristol, Bristol, United Kingdom; ⁷Independent Cancer Patients’ Voice; ⁸University of Warwick, Coventry, United Kingdom; ⁹King’s College London; ¹⁰University of Alberta, Edmonton, AB, Canada; ¹¹Addenbrooke’s Hospital, Cambridge, United Kingdom and ¹²Mount Vernon Cancer Centre, Northwood, Middlesex, United Kingdom.

Body: Background
There is uncertainty about the benefit of chemotherapy for some patients with ER-positive HER2-negative early breast cancer. Multi-parameter assays of gene expression may enhance the value of chemotherapy through personalised treatment decisions. An economic evaluation was undertaken in the context of the feasibility phase of an RCT (OPTIMA prelim) designed to validate prospectively the use of such an assay as a treatment decision tool in the UK National Health Service (NHS). The aim of the economic evaluation was to confirm value in an ongoing RCT and optimise its design for economic endpoints. Comparators included (i) All patients treated with chemotherapy, (ii) Oncotype DX, (iii) MammaPrint/BluePrint and (iv) Prosigna.

Methods
A model-based cost-effectiveness analysis was conducted to the standards of the UK National Institute for Care Excellence (NICE) reference case. A Markov model was constructed to simulate the care pathway of a cohort of patients with characteristics identified in the OPTIMA prelim study or, where unavailable, from the published literature. The costs (GBP) and benefits (QALYs) were estimated over a time horizon of the patient life-time. Alternative scenarios of recurrence rates and chemotherapy effect were explored in patients identified high or low risk by the tests and treated with and without chemotherapy. Scenarios included estimates based on the SWOG-8814 trial, the EBCTCG and outcomes forecasted using Adjuvant! Online. Uncertainty introduced by discrepancy in patient selection between tests was modelled using a Bayesian decision analytic framework. Probabilistic sensitivity analysis and value of information analysis was conducted using Monte Carlo simulation.

Results
There were 285 randomised patients. Multi-parameter analyses were performed on tumour samples and baseline factors were included in the model. The cost-effectiveness of all tests was uncertain. Uncertainty was predominantly driven by assumptions about long term recurrence rates in test-selected groups and the ability of tests to predict benefit from chemotherapy. The relationship between recurrence-free survival and life expectancy in test-selected groups and in patients who did or did not receive adjuvant chemotherapy was also important. The incremental cost-effectiveness ratio (ICER) for Oncotype DX compared with chemotherapy for all was cost-effective in many scenarios, ranging from GBP26,000 per QALY to resulting in increased QALYs with cost savings (dominate), depending on assumptions. The value of information analysis placed high societal value in further research into recurrence-free survival for test-directed chemotherapy, irrespective of the test evaluated.

Conclusion
There is substantial value in prospective comparative research into all tests evaluated, including long term outcomes, to resolve uncertainties in the clinical and economic optimal choice of test.

Acknowledgements
This project was funded by the National Institute for Health Research Health Technology Assessment (NIHR HTA) Programme (project number 10/34/01). The views and opinions expressed therein are those of the authors and do not necessarily reflect those of the HTA programme, NIHR, NHS or the Department of Health.
Title: Integrative proteomic and gene expression analysis identify annexinA1 and caveolin1 as predictive biomarkers for adjuvant trastuzumab resistance

Amir Sonnenblick1, Sylvain Brohéé1, Debora Fumagalli1, Christine Desmedt1, Martine Piccart1, Patrick Neven2, Pirkko-Liisa Kellokumpu-Lehtinen3, Heikki Joensuu4 and Christos Sotiriou1. 1Jules Bordet Institute, Brussels, Belgium; 2KU Leuven, University Hospitals, Leuven, Belgium; 3University Hospital of Tampere, Tampere, Finland and 4Helsinki University Central Hospital and Helsinki University, Helsinki, Finland.

Body: Purpose
Trastuzumab is a remarkably effective therapy for human epidermal growth factor receptor 2 (HER2) positive breast cancer (BC) patients. However, not all women with HER2 amplified tumours benefit from trastuzumab. Here we developed a novel in silico bioinformatics approach integrating proteomic and gene expression data to uncover novel predictive biomarkers associated with trastuzumab response in early BC.

Patients and methods
By integrating RNA and protein expression data from RNA microarray and Reverse-Phase Protein Arrays (RPPA) in HER2+ BC from the TCGA repository, we developed several gene expression metagenes that reflect the level of pathway activation based on the expression of several proteins and/or phosphorylated-proteins relevant in cancer. Next we assessed the ability of these metagenes to predict benefit to trastuzumab using available gene expression data from the randomised phase III FinHer trial. 202 HER2+ BC had sufficient RNA for gene expression analysis. The results were further validated using an independent series of 94 HER2+ BC treated with trastuzumab.

Results
Among the 139 metagenes developed with the RPPA expression levels, 40 metagenes passed our internal validation threshold using the TCGA data set and were further screened for their association with trastuzumab benefit in the FinHer study. A significant association with benefit to trastuzumab was observed with 5 metagenes. Tumors with low Annexin1 (ANXA1-GS) and caveolin1 (Caveolin1-GS) metagene levels showed a significant benefit from trastuzumab (hazard ratio for distant recurrence [HR], 0.32 [95 % CI 0.13-0.83]; P = 0.02, HR 0.38 [95 % CI 0.15-0.94]; P = 0.036 respectively). Similarly, tumour with low expression level of the Aktp473-GS and DJ-1-GS metagenes, capturing Akt/mTOR pathway activation, were also associated with benefit to trastuzumab (HR for distant recurrence, 0.38 [95 % CI 0.1-0.87]; P = 0.02; HR for distant recurrence, 0.45 [95 % CI 0.2-0.99]; P = 0.048 respectively), whereas such observation was not seen for the mTORp2448-GS metagene. Of interest, none of the Aktp473-GS and mTORp2448-GS metagenes were associated with PIK3CA mutation status in the FinHer data set. As expected, high lymphocyte-specific protein tyrosine kinase (Lck-GS) metagene reflecting tumour-infiltrating lymphocytes, correlated with trastuzumab benefit (HR for distant recurrence, 0.4 [95 % CI 0.17-0.97]; P = 0.04). The predictive values of ANXA1-GS and Caveolin1-GS were successfully validated in an independent cohort of 94 subjects who received adjuvant trastuzumab (Log rank: P=0.02, P=0.056 respectively).

Conclusion
Our findings identify novel mechanisms to trastuzumab resistance and suggest that agents targeting ANXA1, caveolin1 or pAkt473 may offer new ways to overcome resistance to trastuzumab.
Title: Is there prognostic significance of tumor cellularity in primary non-treated breast carcinoma?

Emily S Reisenbichler¹, William Dupont¹, Plummer Dale¹ and Omar Hameed¹. ¹Vanderbilt University, Nashville, TN.

Body: Background: Many factors such as tumor size, grade, lymph node and receptor status, either independently or in combination, as with the Nottingham Prognosic Index (NPI), are known to predict outcomes in non-treated breast cancer. With the growing use of neoadjuvant therapies, additional prognostic indicators have been identified for evaluating treated carcinomas. Many post-treatment methods of analysis rely on tumor cellularity (TC) either alone, as in the Miller-Payne system, or in combination with other tumor features, as in the Residual Cancer Burden (RCB) to predict distant relapse-free survival (RFS). It is not clear however, whether TC can predict outcomes in non-treated breast carcinoma. The goal of this study was to evaluate the prognostic value of TC in this particular setting.

Design: TC (%), excluding foci of necrosis and in-situ carcinoma, was determined from histologic review of a representative tumor section in the primary excision of 366 invasive breast carcinomas and categorized into quartiles. Prior detailed histology review included tumor size (TS), histological type and grade, receptor and lymph node status, RFS and overall survival (OS). Nottingham Prognostic Index (NPI) was calculated for each case (0.2 x tumor size (cm) + lymph node stage (1, node negative; 2, 1-3 positive nodes; 3, ≥ 4 positive nodes) + histologic grade).

Results: Mean patient age was 58 yr (range, 21-91) and median follow-up was 87 mo (range, 0.7-165). Invasive ductal carcinoma of no special type constituted 80% of cases, invasive lobular carcinoma 10%, and other special types of carcinoma, 10%. Nottingham grades I, II and III, represented 25%, 41% and 32% of the cases, respectively (unknown in 4). Mean NPI was 3.93 (range, 2.06–6.8). Estrogen receptor was positive in 66% and negative in 25% of cases (unknown in 9%). TC ranged from 2-99% (mean 47.6%). As expected, NPI was predictive of OS (p=0.000; hazard ratio 1.726; 95% confidence interval 1.45-2.05) and RFS (p=0.000; hazard ratio 2.011; 95% confidence interval 1.62-2.50). TC, unadjusted for other covariates was not predictive of OS or RFS (Table 1). The same analysis of ER positive and negative subgroups continued to show no relation of TC to OS or RFS (Table 2). When adjusted for NPI, TC still showed no significant relation to OS or RFS (data not shown).

Table 1

<table>
<thead>
<tr>
<th>TC Quartile</th>
<th>Hazard Ratio*</th>
<th>P Value</th>
<th>95% Confidence Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS, unadjusted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.779</td>
<td>0.315</td>
<td>0.48-1.27</td>
</tr>
<tr>
<td>3</td>
<td>1.073</td>
<td>0.769</td>
<td>0.67-1.73</td>
</tr>
<tr>
<td>4</td>
<td>0.978</td>
<td>0.934</td>
<td>0.58-1.65</td>
</tr>
<tr>
<td>RFS, unadjusted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.968</td>
<td>0.913</td>
<td>0.54-1.75</td>
</tr>
<tr>
<td>3</td>
<td>0.921</td>
<td>0.798</td>
<td>0.49-1.73</td>
</tr>
<tr>
<td>4</td>
<td>1.038</td>
<td>0.913</td>
<td>0.53-2.01</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Estrogen Positive Carcinomas</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>TC Quartile</td>
<td>Hazard Ratio*</td>
<td>P Value</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td>OS, unadjusted</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.824</td>
<td>0.581</td>
<td>0.42-1.64</td>
</tr>
<tr>
<td>3</td>
<td>1.224</td>
<td>0.535</td>
<td>0.65-2.21</td>
</tr>
<tr>
<td>TC Quartile</td>
<td>Hazard Ratio*</td>
<td>P Value</td>
<td>95% Confidence Interval</td>
</tr>
<tr>
<td>------------</td>
<td>---------------</td>
<td>---------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>OS, unadjusted</td>
<td>2</td>
<td>1.090</td>
<td>0.839</td>
</tr>
<tr>
<td>3</td>
<td>0.708</td>
<td>0.414</td>
<td>0.31-1.62</td>
</tr>
<tr>
<td>4</td>
<td>0.488</td>
<td>0.138</td>
<td>0.19-1.26</td>
</tr>
<tr>
<td>RFS, unadjusted</td>
<td>2</td>
<td>3.048</td>
<td>0.031</td>
</tr>
<tr>
<td>3</td>
<td>1.212</td>
<td>0.739</td>
<td>0.39-3.76</td>
</tr>
<tr>
<td>4</td>
<td>0.820</td>
<td>0.759</td>
<td>0.23-2.91</td>
</tr>
</tbody>
</table>

*Relative to 1st quartile

Conclusion: Despite its utility in the neoadjuvant setting, TC does not offer the same prognostic value in the setting of untreated tumors and is not predictive of OS or RFS in primary non-treated carcinomas.
Title: Bone mineral density change on aromatase inhibitors as a predictor of breast cancer recurrence

Hilary L Martin¹,², John A Davidson¹,², Francis Yap², Kim Chung², Muhammad A Khattak¹ and Andrew D Redfern¹,². ¹Royal Perth Hospital, Perth, WA, Australia and ²University of Western Australia, Perth, WA, Australia.

Body: Background
Aromatase inhibitors (AIs) reduce the risk of breast cancer recurrence in hormone receptor positive breast cancers by blocking the production of estrogen. Low estrogen states are also associated with reduction of bone mineral density. AIs have been shown to lead to reduction in bone mineral density although the degree of change in bone mineral density varies between individuals. In this study we investigate the association between change in bone mineral density and recurrence in patients treated with AIs.

Methods
This was a retrospective cohort study utilizing a single centre breast unit database. 327 patients were identified who had an initial bone density T score result at time of commencement of AI and a subsequent result after commencement of AI treatment. There were 145 patients who had the bone density raw score available in g/cm². Logistic regression (Stata 9) was used to predict recurrence. Two pathological prognostic factors, tumor size and number of lymph nodes, were shown to predict recurrence in this data set and were therefore used in multivariate testing. Baseline and sequential data on lumbar spine and hip T score and bone mineral density (BMD) in the same patient were analyzed for predicting recurrence and hazard of failure using a Cox model. Bone density in the models was adjusted to show either an alteration of T score of 1.0 or in BMD of 0.01g/cm².

Results
The mean lumbar T score difference was a reduction of 0.26 in sequential measurements (95% CI 0.2 to 0.31). No baseline T score or BMD showed any significance in predicting recurrence or the hazard of recurrence. Logistic regression modeling showed a reduction in lumbar spine T score following commencement of AI of 1.0 would decrease the odds of recurrence by 0.28 (95%CI 0.1 to 0.75). A 0.01g/cm² decrease in lumbar BMD would decrease the risk of recurrence by 0.79 (95% CI 0.68 to 0.94). Hip T score if decreased by a 1.0 would decrease the odds of recurrence by 0.27 (95% CI 0.08 to 0.86) and a 0.01g/cm² decrease in hip BMD would decrease the odds of recurrence by 0.81 (95% CI 0.66 to 0.99). The hazard for recurrence from commencement of AI was also significant in the lumbar spine data for a decrease of 1.0 in the second T scores (HR 0.2 p 0.002) and for the lumbar BMD reduction of 0.01g/cm² (HR 0.78 p<0.0001). The hip T scores and BMD did not reach statistical significance for predicting the hazard for recurrence.

Conclusion
Our data supports the hypothesis that reduction in bone density post commencement of aromatase inhibitor therapy is associated with reduced recurrence of breast cancer. It is likely that this association is the result of the effect of low circulating estrogen, resulting both in reduced risk of recurrence and lower bone density. This effect on bone density may prove a useful surrogate to measure efficacy of aromatase blockade and consequent anticancer effect on residual breast cancer.
Title: Deconstructing the TNM staging system for breast cancer

Jigar A Patel¹, Matthew T Hueman¹, Dechang Chen² and Donald E Henson². ¹Walter Reed National Military Medical Center, Bethesda, MD and ²Uniformed Services University of the Health Sciences, Bethesda, MD.

Body: Background
The TNM staging system is a standard classification for recording extent of disease in breast cancer. However, with progress in understanding tumor biology, it is unknown how new prognostic factors that will eventually be integrated with the TNM will affect its predictive ability. Our objective was to show the impact on 10-year survival rates for breast cancer as different combinations of prognostic factors are integrated into the TNM.

Methods:
Data were obtained from the Surveillance, Epidemiology, and End Results (SEER) Program of the National Cancer Institute for the years 1991 through 2000. After exclusions, 132,339 cases of female breast cancer were available. An ensemble clustering algorithm was used to calculate survival after including additional prognostic factors listed in SEER in the TNM. Combinations of the following 6 factors were sequentially added to the TNM: tumor grade, ER/PR status, age at diagnosis, racial/ethnic group, and histological tumor type.

Results:
Survival rates amongst some tumors with the same TNM stage varied as new factors were integrated into the TNM. Factors associated with favorable outcome usually were associated with better survival than factors associated with less favorable outcome for each stage group with varying degrees. There were 4 different tumor combinations that represented 4 different TNM stages that all corresponded to a 90% 10-year survival when additional factors were added to the TNM stage. Integration of additional prognostic factors led to a crossover in survival of some stage groups. In one combination (T1, N2, grade 1, ER+, PR+, age <50: 131111) patients who were assigned stage IIIA had a 10-year rate of 90%, which qualifies for a stage I category.

Survival Crossover in TNM Staging

<table>
<thead>
<tr>
<th>10-year Survival (%)</th>
<th>Prognostic Factor Combination</th>
<th>TNM Stage</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>113</td>
<td>IA</td>
<td>18993</td>
</tr>
<tr>
<td>90</td>
<td>112222</td>
<td>IA</td>
<td>2246</td>
</tr>
<tr>
<td>90</td>
<td>21211</td>
<td>IIA</td>
<td>5101</td>
</tr>
<tr>
<td>90</td>
<td>122112</td>
<td>IIA</td>
<td>4019</td>
</tr>
<tr>
<td>90</td>
<td>31111</td>
<td>IIB</td>
<td>682</td>
</tr>
<tr>
<td>90</td>
<td>131111</td>
<td>IIA</td>
<td>82</td>
</tr>
<tr>
<td>58</td>
<td>2232221</td>
<td>IIB</td>
<td>1103</td>
</tr>
<tr>
<td>58</td>
<td>22322213</td>
<td>IIB</td>
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</tr>
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<td>3331111</td>
<td>IIA</td>
<td>128</td>
</tr>
<tr>
<td>58</td>
<td>14211111</td>
<td>IIIC</td>
<td>64</td>
</tr>
</tbody>
</table>

**Abbreviated table. Prognostic Factor Combination in order from left to right: T, N, grade, ER status, PR status, age, race, histological type. * T1 = 1; T2 = 2; T3 = 3; N0 = 1; N1 = 2; N2 = 3; N3 = 4; Grade 1 = 1; Grade 2 = 2; Grade 3 = 3; ER+ = 1; ER- = 2; PR+ = 1; PR- = 2; Age <= 50 = 1; Age >50 = 2;

Conclusions:
Integrating new prognostic factors into the TNM always changed the outcome. Survival rates, therefore, are relative and depend on the selection of prognostic factors. Adding new factors selected different cohorts from the population which had a heterogeneous population of cancer survivors. These cohorts usually had different survival rates compared with the overall
population from which they were drawn. Integrating combinations of prognostic factors revealed frequent crossover of stage
groups at 10 years, which is a violation of a staging system and could impact the interpretation of clinical trials.
Title: NSAS BC02 substudy of chemo-induced amenorrhea (CIA) in premenopausal women who received either taxane alone or AC followed by taxane as a postoperative chemotherapy

Fumikata Hara¹, Hirofumi Mukai², Toru Watanabe³, Yukari Uemura⁴ and Yasuo Ohashi⁵. ¹NHO Shikoku Cancer Center, Matsuyama, Ehime, Japan; ²National Cancer Center Hospital East, Kashiwa, Chiba, Japan; ³Hamamatsu Oncology Center, Hamamatsu, Shizuoka, Japan; ⁴University of Tokyo, Bunkyo, Tokyo, Japan and ⁵Chuo University, Bunkyo, Tokyo, Japan.

Body: Background: Chemotherapy has a direct cytotoxic effect to breast cancer cells as well as ovarian suppression. In NSABP-B30 study, Swain, et al demonstrated that CIA contributed to reduce recurrence and prolong overall survival in premenopausal women with ER positive breast cancer. Thus far, incidence of CIA by anthracycline and cyclophosphamide (AC), or CMF has been reported. However, there has been no report on CIA by taxane alone therapy. Therefore, it is critically important to evaluate the incidence of the CIA in NSAS-BC02 (Watanabe T, ASCO2009) which compared taxane alone (q3w Docetaxel 75mg/m2 x8: DTX:, q3w Paclitaxel 175mg/m2 x8: PTX) to AC -> taxane (q3wAC 60/600mg/m2 x4 -> DTX x4: ACD, q3wAC x4 -> PTX x4: ACP) in postoperative patients with node-positive breast cancer. In addition, we examined the relationship between CIA and prognosis in this substudy.

Methods: Menstrual status of all women participating in NSAS BC02 was assessed at study entry, every cycle during chemotherapy, at 2 months after protocol treatment, and then at every 6 months until 5 years. After 5 years, menstrual status was assessed annually. Women who were having regular menstrual cycles (premenopause) or irregular menstrual cycle (perimenopause) at study entry were included in this CIA substudy. We defined CIA as having no menstrual cycle for at least 6 months after chemotherapy.

Results: Of the 1049 women enrolled in NSAS BC02, 395 were analyzed, including 315 with premenopause and 80 with perimenopause. Median age was 44.2 years old (42-62). Mean body mass index was 22.7 (15.4-38.4). Tumor characteristics were pathological stage I/IIA/IIB/III A 12.7%/39.0%/37.0%/11.4%, ER positivity 56.0% and PgR positivity 54.4%. Of 395 women, 287 (72.7%) was CIA due to protocol treatment. Regarding the type of protocol regimen, proportion of the CIA was 76.9% in ACP, 75.2% in ACD, 62.8% in PTX and 75.2% in DTX. There was no significant difference of CIA frequency between AC followed by taxane and taxane alone (76.01 vs 69.4%, respectively; p=0.14). Predictive factors of CIA were ACD against PTX (odds ratio (OR): 2.15), age increase by 5 years (OR: 1.50), and ER negativity (OR: 2.08) according to logistic regression analysis. In terms of effect to prognosis, CIA was an independent prognostic factor for disease-free survival (DFS) and overall survival in overall population of substudy according to multivariate Cox analysis. To eliminate guarantee time bias (GTB) (Giobbie-Hurder et al. JCO 2013), we used time-dependent Cox model. As a result, CIA was not statistically significant prognostic factor of DFS, even in the subgroup analysis of both ER positive and ER negative patients (Table 1).

Conclusion: Although there has been no data on CIA by taxation alone regimen, eight cycles of taxation treatment caused a high frequency of CIA in premenopausal women with breast cancer (PTX62.8% and DTX75.2%). It would be cautious to conclude that CIA was statistically significant association with prognosis, because it might be due to GTB.

Table 1: The effect of CIA on DFS by time-dependent Cox model

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Hazard ratio</th>
<th>95% Confidence interval</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.77</td>
<td>0.52-1.16</td>
<td>0.21</td>
</tr>
<tr>
<td>ER positive</td>
<td>0.60</td>
<td>0.31-1.16</td>
<td>0.13</td>
</tr>
<tr>
<td>ER negative</td>
<td>0.82</td>
<td>0.47-1.43</td>
<td>0.49</td>
</tr>
</tbody>
</table>
**Title:** The 70-gene signature affects adjuvant systemic treatment decisions in breast cancer patients: A population-based, observational study

Anne Kuijer¹, Annelotte CM van Bommel², Margriet van der Heiden- van der Loo³, Carolien A Drukker⁴ and Thijs van Dalen¹.
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**Body:**

**Background**

Gene signatures (GS), such as the 70-gene signature (MammaPrint™), are used as an adjunct to clinicopathological factors to predict outcome in breast cancer patients. According to the current Dutch national guidelines GS can be used in case of ambivalence regarding the benefit of adjuvant chemotherapy (ACT). While the impact of GS on the individual patient is well established, less is known about the impact on predefined patient cohorts in terms of an increase or decrease of the proportion of patients who receive ACT.

**Methods**

Patients surgically treated for primary breast cancer between November 2011 and April 2013 were selected from the Netherlands Cancer Registry. The administration of ACT in these patients was evaluated in relation to the use of the 70-gene signature (In the Netherlands the 70-gene signature is the most frequently used gene expression profile). Based on the Dutch guidelines clinicians might be ambivalent regarding the administration of ACT in the following subgroups: patients < 70 yrs with pN0, BRI tumours >2cm (group A), patients <70 yrs. with pN0, BRII tumours >1cm (group B) and patients < 70 yrs classified as pN1micro, BR I or II (group C).

**Results**

A total of 13,122 patients were identified in the Cancer Registry. The 70–gene signature was used in 794 patients; 19 in group A, 227 in group B, 62 in group C, and 456 (57.4%) did not fulfil the ambivalence criteria. In the latter patients, clinicopathological characteristics were contributed as follows: BR III tumours > 1cm (n=204), age > 70 (n=71), tumours<1cm (n=88), HER2+ (n=92), ER- (n=83) and N+ (n=166). In the predefined groups A, B and C, ACT was administered less when the 70-gene signature had been used (P= 0.019, 0.016 and 0.117 respectively; see table 1). The administration of ACT was in line with the test result of the 70-gene signature in 89%, 86% and 84% of the patients in group A, B and C respectively. In categories other than the predefined A, B and C an inverse relation was seen: more ACT was given when a GS was used (38% without and 54% with a GS) and adherence to the gene expression test result was lower.

**Table 1:** administration of adjuvant chemotherapy in relation to the use of MammaPrint

<table>
<thead>
<tr>
<th>Group</th>
<th>No MammaPrint</th>
<th>N</th>
<th>Chemotherapy (%)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group A</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MammaPrint</td>
<td>122</td>
<td>61 (47%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low riks/High risk</td>
<td>19</td>
<td>4 (21%)</td>
<td>0.019</td>
<td></td>
</tr>
<tr>
<td>ACT in line with GS</td>
<td>15/3</td>
<td>89%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group B</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MammaPrint</td>
<td>1229</td>
<td>507 (41%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low/ risk/High risk</td>
<td>226</td>
<td>75 (33%)</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>ACT in line with GS</td>
<td>125/74</td>
<td>86%</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Group C</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MammaPrint</td>
<td>397</td>
<td>209 (53%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low risk/High risk</td>
<td>62</td>
<td>26 (42%)</td>
<td>0.117</td>
<td></td>
</tr>
</tbody>
</table>
Conclusion
The proportion of patients who receive ACT decreased when a GS was used in predefined cohorts of patients for whom
ambivalence exists regarding the use of ACT. The majority of patients for whom a GS was used did not fit these predefined
categories and in subsets an inverse relation was seen: the use of a gene signature was associated with a higher chance of
receiving ACT.
Title: Predictive and prognostic value of triple-negative breast cancers molecular subtypes

Helene Bonsang-Kitzis¹, Cecile Laurent¹, Benjamin Sadacca¹, Alice Pinheiro¹, Matahi Moarii¹ and Fabien Reyal¹,². ¹RT2Lab Team, Institut Curie, Paris, France and ²Institut Curie, Paris, France.

Body: Triple-negative breast cancer (TNBC), defined by the absence of estrogen and progesterone receptor, and a lack of HER2 overexpression/amplification, corresponds to 15%-20% of breast cancers and is an aggressive disease. TNBC represents an important clinical challenge because no major improvement in the treatment scheme has recently happened in this subgroup. Robust tools to classify TNBCs are required in order to improve the prognostic determination as to better predict the response to therapy.

Materials and Methods
To establish a robust gene-expression classification we downloaded 21 publicly available datasets that contained raw gene expression micro-arrays of 3247 primary human breast cancer samples. The prognostic performance of this classification was tested using the clinical and gene-expression datas from METABRIC (n=1992). The predictive performance was tested using the clinical and gene-expression datas from 8 publicly available datasets established in order to analyse anthracycline with or without taxane-based neoadjuvant chemotherapy (n=996). A four steps methodology was applied: 1) the selection of TNBC samples was performed by bimodal filtering on ER-HER2 and PR expression in each individual dataset, 2) the selected samples were normalized all together (EMA), 3) the most variant genes (SD>0.8) were selected, 4) 4 clusters of genes were identified. A gene selection was performed into each gene cluster based on String (http://string90.embl.de/) connections >0.7 and gene-expression correlations >0.5. Each gene cluster was transformed as a metagene. Prognostic and predictive performance of each metagene was tested using standard methodology.

Results
Through GE analysis of 557 triple-negative breast cancers from 21 public datasets we identified a 6 metagenes signature (167 genes) enriched in distinct gene ontologies: two in immunity genes, one in proliferation genes, one in metabolism genes, two in matrix genes. This signature was particularly robust to identify TNBC subtypes across many data sets (n=1003 samples), despite the technology differences (Affymetrix A, Plus2 and Illumina).

Two metagenes had a prognostic or a predictive value. In multivariate analysis, a low Immunity2 metagene expression was associated with a poor disease free survival (HR=2.68 [1.59-4.52], p=0.0002) as a NPI score > 5.4 (HR=2.30 [1.36-3.89], p=0.002). In multivariate analysis, a low expression of the proliferation metagene was predictive of a lower response to neoadjuvant chemotherapy (OR=0.39 [0.21-0.70], p=0.002).

Conclusion
We identified a 6 metagenes-signature (167 genes) validated over 1000 TNBC samples. Two metagenes had a prognostic or a predictive value. Low expression level of Immunity2 metagene was associated with a poor prognosis and low expression level of Proliferation metagene was associated with a low response rate to neoadjuvant chemotherapy.
Title: Activated form of the estrogen receptor α (ER) in breast cancer (BC) and its correlation with prognosis

Erard Gilles¹, Jacques Bosq², Charline Alleaume³, Alexander Zukiwski⁴, Emile Hutt⁵ and Jacques Bonneterre⁵. ¹Invivis Pharmaceuticals, Bridgewater, NJ; ²Gustave Roussy Institute, Villejuif, France; ³Biodosis, Romainville, France; ⁴Arno Therapeutics, Flemington, NJ and ⁵Centre Oscar Lambret, Lille, France.

Background: About 50% of ER positive (ERpos) BCs are resistant to hormone treatment. In absence of ligand, ERs are evenly distributed in nuclei in normal tissue. Upon ligand binding, ERs dimerizes and form a discrete focal subnuclear distribution pattern (FDP), which is associated with transcriptional activation of ER and can be visualized with high powered microscopy. We have developed an IHC method to characterize the FDP in archival BC specimens (ASCO 2013 abst#592). We hypothesized that, in BC, the presence/absence of FDP of ER could predict anti-estrogen (anti-E) activity. We could determine two tumor phenotypes for ERpos tumors: a diffuse nuclear ER staining or "D-ER" corresponding to the expression of non-functional ER, which is the pattern observed in vitro or in vivo when no ligand are bound to steroid receptors (SR); D-ER thus is thought to predict lack of treatment effect of anti-E. And an aggregated nuclear pattern which corresponds to a similar pattern observed in vitro or in vivo when ligand is bound to ER; A-ER would suggest that ER is activated and a potential target to anti-Es.

Methods: A previously reported study (ASCO 2013 abst #592) was expanded from 254 evaluable cases to 755 with paraffin embedded formalin fixed (PEFF) BC specimens with clinical and pathology data. Specimens were analyzed for standard HES, ER, progesterone receptor (PR) and Ki67. The A-ER and D-ER nuclear patterns were analyzed at 1000x magnification.

Results: Mean age; 57 (17 -89). Histology: ductal 85% lobular 13%, other 2%; 82% ERpos and 78% either PRApos or PRBpos, 10% ERpos and 6% PRpos only. 92% of ERpos cases had received anti-Es; Adj. Chemotherapy 36%, Stage: I 47%, II 45%, III 8%. Grade: I 25%, II 52%, III 23%. Median follow up 42 months. ER status was D-ER in 71% and A-ER in 29% of the specimens. With DFS defined as time to PD or death (5 year cut off), 125/755 events were observed. ERpos was better than ERneg (HR=0.36, p = 0.00001). Within ERpos tumors group, in univariate analysis, a time-dependent Cox model showed that A-ER pattern was associated with better DFS vs D-ER pattern (HR = 0.03, p=0.02, time interaction = 0.01). A-ER was not correlated with SBR Grade, and was associated with its anisonucleosis (Aniso) index (0.02), with histology (ductal, 0.005) but not age, stage or HER2 status. Ki67 testing is ongoing. In monovariate analysis stage (0.00004), grade (p < 10-6), PR (p < 10-6) were prognostic on DFS, but not histology and HER2. In a time-dependent multivariate Cox model, A-ER remained an independent predictor (p = 0.013), with grade (p =0.001 with Mitotic Index 0.002, Differentiation 0.023, Aniso NS), stage 0.001, time interaction 0.009), PR and HER2 NS.

Conclusions: This study supports the hypothesis that anti-Es are mainly active in BC with A-ER pattern, which is targetable by anti-Es. Independent statistical significance was reached after adjusting for well-established prognostic factors. Given the 10-year hormonal treatment adjuvant recommendation guidelines, a better assay than the simple ER status determination would have important implications in BC management.
Title: Prognostic significance of the interferon metagene in node-negative breast cancer depends on the molecular subtype

Marcus Schmidt¹, Leonie van de Sandt², Karolina Edlund³, Isabel Sicking¹, Marco Battista¹, Anne-Sophie Heimes¹, Antje Lebrecht¹, Gerald Hoffmann¹, Mathias Gehrmann⁴, Jörg Rahnenführer² and Jan G Hengstler². ¹University Hospital Mainz, Mainz, Germany; ²Technical University Dortmund, Dortmund, Germany; ³Leibniz Research Centre for Working Environment and Human Factors (IfADo) at Dortmund TU, Dortmund, Germany and ⁴Bayer GmbH, Leverkusen, Germany.

Body: Background: Interferons are crucial for adaptive immunity and play an important role as central coordinators of tumor-immune system interactions. We examined the subtype specific prognostic significance of an interferon (IFN) metagene in node-negative breast cancer.

Methods: Using microarray based gene-expression data, we identified co-regulated genes related to biological processes. After hierarchical clustering, we defined an interferon (IFN) metagene which was composed of 36 interferon-stimulated genes. The subtype specific prognostic role of the IFN metagene was analysed in four previously published cohorts (Mainz, Rotterdam, Transbig, Yu) of node-negative breast cancer patients not treated with adjuvant therapy (n=824). A meta-analysis of previously published cohorts was performed using a random effects model. Prognostic significance of the IFN metagene for metastasis-free survival (MFS) was examined in different molecular subtypes: luminal A (ER+/HER2-/aurora kinase A [AURKA]low, luminal B (ER+/HER2-/AURKAhigh), basal-like (ER-/HER2-), and HER2+.

Results: Prognostic significance of the IFN metagene was restricted to the HER2+ positive molecular subtype (HR 0.50, 95% CI 0.28-0.88, P=0.0056). Prognostic effects were not seen in luminal A (HR 1.00, 95% CI 0.65-1.53, P=0.8819), luminal B (HR 1.00, 95% CI 0.76-1.32, P=0.9770) or basal-like (HR 0.87, 95% CI 0.66-1.15, P=0.3282) carcinomas of the breast.

Conclusions: The prognostic significance of the interferon metagene in node-negative breast cancer is subtype specific and confined to the HER2+ molecular subtype. A higher expression of the IFN metagene is associated with improved outcome in HER2+ breast cancer.
Title: Low body mass index (BMI) is associated with poor survival in Japanese patients with early breast cancer; an exploratory analysis of prospective randomized phase III trials N-SAS BC02 and 03

Body: Background: Obesity is reported to be associated with worse prognosis in early breast cancer. However, there is little data regarding the impact of low BMI on survival in patients with breast cancer. As obesity is rare and low BMI is relatively common in Japanese population compared to Caucasians, Japanese cohort is suitable to assess the impact of low BMI on survival in patients with early breast cancer. Recently an exploratory analysis of a small Japanese randomized phase II trial (JFMC 34-0601) suggested that low BMI was associated with a decreased overall response rate to neoadjuvant endocrine therapy with exemestane. We further explored the impact of low BMI on survival in patients with early breast cancer using a dataset of randomized phase III trials in Japan.

Methods: Patients included in prospective randomized phase III trial N-SAS BC02 and BC03 were retrospectively analyzed. N-SAS BC02 investigated four arms of adjuvant chemotherapy consisted of taxane alone or in combination with anthracycline-containing regimen (median follow up of 6.1 years). NSAS BC03 compared anastorozole with tamoxifen as adjuvant endocrine therapy (median follow up of 6.4 years). The correlation of BMI and overall survival was exploratory analyzed. This study was supported by the Public Health Research Center Foundation CSPOR.

Results: A total of 1726 patients were included in our study. Median age was 56 (24 – 82) years, 71.2% of tumors were ER positive, and 9.7% were HER2 overexpressed. Lymph node metastases were observed in 76% of patients. Mean value of BMI was 23.3 and only 4.6% of patients had BMI over 30. 33.1% of patients had BMI under 22 and 4.8% had BMI under 18.5. In the univariate Cox proportional hazard model, lower BMI was significantly associated with worse prognosis (BMI<27 vs >27, HR 0.55, 95% CI 0.32 – 0.93, p = 0.025). The same trend was observed in multivariate analysis (HR 0.61, p = 0.064).

Conclusion: We confirmed that obese patients were relatively rare in Japanese patients with early breast cancer. In this non-obese population, lower BMI was correlated with worse prognosis. However these results should be cautiously interpreted. Our findings suggest that there may be an optimal BMI in patients with early breast cancer and it should be confirmed by another cohort.
Title: Functional subtyping with BluePrint 80-gene profile identifies two distinct triple positive subtypes with and without trastuzumab/chemo-sensitivity: Implications for treatment from the NBRST registry

Pat Whitworth¹, Jennifer Beatty², Paul Baron³, Paul Richards⁴, James Pellicane⁵, Angela Mislowsky⁶, Charles Nash⁷, Laura Lee⁸, Mary Murray⁹, Femke de Snoo¹⁰, Lisette Stork-Sloots¹⁰, Sarah Untch¹⁰, Mark Gittleman¹¹, Stephanie Akbari¹² and Peter Beitsch¹³.
¹Nashville Breast Center, Nashville, TN; ²Breast Place, Charleston, SC; ³Breast & Melanoma Specialists of Charleston, Charleston, SC; ⁴Blue Ridge Cancer Care, Roanoke, VA; ⁵Virginia Breast Center, Midlothian, VA; ⁶Coastal Carolina Breast Center, Murrells Inlet, SC; ⁷Northeast Georgia Medical Center, Gainesville, GA; ⁸Comprehensive Cancer Center, Palm Springs, CA; ⁹Akron General Hospital, Akron, OH; ¹⁰Agendia Inc, Irvine, CA; ¹¹Breast Care Specialists, Allentown, PA; ¹²Virginia Hospital Center, Arlington, VA and ¹³Dallas Surgical Group, Dallas, TX.

Body: Background

Classification by molecular subtype can aid in the selection of therapy for patients with breast cancer. However at present, the methodology for molecular subtyping is not standardized and the methodology and interpretation of results vary between different laboratories. Subtype is being assigned using conventional immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) (“conventional subtype”) or molecularly using gene expression profiling. The aim of the current prospective NBRST study is to compare chemosensitivity as defined by pathological Complete Response (pCR), or endocrine sensitivity as defined by partial response (PR) using the 80-gene BluePrint functional subtype profile vs. conventional IHC/FISH subtyping.

Methods

MINDACT is an international, prospective, randomized, phase III trial investigating the clinical utility of MammaPrint in selecting patients with early BC for adjuvant chemotherapy (CT), which enrolled 6,694 patients. Molecular subtyping data were obtained by MammaPrint and BluePrint (Agenda, Amsterdam, the Netherlands) on frozen samples (n=6,694) classifying patients in the following subtypes: Luminal A (Luminal-type/MammaPrint Low Risk); Luminal B (Luminal-type/MammaPrint High Risk); HER2-type; and Basal-type. ER, PgR, HER2 and Ki67 protein status were centrally assessed on FFPE blocks by IHC and/or FISH in the European Institute of Oncology, Milan, Italy (n=5,740; 86%). Patients were also classified according to the St. 2013 Gallen recommendations [Goldhirsch et al. 2013], which recognizes an additional category (Luminal B-like HER2+).

Results

Ki67 is often used as biomarker to distinguish Luminal A from Luminal B subgroups. The concordance between MammaPrint and centrally assessed Ki67 in Luminal-type patients is 60%, with a κ score of 0.26 (95% CI 0.24– 0.28) indicating that Ki67 and MammaPrint cannot reliably substitute for each other. When using a cut-point of 20% instead of 14% the concordance increased to 78%, with a κ score of 0.44 (95% CI 0.41–0.47).

There is a relatively large group of clinical HER2+ cases that are BluePrint Luminal-type (208 out of 541; 38%) indicating that tumor expression of the Luminal profile is dominant compared with expression of the HER2 profile. These patients have high IHC ER results and all except for 1 fall into the group that St Gallen separately defines as Luminal B-like HER2-positive. These patients may have lower response to trastuzumab [von Minckwitz et al. JCO 2012]. 98 out of 622 BluePrint Basal-type patients are clinical Luminal HER2-. 2/3 of these patients have low centrally assessed IHC PR expression and 1/3 have low centrally assessed ER expression (≥1% and <10%).

Conclusions

Molecular subtyping using BluePrint and MammaPrint leads to a reclassification of 22% (113/515) of tumors. The re-classification of patients leading to re-assignment to more responsive vs. less responsive groups is most prominent in classically assessed triple positive patients where 46% of patients are re-assigned to the less responsive BP Luminal-type group (pCR rate of 7%) vs. 46% of patients assigned to the responsive BP HER2-type group (pCR rate of 49%). These findings confirm the more accurate identification of molecular subgroups for treatment decision by BluePrint functional subtype classifier which may therefore serve as a better guide for neo-adjuvant treatment than standard, local IHC/FISH assay.
**Title:** Serum–based test to identify patients with early relapse treated with adjuvant hormonal therapy

Heidi Fiegl¹, Christian Marth¹, Krista Meyer², Julia Grigorieva² and Heinrich Roder². ¹Innsbruck Medical University, Innsbruck, Tyrol, Austria and ²Biodesix, Boulder, CO.

**Body: Background**
Prediction of relapse in hormone receptor-positive patients treated with adjuvant hormonal therapy is an area of active research. Several tissue based genomic tests have been developed and are used in clinical practice [e.g. OncotypeDX (Genomic Health), MammaPrint (Agendia)] to evaluate the risk of recurrence in early stage breast cancer. However, a cost–effective serum based test, that would allow identification of patients at high risk of early relapse, is of clinical interest.

**Methods**
We used MALDI ToF Mass spectrometry to obtain mass spectra from pre-surgery serum samples from 499 patients treated with adjuvant hormonal therapy. Spectra were subjected to pre-processing and 84 peaks (features) were selected for the analysis. We used a novel proprietary approach, utilizing recent advances in learning theory, to create a diagnostic test to classify patients as Early Relapse or No Early Relapse. The method creates many multivariate classifiers that are filtered for performance and combined using logistic regression with dropout regularization into a single master classifier. To avoid bias introduced through a particular split of training and test, many realizations of the development set are created. The performance of each master classifier is examined and the classifiers are combined using a majority vote procedure to serve as the final test. The method allows the use of smaller training sets and minimizes overfitting.

**Results**
22 out of 499 patients had an early relapse in < 5 years. Samples from these patients were matched, mindful of treatment and HER2 status, with 22 samples from patients without relapse with the longest duration of relapse free survival (RFS), to serve as the development set. The remaining samples, including those from patients with late relapses (> 5 years), were set aside for additional testing. The performance of the 200 master classifiers created from the 200 test/training splits of the development set was evaluated showing a median overall accuracy of 70%, specificity of 73%, sensitivity of 67%, and hazard ratio (HR) of 3.5. The final classifier was created using the 200 master classifiers from the realizations of Training and Test splits which were combined using the majority vote procedure. Classification of patients in the development set from the majority vote of master classifiers resulted in a significant separation in survival curves (log-rank p<0.0001, HR 6.2 Median RFS Early Relapse 2.6 years, No Early Relapse not reached) with overall accuracy of 79%, specificity of 82%, and sensitivity of 77%. In the combined population of all patients, the separation was also significant (log-rank p=0.028, HR 2.0, median RFS not reached in both classifications). In the multivariate analysis of the overall population, classification remained significant (p=0.007, adjusted HR 3.5) along with menopausal status, nodal status, and tumor size.

**Conclusions**
We created a classifier from pre-surgery serum samples that can identify patients at risk of early relapse (<5 years) when treated with adjuvant hormonal therapies. Such a test would have clinical utility in identifying patients who may need a revised adjuvant treatment strategy.
Title: Age independently predicts opposite disease-specific survivals in luminal A breast cancer patients diagnosed at younger and older than 50 years of age

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Body: BACKGROUND
It has been reported that women diagnosed with invasive breast cancer (IBC) in their 50s or 60s have better survival than either younger or older women. Young women with IBCs often have a more aggressive phenotype which contributes to worse survival. But there is supporting evidence in the literature for both sides whether age alone is an intrinsic driver of the poor outcome in young women. The availability of large-scale IBC data provided a good opportunity to address this issue.

METHODS
Clinicopathologic and gene expression data from 2 public datasets, including METABRIC from the European Genome-Phenome Archive (n=1992) and The Cancer Genome Atlas-Breast Cancer project (TCGA-BC, n=980) from National Cancer Institute, were used in this study. PAM50 was used to derive intrinsic subtypes based on microarray and RNA-Seq data. For a given phenotype, Fisher’s exact test was used for its association with age, and 2-sample test for equality of proportions with continuity correction between young and older patients. Kolmogorov-Smirnov’s test was used for equality of age distributions between phenotypes. Disease-specific survival (DSS) was examined for relationship with age, adjusted for race, AJCC stage, nodal metastasis, tumor size, grade, and subtype where applicable. Kaplan-Meier estimate and log-rank test were used to generate and compare survival curves, respectively. Cox proportional hazards model was used for univariate and multivariate analyses and to calculate hazard ratios (HRs).

RESULTS
Firstly, we confirmed that more aggressive IBCs were enriched in younger patients. Younger patients (<50 years) were diagnosed with more basal-like subtype (P<0.05) and more node+ diseases (P<0.05) compared with their older counterparts (≥50 years) in both datasets. Secondly, we found no significant difference in DSS between these 2 age groups after adjusting for subtype and other clinicopathologic variables. Finally, we examined the effect of age on DSS within the younger and older patient groups separately. After adjusting for the effects of other clinicopathologic variables in the METABRIC dataset, the continuously increasing age was associated with better (HR=0.897, P=0.039) and worse (HR=1.033, P=0.0079) DSS in Luminal A subtype in the younger and older patient groups, respectively. Age was also associated with worse DSS in Luminal B (HR=1.018, P=0.086) and all (HR=1.014, P=0.030) subtypes in older patients. The results were validated in the TCGA-BC data (younger: HR=0.877 and P=0.022 in Luminal A; older: HR=1.070 and P=0.066 in Luminal A, HR=1.081 and P=0.034 in Luminal B, HR=1.055 and P=0.0052 in all patients).

CONCLUSION
Our results suggested that age was an independent predictor of better DSS in younger patients, but worse DSS in older patients, especially those with Luminal A subtype. The opposite effects of age on DSS in younger and older patients warrant further molecular studies.

The views expressed in this abstract are those of the authors and do not reflect the official policy of the Department of Defense, or U.S. Government.
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Average Grade: 7.40

Title: Unique characteristics and failure patterns of metaplastic breast cancer in contrast to invasive ductal carcinoma: A retrospective multicenter case-control study (KROG 13-07)

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Body: Purpose: This retrospective study was performed to investigate the need for management modification in metaplastic breast cancer (MBC) by evaluation of characteristics and failure patterns compared to invasive ductal carcinoma (IDC).

Methods and Materials: We performed this multicenter study taking MBC and randomly assigned IDC cases matched for age (± 3 years), pathologic stage (T and N), loco-regional treatment methods [surgery ± radiation therapy (RT)] and period of treatment (± 6 months) that occurred from January 1999 to November 2011 in the six institutions of the Korean Radiation Oncology Group (KROG).

Results: A total of 144 female MBC patients were enrolled. The median follow-up was 51 months (range, 1 to 186 months). The rates of positivity for ER (P<0.001), PR (P<0.001), and HER-2 (P=0.003) were significantly lower in MBC patients.

Characteristics of patients with metaplastic breast cancer (MBC) and invasive ductal carcinoma (IDC)

<table>
<thead>
<tr>
<th>Variables</th>
<th>MBC (n=144)</th>
<th>IDC (n=144)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histologic grade (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>5 (3.5)</td>
<td>17 (11.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>II</td>
<td>18 (12.5)</td>
<td>63 (43.8)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>109 (75.7)</td>
<td>63 (43.8)</td>
<td></td>
</tr>
<tr>
<td>Nuclear grade (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>3 (2.1)</td>
<td>10 (6.9)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>II</td>
<td>17 (11.8)</td>
<td>47 (32.6)</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>89 (61.8)</td>
<td>58 (40.3)</td>
<td></td>
</tr>
<tr>
<td>Molecular subtype (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal A</td>
<td>12 (8.3)</td>
<td>80 (55.6)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Luminal B</td>
<td>1 (0.7)</td>
<td>6 (4.2)</td>
<td></td>
</tr>
<tr>
<td>HER-2 enriched</td>
<td>2 (1.4)</td>
<td>9 (6.3)</td>
<td></td>
</tr>
<tr>
<td>Triple negative</td>
<td>123 (85.4)</td>
<td>42 (29.2)</td>
<td></td>
</tr>
</tbody>
</table>

During follow-up, recurrence developed in 22 (15.3%) MBC and 6 (4.2%) IDC patients (P=0.002).

Patterns of primary site recurrence according to histology and molecular subtype

<table>
<thead>
<tr>
<th>Site of first recurrence</th>
<th>MBC (n=144)</th>
<th>IDC (n=144)</th>
<th>P-value</th>
<th>TN-MBC (n=123)</th>
<th>Other (n=161)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local-n(%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Axilla</td>
<td>4 (2.8)</td>
<td>1 (0.7)</td>
<td>0.37</td>
<td>4 (3.3)</td>
<td>1 (0.6)</td>
<td>0.17</td>
</tr>
<tr>
<td>Internal mammary</td>
<td>4 (2.8)</td>
<td>1 (0.7)</td>
<td>0.37</td>
<td>4 (3.3)</td>
<td>1 (0.6)</td>
<td>0.17</td>
</tr>
<tr>
<td>Supraclavicular</td>
<td>3 (2.1)</td>
<td>0</td>
<td>0.25</td>
<td>3 (2.4)</td>
<td>0</td>
<td>0.08</td>
</tr>
<tr>
<td>Total</td>
<td>9 (7.6)</td>
<td>2 (1.4)</td>
<td>0.06</td>
<td>11 (8.9)</td>
<td>3 (1.8)</td>
<td>0.01</td>
</tr>
</tbody>
</table>
The median time to recurrence of MBC and IDC was 15 months and 24 months, respectively. Most instances of recurrence in MBC developed in the triple-negative (TN) subgroup (TN-MBC). In particular, loco-regional recurrence developed exclusively in the TN-MBC subgroup. In the TN-MBC subgroup, the number of risk factors (pT2-3, N1-3) was related to significant differences in overall survival (P=0.001) as well as recurrence-free survival (P<0.001).

**Conclusions:** The MBC had a higher rate of TN, poorer differentiation, and a higher recurrence rate than did the IDC. Considering the unique characteristics and failure patterns, modification of the current management guidelines for MBC might be necessary.
Title: Glucose-regulated protein 78 and C/EBP homologous protein predict disease-free survival and responsiveness to chemotherapy in breast cancer

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Body: Background

Cancer cells are generally under endoplasmic reticulum (ER) stress. Notably, the ability of cells to respond to ER stress is critical for cell survival, and chronic or unresolved ER stress can lead to apoptosis. Glucose-regulated protein (GRP) 78 and C/EBP homologous protein (CHOP) are commonly used as markers of endoplasmic reticulum (ER) stress. As an ER chaperone, GRP78 functions as a potent anti-apoptotic factor and confers drug resistance, whereas CHOP is a key initiating factor of ER stress-related cell death. The clinical implications of GRP78 and CHOP, however, have not been fully studied in breast cancer. In this study, we aimed to investigate the predictive value of GRP78 and CHOP in breast cancer patients who underwent adjuvant chemotherapy.

Methods: An immunohistochemistry screen for GRP78 and CHOP was performed using a tissue microarray (TMA) containing 250 tumors from female patients diagnosed with invasive ductal breast carcinoma at the Fudan University Shanghai Cancer Center. The staining results were scored semi-quantitatively, and a prediction model was constructed to verify the hypothesis.

Results: In this retrospective study cohort, positive GRP78 staining was detected in 52.6% (n = 112; 52.6% positive, 47.4% negative) of tumors, and CHOP staining was present in 56.3% (n = 120; 56.3% positive, 43.7% negative) of cases. In Kaplan-Meier analysis, CHOP correlated with prolonged disease-free survival (DFS; P = 0.001), whereas GRP78 showed an opposite association (P < 0.001). Moreover, in a GRP78-positive subset, CHOP overexpression correlated with a lower risk of recurrence. A further multivariate COX analysis revealed that positive GRP78 staining cases exhibited a higher likelihood for disease events (HR = 4.573; 95% CI: 2.291-9.128; P < 0.001), while CHOP positivity was indicative of lower risk for recurrence (HR = 0.385, 95% CI: 0.215-0.688; P = 0.001). In the receiver operating characteristic (ROC) analysis, the prediction capability of the predictive model combining the above two markers surpassed that of a traditional model (P = 0.0085 for the area under the curve comparison). Within the anthracycline-treatment subgroup, the combined GRP78 and CHOP exhibited similar predictive significance.

Conclusions: Collectively, our findings suggest a tight association between ER stress markers and clinical outcomes for female patients diagnosed with invasive ductal breast carcinoma. Adding these two ER stress markers to traditional prognostic factors provides a more sensitive and accurate predictive model for female patients with invasive ductal breast cancer. Moreover, the combination of GRP78 and CHOP has predictive value for responsiveness to anthracycline-based adjuvant breast cancer chemotherapy. Thus, our findings provide insights into further applications of GRP78 and CHOP, potentially providing additional predictive information for oncologists with regard to choosing treatment regimens.
Title: Prognostic and predictive value of an integrated mRNA-lncRNA signature in triple-negative breast cancer: A comprehensive transcriptome analysis

Yizhou Jiang¹, Yi-Rong Liu¹, Ke-Da Yu¹, Xin Hu¹, Xiao-En Xu¹, Ling Yao¹ and Zhi-Ming Shao¹. ¹Fudan University Shanghai Cancer Center, Shanghai, China.

Body: Purpose
Triple-negative breast cancer (TNBC) is a highly diverse group of disease, and clinical outcome of patients with TNBC is highly variable. Due to the heterogeneity of TNBCs, there is limited molecular signature routinely used for predicting the risk of disease recurrence and benefit of adjuvant chemotherapy. Our study aims to develop and validate a RNA signature, integrating messenger RNAs (mRNAs) and long non-coding RNAs (lncRNAs) together, for TNBC patients to improve risk stratification and avoid unnecessary adjuvant therapy.

Methods
Using transcriptome microarrays, we analyzed 33 paired TNBC and adjacent normal breast tissues, and identified 1,644 mRNAs and 1,047 lncRNAs which were differentially expressed between tumors and normal tissues. We further determined the expression of these mRNAs and lncRNAs in an additional 134 TNBC samples using transcriptome microarrays, and confirmed their associations with patients' recurrence-free survival (RFS). Using the LASSO Cox regression model, we built an integrated mRNA-lncRNA signature incorporating seven mRNAs and three lncRNAs. Prognostic and predictive accuracy of the signature was tested in the training set of 167 TNBC patients and further validated in an independent validation set of 143 TNBC patients.

Results
In the training set, we identified 36 mRNAs and 32 lncRNAs which were tumor-specific and significantly associated with patients' RFS. Using the LASSO Cox regression model, an integrated mRNA-lncRNA signature based on seven mRNAs (CCR4, CTSB, ERO1L, HIF1A, IGFR1R, SRD5A1 and TFF1) and three lncRNAs (n381928, n333541 and TCONS_I2_00013109-XLOC_I2_007048) was developed in the training set and subsequently validated in the validation set. Patients were classified to the high-risk (high risk of recurrence) and low-risk (low risk of recurrence) groups according to their scores in the signature. In the training set, multivariate analysis showed that the predicted high-risk group had higher risk of developing recurrent disease within five years of surgery than the low-risk patients (hazard ratio [HR] = 6.12, 95% confidence interval [CI] 2.76-13.56, P<0.001). In the validation set, the predicted high-risk group also had poorer RFS in multivariate analysis (HR = 5.09, 95% CI 1.82-10.96, P<0.001). Furthermore, analyzing the areas under the time-dependent receiver operating curve for five-year RFS, we proved the integrated mRNA-lncRNA signature had better prognostic value than the seven-mRNA-only signature and clinicopathological risk factors in both the training and validation sets. Finally, Cox proportional hazards models were utilized to test the interaction between adjuvant chemotherapy and different risk groups. Patients in the low-risk group had a more favorable response to adjuvant chemotherapy both in the training and validation sets.

Conclusion
The integrated mRNA-lncRNA signature is a reliable tool for predicting disease recurrence and benefit of adjuvant chemotherapy in TNBC patients. If further validated in larger population, it could facilitate patient counseling and individualize treatment of TNBC.
Title: The thrombin clotting pathway is upregulated in the stroma of pre-invasive breast cancer and further upregulated in aggressive invasive breast cancer phenotypes

Hudhaifah Shaker1,2, Nigel J Bundred1, Harith Albadry3, Sarah L Nicholson3, Susan Pritchard3, Karin Jirström4, Goran Landberg2 and Cliona C Kirwan1. 1University of Manchester, Manchester Academic Health Science Centre, University Hospital of South Manchester, Manchester, United Kingdom; 2Breakthrough Breast Cancer Unit, Cancer Research UK Institute Manchester, Manchester, United Kingdom; 3University Hospital of South Manchester, Manchester, United Kingdom and 4Oncology and Pathology, Lund University, Skane University Hospital, Lund, Sweden.

Body: BACKGROUND
Components of the thrombin (extrinsic) clotting pathway are upregulated in cancer. The clotting pathway factors tissue factor (TF) and Thrombin promote tumour progression through protease activated receptors PAR2 and PAR1 respectively.

AIMS
To determine if tumour expression (epithelial and stromal) of a procoagulant phenotype is associated with aggressive breast cancer phenotypes and reduced survival.

METHODS
Tumour expression of TF, thrombin, PAR1 and PAR2 was determined by immunohistochemistry in two cohorts.

PROSPECTIVE STUDY
Early invasive breast cancer (n=199), ductal carcinoma in situ (DCIS, n=42) and normal breast tissue samples (n=121).

RETROSPECTIVE STUDY
Early invasive breast cancer patients (n=144) with median follow-up of 69 (range 4 to 91) months.

Procoagulant phenotype expression was correlated with tumour grade, proliferation (Ki67), ER and HER2 status (both cohorts), survival and recurrence (retrospective cohort).

RESULTS

PROSPECTIVE STUDY
Epithelium
Thrombin (p<0.01) but not TF, PAR1 or PAR2 was increased in invasive cancer compared to DCIS and normal breast tissue.

Stroma
TF, Thrombin, PAR1 and PAR2 were increased in the stroma of DCIS compared to normal breast stroma (p<0.05, all). In invasive breast cancer, TF was increased in invasive cancer compared to DCIS and compared to normal breast tissue (p<0.01, both). Thrombin, PAR1 and PAR2 were increased in invasive cancer compared to normal breast tissue (p<0.01, all). TF, thrombin, PAR1 and PAR2 were increased in high proliferating (p<0.01, all) and high grade cancer (p<0.01, all). TF (p=0.02) and PAR1 (p<0.01) were increased in ER negative cancer. TF, thrombin and PAR2 was increased in HER2 positive cancer (p<0.01, all).

RETROSPECTIVE STUDY
Stroma
As with the prospective study, thrombin and PAR2 expression was increased in high proliferating cancer (p<0.05, both) and thrombin was increased in high grade cancer (p<0.05). PAR1, TF and thrombin expression was increased in ER negative cancer (p<0.05, all) and PAR2 was increased in HER2 positive cancer (p=0.05).

Overall (OS) and disease-free survival (DFS)
PAR1 stromal expression was an independent predictor of reduced OS (HR 3.3, 95% CI 1.3-8.3, p=0.01) but did not correlate with DFS.

There was no association between epithelial PAR1 expression or epithelial or stromal TF, thrombin or PAR2 expression and DFS or OS.

CONCLUSION
Stromal upregulation of the thrombin pathway occurs in in-situ cancer, implying cancer-stromal communication at the pre-invasive stage. Stromal thrombin pathway components may have a role in the transition of pre-invasive to invasive cancer.
Stromal (but not epithelial) thrombin pathway upregulation is associated with aggressive invasive breast cancer phenotypes and reduced survival. The thrombin pathway may provide a novel therapeutic target, particularly in ER negative, HER2 positive breast cancer.
Title: Linking genotype to clinical outcome in breast cancer by combining NGS and gene chip data

Balazs Gyorffy1,2,3, Lorinc Pongor1 and Mate Kormos1. 1MTA TTK Lendület Cancer Biomarker Research Group, Budapest, Hungary; 2MTA-SE Research Group for Pediatrics and Nephrology and 3Semmelweis University.

Body: Introduction: Next generation sequencing (NGS) provides the possibility to measure mutational status for any part of any gene. However, because of scarce data available to date, linking these mutations to relevant clinical outcome in a large number of patients is not possible.

Aim: Our goal was to combine available genotype data generated by using NGS with gene expression data generated by gene chips to establish a framework to assess the effect of genotype on clinical outcome.

Methods: NGS data generated by the TCGA consortia and publicly available gene chip data obtained from the GEO and EGA repositories were utilized. NGS data was processed using MuTect, SNPeff, GRCh37 and R. RNA-seq data was normalized using DEseq. Gene chip data was MAS5 normalized. Generation of the transcriptomic fingerprint for mutation status was computed by ROC utilizing the RNA-seq data. In the gene chip data, the average expression of significant genes identified was designated as a metagene for the given genotype. Correlation to survival for this metagene was assessed by computing Cox regression and plotting Kaplan-Meier survival plots. Finally, we have set up an online interface to enable running the analysis for any selected gene.

Results: The database contains 332 NGS samples containing mutational status for 22,938 genes and RNA-seq data for 10,987 genes. The gene chip database contains 5,934 patients with 10,987 genes plus detailed clinical characteristics and survival data. We evaluated correlation to outcome for previously identified genes harboring the ten most common somatic mutations in breast cancer. Of these, TP53 (n of mutations out of 332=93, hazard rate=0.51, p<1E-16), AKT1 (n=18, HR=1.6, p=1.6E-15), PIK3CA (n=119, HR=1.5, p=8.5E-12), MAP3K1 (n=20, HR=1.4, p=1.3E-08), CDH1 (n=34, HR=1.3, p=4.4E-07), and RB1 (n=21, HR=1.3, p=7E-06) reached statistical significance while PI3K, PTEN, CDKN1B and GATA3 were not significant or had insufficient number of mutated samples.

Discussion: By connecting genotype to gene expression signature and employing this signature for survival analysis we have set up a pipeline enabling the functional validation of a discovered mutation for any gene in a large breast cancer cohort.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P6-08-32  
**Average Grade:** 5.80

**Title:** Elevated neutrophil lymphocyte ratio predicts survival in breast cancer

Cher Hui Koh¹, Nirmala Bhoo-Pathy²,³, Khoon Leong Ng¹, Mee Hoong See¹, Gie Hooi Tan¹, Suniza Jamaris¹ and Nur Aishah Taib¹. ¹Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia; ²Julius Centre University of Malaya, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia and ³Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, Netherlands.

**Body:** Background: Host inflammatory response affects disease progression and survival in cancer. While the elevation of neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (PLR) have been associated with poor prognosis in colorectal cancer, evidence on prognostic significance of these indicators in breast cancer is sparse. We determined whether the initial, pretreatment NLR and PLR impact survival of patients presenting with primary breast cancer.

**Methods:** Of 2059 consecutive patients, newly diagnosed with breast cancer in University Malaya Medical Centre, Malaysia between January 2000 and December 2008, we only included 1447 patients with available differential blood count at time of diagnosis (~70%). Data on tumor characteristics and treatment were obtained from the hospital-based breast cancer registry whereas vital status was verified with the national mortality registry. Patients were stratified into quintiles of NLR and PLR. Differences in demography, tumor characteristics and treatment patterns between the quintiles were assessed. Relative survival rates (RSR) were estimated using the Malaysian population mortality data. Multivariable Cox regression was used to determine the independent prognostic significance of NLR and PLR.

**Results:** Median age at presentation was 52 years, whereas median tumor size at diagnosis was 3.5 cm with 48% of patients presenting with axillary lymph node involvement. Both NLR and PLR were positively correlated (p<0.001). Compared to patients in the lowest NLR quintile (NLR≤1.38), those in the highest quintile (NLR≥3.95) were younger at diagnosis (median: 49 years versus 56 years; p<0.001), and presented with bigger tumors (median: 5.0 cm versus 3.0 cm; p<0.001), axillary lymph node metastasis (55.2% versus 45.9%; p=0.035), distant metastases at diagnosis (32.0% versus 7.2%; p<0.001), and higher tumor grades (49.6% versus 33.9%; p=0.016). A total of 598 deaths were observed during 9248 person-years of follow-up, corresponding with a 5-year RSR of 70.3% (95%CI: 67.7%-72.8%). Higher NLR quintiles were significantly associated with poorer survival; 5-year RSRs were 75.6% (95%CI: 68.9%-81.4%) in quintile 1, 79.4% (95%CI: 74.5%-83.7%) in quintile 2, 72.3% (95%CI: 66.5%-77.5%) in quintile 3, 65.5% (95%CI: 59.7%-70.9%) in quintile 4, and 51.8% (95%CI: 44.4%-58.7%) in quintile 5. Following adjustment for age, ethnicity, AJCC6 stage, PLR, tumor grade, lymphovascular invasion, hormonal receptor status, locoregional management, chemotherapy, and hormone therapy, the hazard ratios (HR) for the second to fifth quintiles of NLR compared to the first quintile were 1.06 (95%CI:0.80–1.40), 1.15 (95%CI:0.86–1.55), 1.59 (95%CI:1.21–2.10), and 2.42 (95%CI:1.81–3.21) respectively; p for linear trend test <0.001. Patients in the highest PLR quintile were also significantly associated with decreased survival compared to those in the lowest quintile; 5-RSR: 53.2% (95%CI: 46.9%-59.1%) versus 76.9% (95%CI: 70.9%-82.1%), respectively. Nevertheless, this association was not significant following multivariable adjustment (HR: 0.99, 95%CI:0.76-1.29).

**Conclusion:** High NLR seems to be an independent prognostic factor for breast cancer but not PLR. These findings warrant further validation.
Title: Prognostic value of axillary nodal ratio after neoadjuvant chemotherapy of AC followed by docetaxel: A multicenter retrospective cohort study

Se Hyun Kim¹, Jee Hyun Kim¹, Tae-Yong Kim², In Sil Choi³, Yee Soo Chae⁴, Sun Kyung Baek⁵, Seok Yun Kang⁶, In Hae Park⁷, Yoon Ji Choi⁸, Soohyeon Lee⁹, Joo Hyuk Sohn⁶, Yeon-Hee Park⁶, Young-Hyuck Im¹⁰, Jin-Hee Ahn¹¹, Sung-Bae Kim¹¹ and Kyung Hae Jung¹¹. ¹Seoul National University Bundang Hospital, Seong-nam, Korea; ²Seoul National University Hospital, Seoul, Korea; ³SMG-SNU Boramae Medical Center, Seoul, Korea; ⁴Kyungpook National University Hospital, Daegu, Korea; ⁵Kyung Hee University Medical Center, Seoul, Korea; ⁶Ajou University Hospital, Suwon, Korea; ⁷National Cancer Center, Goyang, Korea; ⁸Korea University Ansan Hospital, Seoul, Korea; ⁹Severance Hospital, Seoul, Korea; ¹⁰Samsung Medical Center, Seoul, Korea and ¹¹Asan Medical Center, Seoul, Korea.

Background: The ratio of involved to retrieved lymph nodes (LNR) is suggested as a prognostic factor in operable breast cancer. However, there are conflicting results regarding its clinical significance after neoadjuvant chemotherapy. We investigated the prognostic value of LNR with a thorough evaluation of potential prognostic factors in a large cohort constructed from Health Insurance Review and Assessment Service database of Korea.

Patients and method: This retrospective analysis is based on the data of 814 patients with clinical stage II/III breast cancer treated with four cycles of adriamycin/cyclophosphamide (AC) followed by four cycles of docetaxel (DOC) before surgery. We evaluated the clinical significance of the LNR (3 categories: Low, 0-0.20 vs. Intermediate, 0.21-0.65 vs. High, 0.66-1.00) using Kaplan-Meier method, log-rank test, and Cox proportional hazard regression model.

Result: A total of 799 patients underwent breast surgery (Median age 45, range 16-74; Mastectomy 369, Lumpectomy 380, and Others 50). Axillary lymph node dissection was performed in 704 (88.1%) patients. Pathologic complete response (pCR, pT0/isN0) was achieved in 129 (16.1%) of 799 patients (HR+/HER2-, 34/373 [9.1%]; HER2+, 45/210 [21.4%]; TNBC 50/216 [23.1%]). The mean numbers of involved LN and retrieved LN were 2.70 (range 0-42) and 13.98 (range 1-64), respectively. The mean LNR was 0.17 (Low, 574 [71.8%]; Intermediate, 170 [21.3%]; High, 55 [6.9%]). In univariate analysis, LNR was significantly associated with worse relapse-free survival (3-yr RFS rate 84.8% in low vs. 66.2% in intermediate vs. 54.3% in high; P <0.0001, log-rank test). In multivariate analysis, LNR was not significantly associated with recurrence after adjustment of other clinical factors (Age, histologic grade, intrinsic subtype, ypT-stage, ypN-stage, lymphatic or vascular invasion, and pCR).

<table>
<thead>
<tr>
<th>Multivariate analysis for relapse-free survival</th>
<th>P-value</th>
<th>HR</th>
<th>95%CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGE (&lt;50, ≥50)</td>
<td>0.157</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ypT-stage</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ypN-stage</td>
<td>0.035</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pCR (pT0/isN0)</td>
<td>0.027</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
<td>0.040</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Subtype</td>
<td>&lt;0.0001</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Histologic grade</td>
<td>0.001</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>LNR Low (0-0.20)</td>
<td>0.954</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>LNR Intermediate (0.21-0.65)</td>
<td>0.973</td>
<td>1.01</td>
<td>0.55-1.86</td>
</tr>
<tr>
<td>LNR High (0.66-1.00)</td>
<td>0.797</td>
<td>1.12</td>
<td>0.48-2.59</td>
</tr>
</tbody>
</table>

Conclusion: LNR is not superior to ypN-stage in predicting clinical outcome of breast cancer after neoadjuvant chemotherapy.
Title: p16\textsuperscript{INK4a} expression and chemotherapy toxicity in women with early stage breast cancer

Hyman Muss\textsuperscript{1}, Allison Deal\textsuperscript{1}, Arti Hurria\textsuperscript{2}, Natalia Mitin\textsuperscript{1}, Chad Torrice\textsuperscript{1}, Krishnamurthy Janakiraman\textsuperscript{1}, Trevor Jolly\textsuperscript{1}, Grant Williams\textsuperscript{1}, Shani Alston\textsuperscript{1}, Jerard West\textsuperscript{1}, Laura Zavala\textsuperscript{2}, Vani Katheria\textsuperscript{2} and Norman Sharpless\textsuperscript{1}. \textsuperscript{1}UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC and \textsuperscript{2}City of Hope.

Body: Background: Increased expression of p16\textsuperscript{INK4a}, a molecular marker of aging, is a hallmark of increased cellular senescence in most mammalian tissues. In human peripheral blood T-lymphocytes, expression of p16\textsuperscript{INK4a} increases 10-fold between 20 and 80 years of age (Liu et al, Aging Cell, 2009). We hypothesized that higher "molecular age," as evidenced by increased T-cell expression of p16\textsuperscript{INK4a} at the time of initiation of breast cancer (BC) chemotherapy (CRx), predicts increased treatment-related toxicity.

Methods: Patients (pts) with early breast cancer scheduled to receive neoadjuvant (NA) or adjuvant (Adj) chemotherapy (CRx) had p16\textsuperscript{INK4a} evaluation performed prior to treatment. Expression of p16\textsuperscript{INK4a} mRNA in CD3+ T-lymphocytes was determined using TaqMan real time quantitative reverse transcription polymerase chain reaction. Grade 3 and 4 (G3/4) hematologic (H) and non-hematologic (NH) toxicities (NCI CTCAE version 4) were assessed during and within 4 weeks of completion of CRx. Wilcoxon Rank Sum tests compared p16\textsuperscript{INK4a} between groups.

Results: 93 pts with Stage I-III BC and complete toxicity data during and within 4 weeks after completion of CRx have been accrued. Median age (range) was 52 (25-76). 48 (52%) of pts were hormone receptor (HR) positive, 21 (23%) HER-2 positive, and 24 (26%) triple-negative. 39 (42%) received NA and 54 (58%) received Adj CRx. 57 pts (61%) received an anthracycline containing combination and 21 (23%) cyclophosphamide/docetaxel (TC). 85 pts (91%) received pegfilgrastim. Overall, 69 pts (74%) had a G3/4 toxicity during or \(\leq 4\) wks of CRx, 54% NH and 40% H. 22 pts (24%) were hospitalized for CRx related toxicity and 11 (12%) of these admissions were for neutropenic fever (NF). 23 (25%) reported G3/4 fatigue during CRx and had significantly higher p16\textsuperscript{INK4a} values at baseline than those without fatigue (\(p=0.03\)). There was no significant association of baseline p16\textsuperscript{INK4a} and other NH or H toxicity or hospitalization in this cohort.

Conclusion: In this small sample of pts treated with anthracycline and non-anthracycline containing NA and Adj CRx regimens, there was a significant association of baseline p16\textsuperscript{INK4a} and G3/4 fatigue. The heterogeneity of treatment and use of pegfilgrastim in almost all pts limits the power of this study to find significant relationships between p16\textsuperscript{INK4a} and other toxicities. The cohort for this study is being expanded to further explore p16\textsuperscript{INK4a} as a predictor for G3/4 toxicity.

Support: Breast Cancer Treatment Foundation, New York, NY; University Cancer Research Fund, University of North Carolina, Chapel Hill.
Title: Pre-surgical neutrophil-to-lymphocyte ratio (NLR) is a prognostic indicator of recurrence free and overall survival in breast cancer patients undergoing primary surgery

Derbrenn O Connor¹,², Mark L Griffin¹, Jenna S O'Sullivan², Sean Millar², Jo O'Keeffe¹, Brian R Bird¹,², Sandra Deady³ and Conleth G Murphy¹,². ¹Bon Secours Hospital, Cork, Ireland; ²University College, Cork, Ireland and ³National Cancer Registry of Ireland, Cork, Ireland.

Body: Background
There is growing evidence that elevated neutrophil-to-lymphocyte ratio (NLR) is an independent prognostic indicator associated with poor survival in various cancers including colon cancer, ovarian cancer, esophageal cancer and gastric cancer. Several studies in early breast cancer suggest that NLR at diagnosis may be an independent negative prognostic marker in this population also. The aim of the current study is to evaluate the association between blood NLR immediately prior to surgery and recurrence free and overall survival in breast cancer patients.

Methods
We performed a single institution, retrospective cohort study including all patients treated for invasive breast cancer amenable to primary surgery at our institution between 1st January 2006 and 31st December 2010. Clinical and pathologic details were collected from the patient medical records. Exclusion criteria included prior malignancy, chemotherapy receipt prior to surgery, recent corticosteroid use, systemic autoimmune conditions, recent significant cardiovascular illness, infection or inflammatory condition. NLR was calculated on the most recent complete blood count performed on the day of surgery or at the pre-surgical assessment. Eligible patients were divided into high (≥4) and low (<4) NLR groups.

Results
We identified 357 patients, of whom 223 met eligibility criteria for analysis. At a median follow-up of 55.8 months, 18 patients (8.1%) died and 32 (14.3%) experienced disease recurrence. Kaplan Meier survival curves revealed significantly inferior overall survival (log-rank p=0.003) and recurrence free survival (log-rank p=0.01) in the high NLR group. Univariate Cox proportional hazard regression demonstrated an increased risk of mortality and breast cancer recurrence with pre-treatment NLR ≥4, with hazard ratios of 5.49 (p=0.008, 95% CI 1.56 to 19.37) and 3.68 (p=0.016, 95% CI 1.28 to 10.58) respectively.

Conclusion
This study confirms pre-treatment NLR as a prognostic factor for breast recurrence and death among patients receiving curative surgery for early breast cancer. Strict inclusion criteria reduced the likelihood of confounding due to comorbidities which might affect NLR and be independently associated with poor outcomes. Our study supports the usefulness of NLR as a component of the prognostic assessment of early breast cancer patients.
Title: SA02 trial: Results of a genomics-based prospective cohort in node-positive early breast cancer with « good-prognosis signature » treated with adjuvant chemotherapy

Bertucci François1, Extra Jean-Marc1, Ferrero Jean-Marc2, Bachelot Thomas3, Autret Aurélie1, Boyer-Chammard Agnès1 and Viens Patrice1. 1Institut Paoli-Calmettes, Marseille, France; 2Centre Antoine Lacassagne, Nice, France and 3Centre Léon Bérard, Lyon, France.

Body: Background. Adjuvant chemotherapy (CT) for node-positive (N+) early breast cancer (EBC) is based upon an anthracycline-taxane combination. However, the benefit of taxane is limited to a small population, but associated to morbidity and financial costs, making crucial the identification of patients likely to benefit or not from anthracycline-based regimen without taxane. Using DNA microarrays to profile a retrospective series of 498 patients (pts) treated with adjuvant anthracycline-based CT without taxane, we had identified and validated a gene expression signature (GES) associated with metastatic relapse. The corresponding Relapse Score (RS) sorted the patients (pts) in two groups: the "good-prognosis" group (75% of pts) with a 5-year metastasis-free survival (MFS) of 82%, and the "poor-prognosis" group (25% of pts) with a 56% 5-year MFS. We present here the results of a prospective multicentric national cohort of 175 pts, SA02, initiated to analyze anthracycline-based adjuvant CT without taxane in N+ EBC pts with a "good-prognosis" RS, with the aim of confirming their good prognosis in term of 5-year MFS.

Methods. Women with surgical EBC were screened for inclusion in 4 French hospitals. After diagnosis of lymph node involvement, frozen tumor samples were used for hybridization on Affymetrix U133 Plus 2.0 microarrays, and the RS was defined. RS-based "good-prognosis" pts were treated in the SA02 cohort and received 6 FEC100 cycles (Fluoro-uracile 500 mg/m², Epirubicin 100 mg/m², Cyclophosphamide 500 mg/m², every 21 days), followed by adjuvant radiotherapy, hormone therapy and/or trastuzumab according to standard guidelines. Pts with a "poor-prognosis" or non-evaluable RS were not included in the cohort. We present here an analysis with a median follow-up of 50 months.

Results: Between May 2007 and May 2010, samples from 175 eligible N+ EBC pts were collected for gene profiling. The profiling failed for 54 samples (31%), due to insufficient RNA amount, poor RNA quality, poor labeling performance, or forgotten. 102 pts (84%) were defined as "good-prognosis", while only 5 pts (4%) were defined as "poor-prognosis", and 14 pts (12%) could not be assigned a prognostic group. The percentage of "good-prognosis" RS was higher than observed in the initial retrospective series. On the 102 "good-prognosis", 88 were included in the SA02 cohort to receive 6 FEC100 cycles, 14 pts were not included in the cohort due to investigator or patient’s decision. The mean time from the date of surgery to the onset of chemotherapy was 5.4 +/-1.5 weeks. With a median follow-up of 50 months, the 2-year and 4-year MFS are 98% (95% CI 91-99) and 96% (95% CI 89-99). The 2-year and 4-year overall survival are 100% and 99% (95% CI 92-100).

Conclusion. This analysis confirms that genomic analyses are feasible in clinical practice. The MFS results with a median follow-up of 50 months are within the expected hypothesis; follow-up is needed to confirm or not these results at 5 years. Final follow-up data will be available in 2015.
Title: Cardiorespiratory fitness (VO$_{2\text{max}}$) before, during and after adjuvant treatment in breast cancer patients

Hanne Frydenberg$^1$, Tora J Bettum$^1$, Trygve Lofterød$^1$, Elisabeth Edvardsen$^{2,3}$, Vidar G Flote$^1$, Sissi E Finstad$^4$, Gro F Bertheussen$^5$, Ellen Schlichting$^6$, Anne McTiernan$^7$ and Inger Thune$^{1,8}$. $^1$Cancer Center, Oslo University Hospital, Oslo, Norway; $^2$Norwegian School of Sport Sciences, Oslo, Norway; $^3$Oslo University Hospital, Oslo, Norway; $^4$Norwegian Directorate of Health, Oslo, Norway; $^5$St Olav University Hospital of Trondheim, Trondheim, Norway; $^6$Cancer Center, Section for breast Surgery, Oslo University Hospital, Oslo, Norway; $^7$Fred Hutchinson Cancer Research Center, Seattle, WA and $^8$Institute of Community Medicine, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway.

Body: Background: Breast cancer treatment may result in reduced exercise capacity that may in turn lead to reduced maximum oxygen consumption (VO$_{2\text{max}}$). However, whether physical exercise can counteract any observed decline in VO$_{2\text{max}}$ in breast cancer patients undergoing adjuvant breast cancer treatment, is less known.

Material & methods: The women participating in the Norwegian Energy Balance and Breast Cancer Aspect (EBBA)-II pilot study, were aged 35-75 years and diagnosed with stage I-II breast cancer. Performing a maximum exercise test on a treadmill (modified Balke protocol), VO$_{2\text{max}}$ was assessed at four times; preoperative, 6, 12 and 24 months postoperative. The patients were randomized postoperative to a control group (n=31) or an intervention group (n=29) stratified by menopausal status. The 12 months exercise intervention program consisted of group-based exercise, 60 minutes twice a week and a minimum of 60 minutes of individual exercise. Regression models were used to study the associations between treatment regime and VO$_{2\text{max}}$.

Results: Breast cancer patients (n=60) with a mean age at diagnosis of 55.3 years (38.0-69.0 years), had a mean body mass index of 25.1 kg/m2, and a mean preoperative VO$_{2\text{max}}$ of 32.4 ml/min/kg. Comparing the intervention group to the control group, the intervention group maintained VO$_{2\text{max}}$ throughout the treatment period, and improved their VO$_{2\text{max}}$ with 7.8 % from 12 to 24 months postoperative ($p=0.117$), while the control group had a 15% reduction in VO$_{2\text{max}}$ 6 months after surgery ($p<0.001$), which improved 14 % at 12 months and additionally 6 % at 24 months postoperative ($p=0.025$). Among those patients receiving chemotherapy (60%), and being in the control group, a decline in VO$_{2\text{max}}$ of 22.9 % ($p<0.001$) at 6 months postoperative was observed. In comparison, patients in the intervention group who received chemotherapy had a 4.5 % reduction in VO$_{2\text{max}}$ at 6 months postoperative ($p = 0.159$). Thereafter, in the control group, VO$_{2\text{max}}$ improved with 21.6 % at 12 months postoperative ($p=0.006$), while in the intervention group VO$_{2\text{max}}$ improved with 13.4 % 24 months postoperative ($p=0.038$). Patients in the intervention group who did not receive any chemotherapy increased their VO$_{2\text{max}}$ by 6% 6 months postoperative ($p=0.174$), while patients in the control group who did not receive any chemotherapy had a reduction in VO$_{2\text{max}}$ of 2.1 % at 6 month postoperative ($p=0.630$).

Conclusion: Our findings suggest that systematic physical training may counteract a decline in VO$_{2\text{max}}$ in breast cancer patients receiving adjuvant treatment, including chemotherapy, and is of clinical interest, but needs to be replicated in larger studies.
**Title:** Surgery time interval and molecular subtype influence Ki67 change after core needle biopsy in breast cancer patients

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**Body:** Background: Core needle biopsy (CNB) is accuracy to evaluate estrogen receptor (ER), progesterone receptor (PR), and HER2 status in breast cancer, while concordance rate for Ki67 test between CNB and subsequent surgical removal samples (SRS) was relatively low. A comprehensive analysis was performed to find out whether some factors were associated with Ki67 value change.

Study design: A retrospective study was carried out on 276 patients with paired CNB samples and SRS. ER, PR, HER2, and Ki67 results were used to construct molecular subtypes. Ki67 ≥ 20% was defined as high expression and its value change was calculated as SRS minus CNB. Five subtypes were classified as follows: Luminal A, Luminal B-HER2-, Luminal B-HER2+, Triple negative (TN), and HER2+. Clinico-pathological factors as well as surgery time interval (STI) were collected. ANOVA analysis was used to analyze association between factors and Ki67 change.

Results: Mean STI after CNB was 4.5 (1-37) days. A good agreement was achieved for ER, PR, HER2, and molecular subtype evaluation between CNB and SRS. However, Ki67 expression level was much higher in SRS compared with CNB samples: 29.1% vs. 26.2% (P < 0.001). Both univariate and multivariate analysis demonstrated that STI and molecular subtype were associated with Ki67 change. Breast cancer patients with longer STI had a higher Ki67 increase: -1.1% within 1-2 days, 2.1% with 3-4 days, and 5.6% more than 4 days, respectively (P = 0.007).

### Ki67 expression and change value at CNB and SRS in different surgery time intervals

<table>
<thead>
<tr>
<th></th>
<th>No.</th>
<th>Median Ki67% (IQR)</th>
<th>Mean Ki67%(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All population</strong></td>
<td>276</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNB</td>
<td>20 (10, 40)</td>
<td>26.2 (22.0)</td>
<td></td>
</tr>
<tr>
<td>SRS</td>
<td>25 (10, 40)</td>
<td>29.1 (22.0)</td>
<td></td>
</tr>
<tr>
<td>Ki67 change*</td>
<td>0 (-4.5, 10)</td>
<td>2.9 (13.2)</td>
<td></td>
</tr>
<tr>
<td><strong>1-2 days</strong></td>
<td>55</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNB</td>
<td>20 (10, 40)</td>
<td>27.7 (21.0)</td>
<td></td>
</tr>
<tr>
<td>SRS</td>
<td>20 (10, 40)</td>
<td>26.6 (19.1)</td>
<td></td>
</tr>
<tr>
<td>Ki67 change*</td>
<td>0 (-10, 5)</td>
<td>-1.1 (11.0)</td>
<td></td>
</tr>
<tr>
<td><strong>3-4 days</strong></td>
<td>113</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNB</td>
<td>20 (10, 50)</td>
<td>28.4 (24.6)</td>
<td></td>
</tr>
<tr>
<td>SRS</td>
<td>25 (10, 50)</td>
<td>30.5 (23.0)</td>
<td></td>
</tr>
<tr>
<td>Ki67 change*</td>
<td>0 (-5, 10)</td>
<td>2.1 (15.2)</td>
<td></td>
</tr>
<tr>
<td><strong>≥ 5 days</strong></td>
<td>108</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNB</td>
<td>17.5 (10, 30)</td>
<td>23.2 (20.8)</td>
<td></td>
</tr>
<tr>
<td>SRS</td>
<td>25 (10, 40)</td>
<td>28.8 (22.4)</td>
<td></td>
</tr>
<tr>
<td>Ki67 change*</td>
<td>5 (0, 10)</td>
<td>5.6 (11.4)</td>
<td></td>
</tr>
</tbody>
</table>

*Ki67 change, SRS minus CNB; Abbreviation: CNB, core needle biopsy; IQR, inter quartile range; SRS, surgical removal samples; SD, standard deviation.

Luminal A tumors experienced more Ki67 elevation than Luminal B-HER2- diseases (5.2% vs 1.5%, P = 0.007). For TN or
HER2+ patients, Ki67 change was apt to be 0 with STI $\leq$ 4 days, while more than 7% absolute Ki67 increase was noticed in patients with STI $\geq$ 5 days.

Conclusions: STI and molecular subtype are significantly associated with Ki67 change after CNB in breast cancer patients. TN and HER2+ patients seem to experience a high Ki67 increase with prolonged STI, which warrants further study to validate this change.
Title: Influence of lifestyle factors and tumor cell dissemination in 632 early breast cancer patients

Bahriye Aktas¹, Anna Frackenpohl¹, Siegfried Hauch², Johann Kraus³, Hans Armin Kestler³, Rainer Klaus Kimmig¹ and Sabine Kasimir-Bauer¹. ¹University Hospital, Essen, Germany; ²AdnaGen AG, Langenhagen, Germany and ³University Ulm, Ulm, Germany.

Body: Introduction: Influence of lifestyle behaviour in risk of developing breast cancer is supported by several lines. Data from 632 early breast cancer (EBC) patients were collected to evaluate the influence of lifestyle factors in progression free survival (PFS) and overall survival (OS). Results of disseminated tumor cell (DTC) in bone marrow and circulating tumor cells (CTC) in blood were available as well. A complete pathological data set and medical history were obtained. It was the purpose of the present study to correlate these data to compare the findings.

Methods: We evaluated 629 bone marrow samples and 606 blood samples from EBC patients treated between 2004 to 2010 at the time of first diagnosis. All samples underwent immunomagnetic enrichment using the AdnaTest BreastCancerSelect (AdnaGen AG, Germany) within 4 hours after blood withdrawal followed by RNA isolation and subsequent gene expression analysis by reverse transcription and Multiplex-PCR in separated tumor cells using the AdnaTest BreastCancerDetect. CTCs were analyzed for the three breast cancer associated markers: GA733-2, Muc-1, Her-2 and actin as an internal PCR control. BM aspirates were analyzed for DTCs by immunocytochemistry using the pan-cytokeratin antibody A45-B/B3. Lifestyle data including menopausal status, BMI, usage of Metformin, hormone replacement drugs, beta blockers and Bisphosphonates were collected by accessing the patient files. Histological data of the primary tumor were available for each patient.

Results: The overall detection rate for CTCs was 15.41% (88/571 patients) and for DTC was 38.5% (242/628 patients), respectively. The mean BMI of 428 patients was 26.4 in 81/574 premenopausal, 426/574 postmenopausal and 67/574 perimenopausal patients. Medical history of smoking (128/394 patients), using hormone replacement therapy (92/354 patients), alcohol consumption (68/378 patients), having allergies (188/419), using Metformin (22/389 patients), taking beta blockers (83/392 patients) and Bisphosphonates (201/526 patients) were compared to PFS and OS as well as the histological data of the primary tumor.

Conclusion: Lifestyle factors seems to influence the outcome in our cohort of EBC patients as shown in previous studies. Final data and results regarding to tumor cell dissemination compared to lifestyle behaviour will be available for the SABCS 2014.
Survival of metastatic hormone receptor (HR) positive/HER2 negative; HER2+; and triple negative (TN) breast cancer based on initial presentation

Wendie-Lou D den Brok¹, Caroline Speers¹, Gondara Lovedeep¹, Emily Baxter¹, Scott Tyldesley¹ and Lohrisch Caroline¹. ¹British Columbia Cancer Agency (BCCA), Vancouver, BC, Canada.

Body: Introduction: Median overall survival (OS) for patients (pts) with metastatic breast cancer (MBC) is described as 2-3 years, but few reports compare OS for de novo versus relapsed disease. The recognized clinical subtypes of breast cancer (HR positive[+] /HER2 negative; HER2+; TN) are known to have variable OS clinically, but this has not been systematically documented. We hypothesized that pts relapsing from a non MBC stage (relapsed) might have different OS than those presenting initially with metastases (de novo), and that OS would differ for the three clinical biomarker subgroups.

Methods: Using the Breast Cancer Outcomes Unit database, we identified all women diagnosed with MBC, de novo or relapsed, in British Columbia between 01/2001 and 12/2009 and referred to the BC Cancer Agency. Review of medical records confirmed ER, PR and HER2 status. Survival from MBC diagnosis was calculated for relapsed vs de novo in the three biomarker subgroups.

Results: After excluding pts with a synchronous or prior contralateral disease, we identified 3645 women with known ER. Median follow up was 91 months. HER2 known (n=3010) and unknown (n=635, 17%) cases had the same median OS (17 months [m]) and HR status (72% ER+). Trastuzumab (T) was standard in MBC during this era, but fewer than 10% had adjuvant T (introduced mid 2005) which explains the high number of HER2+ MBC cases. We previously reported longer OS for HER2+ disease relapsing after adjuvant therapy without T (older cohort) than after adjuvant T (SABCS 2013 Lohrisch). HER2 unknown pts were excluded from further survival analyses. Three percent of cases with known PR were ER negative/PR +. Therefore ER was used as the main determinant of HR status.

For the entire cohort and all biomarker groups, OS was longer for de novo than for relapsed MBC.

Overall Survival - relapsed vs de novo MBC for each biomarker subtype

<table>
<thead>
<tr>
<th>Subgroups</th>
<th>Relapsed MBC</th>
<th>De novo MBC</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N dead/total</td>
<td>Median OS, m</td>
<td>N dead/total</td>
</tr>
<tr>
<td>All cases with known ER</td>
<td>2124/2311</td>
<td>15</td>
<td>593/699</td>
</tr>
<tr>
<td>HR+/ HER2 neg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>821/912</td>
<td>24</td>
<td>290/364</td>
</tr>
<tr>
<td>PR known</td>
<td>414/477</td>
<td>20</td>
<td>251/319</td>
</tr>
<tr>
<td>PR unknown</td>
<td>407/435</td>
<td>28</td>
<td>39/45</td>
</tr>
<tr>
<td>TNBC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>368/391</td>
<td>9</td>
<td>110/126</td>
</tr>
<tr>
<td>HER2+</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All</td>
<td>935/1008</td>
<td>11</td>
<td>193/209</td>
</tr>
<tr>
<td>ER neg</td>
<td>268/278</td>
<td>7</td>
<td>58/60</td>
</tr>
<tr>
<td>ER+</td>
<td>667/730</td>
<td>15</td>
<td>135/149</td>
</tr>
</tbody>
</table>

OS was longest for denovo HR+/HER2 negative (34m) and shortest for HER2+/ER negative (7m) and TN (9m) relapsers. The difference in OS for relapsed vs denovo HER2+ disease was significant for HR+ but not HR negative cases, likely due to small numbers in the latter subset.
Conclusion: Relapsers experience shorter OS than their denovo biomarker counterparts, possibly due to the selective pressure of adjuvant therapy on disease biology. When restricted to pts who received systemic therapy for MBC, OS figures may be higher. Novel therapies may decrease the total number of relapers, and improve OS in MBC for all, but are unlikely to narrow the OS gap between relapsed and denovo groups. Trials exploring therapies for MBC of all biomarker types should therefore stratify by stage at initial diagnosis.
Title: Distribution patterns of mammographic calcification can predict outcome in women with breast cancer treated with breast conserving surgery

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Body: Background. The impact of calcification in patients with breast carcinoma treated with breast-conserving surgery (BCS) is unclear. We sought to determine the short and long-term outcomes of patients with calcification treated by BCS.

Methods. The records of 409 patients with breast carcinoma treated with BCS from 2004 through 2008 were reviewed. The results of mammograms and breast untrasound (BUS) tests were available for all the patients. Patients were classified as those without calcification on both mammograms and BUS, those with calcification on mammograms, and those with calcification on BUS but not on mammograms; the latter two groups were called collectively as patients with calcification. Survival analyses were performed with respect to morphologic types and distribution patterns of calcification. Median follow-up was 85 months.

Results. Pathologic characteristics of patients with or without calcification were not statistically different with respect to tumor size, histologic grade, regional lymph node metastasis, margin status, hormonal receptor expression, and Her-2 status. Survival analyses found that patients with calcification had significantly higher risk of local recurrence, distant metastasis, and breast cancer-associated death after BCS than those without calcification (relative risk [RR] and 95% CI; 2.46, 1.11-5.44; 2.24, 1.19-4.24; 2.50, 1.06-5.86, respectively). Subgroup analyses according to morphologic types of calcification revealed that local relapse free survival(LRFS), disease free survival (DFS), and overall survival were significantly lower in patients with microcalcification and pleomorphic calcification than in patients with large/coarse calcification, BUS calcification, and those without calcification. Further survival analyses were performed by the distribution patterns of calcification. Results showed that patients with calcification of liner and segmental distribution, or calcification spreading along the ducts, had significantly lower LRFS (RR = 6.20, 95% CI, 2.26-16.98), DFS(RR = 6.81, 95% CI, 2.86-16.20), and OS (RR = 9.14, 95% CI, 2.53-33.00), compared with those without calcification. Patients with mammographic calcification of clustered distribution also showed trends of lower LRFS, DFS, and OS (P > 0.05). Tumors of patients with calcification of liner/segmental distribution were more often accompanied with extensive intraductal component (EIC), compared with tumors of patients without calcification, with BUS calcification, and with calcification of clustered distribution (P < 0.001). The rates of local recurrence and breast cancer-associated death were significantly higher in patients with EIC than in those without EIC. The rates of isolated distant metastasis in patients with or without EIC were not statistically different (P = 0.12).

Conclusion. Patients with calcification, especially those with calcification spreading along the ducts, have higher risk of local failure after BCS, which have negative impacts on long-term survival. Calcification found in BUS tests does not influence the short and long-outcome of patients treated with BCS. Existence of EIC is a predictive factor of local failure in patients with calcification treated with BCS.
Title: Association of metabolic syndrome, its components and multigene assays for recurrence risk

Hanh P Mai¹, Stephanie Kliethermes¹, Shikha Jain¹, Shelly S Lo¹, Ellen R Gaynor¹, Kathy S Albain¹ and Patricia Robinson¹.
¹Loyola University Medical Center, Maywood, IL.

Body: Background:
There is an association of metabolic syndrome (MS) and its constituents (obesity; diabetes mellitus, DM; hypertension, HTN; hyperlipidemia, HL) with breast cancer (BC) causation and outcomes. Previously we showed an impact of obesity on tumor biology as defined by a multigene assay. Our objective was to study the association between MS, its components and tumor biology, as determined by the 70 gene signature (70-GS) and the 21-gene Recurrence Score (RS).

Methods:
Consecutive patients with newly diagnosed ER+, lymph node-negative BC from 2005-2012 were studied. A 70-GS was done for those pts with tumors that had either low or intermediate RS. Pearson’s Chi-square tests for univariate analyses and logistic regression for multivariate analysis were used.

Results:
Low or intermediate RS were found on tumors from 151 pts of which 133 had a 70-GS. The MS was present in 23/104 (22%) pts with intermediate RS and 21/46 (46%) pts with low RS (p=0.004). DM, HTN, and obesity were each inversely associated with 21-gene RS in univariate analyses (p=0.002, p=0.003, p=0.004 respectively, see Table). However, MS and its individual components were not significantly associated with the 70-GS. Upon adjustment for age and race, the association between MS and RS was not significant (OR=0.64; p=0.28); however, DM (OR=0.35; p=0.01), HTN (OR=0.44; p=0.05) and obesity (OR=0.35; p=0.01) remained significantly inversely associated with RS. Independent of age and race, patients with DM, HTN, or obesity were more likely to be in the low-risk 21-gene RS group.

Table

<table>
<thead>
<tr>
<th>Metabolic Syndrome</th>
<th>High Risk 70-GS</th>
<th>Low Risk 70-GS</th>
<th>p-value</th>
<th>Intermediate 21-gene RS</th>
<th>Low 21-gene RS</th>
<th>p-value</th>
</tr>
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<td>54(51%)</td>
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Conclusions and Implications:
The association of MS and its components differs for the 70-GS and RS assays. There is significant association between MS (and DM, HTN, obesity) and RS, but no association with 70-GS. Patients with MS (and components) are more likely to have a biology (low RS) known to have less chemotherapy benefit. Given the reported impact of MS on BC incidence and outcomes
along with the global increased MS incidence, the MS may have profound impact on BC biology and treatment outcomes in the coming years. It is also possible that multigene assays currently in use for prognosis and prediction may need refinement in the presence of MS and/or its components.
Histological grade provides significant prognostic information in the discrimination between luminal A-like and luminal B-like HER-2 normal subtypes of breast cancer according to St Gallen 2013

Anna Ehinger¹,², Per Malmström¹,³, Pär-Ola Bendahl¹, Christopher W Elston⁴, Anna-Karin Falck¹,⁵, Carina Forsare¹, Dorthe Grabau¹,⁶, Lisa Rydén¹,⁷, Olle Stål⁸ and Mårten Fernö¹. ¹Division of Oncology and Pathology, Lund Cancer Center at Medicin Village, Lund University, Lund, Sweden; ²Blekinge County Hospital, Karlskrona, Blekinge, Sweden; ³Skåne University Hospital, Lund, Sweden; ⁴Nottingham University Hospitals NHS Trust, Nottingham, United Kingdom; ⁵Helsingborg Hospital, Helsingborg, Sweden; ⁶Skåne University Hospital, Lund, Sweden; ⁷Skåne University Hospital, Lund, Sweden and ⁸Linköping University, Faculty of Health Sciences, Linköping, Sweden.

Objective: According to St Gallen recommendations from 2013, estrogen receptor (ER), progesterone receptor (PR), HER-2, and Ki-67 defines two subtypes of ER-positive and HER-2 normal breast cancer (BC): Luminal A-like and Luminal B-like. Patients with Luminal B-like BC are often recommended chemotherapy in addition to endocrine therapy, whereas endocrine therapy may be sufficient for patients with Luminal A-like BC. Histological grade (G) 1, 2 and 3 are not included in the St Gallen recommendations. Our unpublished data from a series of 161 premenopausal N0 BC patients with long-term follow-up show that the classification of Luminal A-like vs Luminal B-like HER-2 normal BC is strongly associated to G. Luminal A-like BC is often G1 or G2 and Luminal B-like BC is usually G2 or G3. We also found that the few G3 (n=6) Luminal A-like cases had a prognosis more similar to Luminal B-like and that the few G1 (n=2) Luminal B-like HER-2 normal cases had a prognosis more similar to Luminal A-like. The aim of this study is to evaluate in other cohorts if these findings can be confirmed.

Methods: G and St Gallen subtypes were evaluated in three BC cohorts from altogether 547 pre- and postmenopausal chemotherapy naïve T1-2N0-N1M0 patients. The endpoint was distant disease-free survival with 10 years of follow-up. We compared the Luminal A-like and the Luminal B-like HER-2 normal subtype definition according to the original St Gallen recommendation from 2013 based on ER, PR, HER-2, and Ki-67 with our proposal, where ER-positive, HER-2 normal, G1 BC is defined as Luminal A-like, independent of Ki-67 and PR, and ER-positive, G3 BC is defined as Luminal B-like, independent of Ki-67 and PR (St Gallen 2013+G). The importance of Ki-67 and PR for subtyping was thus restricted to G2 BC.

Results: The hazard ratio (HR) between Luminal B-like HER-2 normal (n=185) and Luminal A-like (n=362) defined according to St Gallen 2013+G, was 2.3 (95% confidence interval (CI) 1.6-3.4; p<0.0001) compared to 1.6 (95% CI: 1.1-2.4; p=0.025) according to St Gallen 2013. Twenty-five patients, classified as Luminal B-like HER-2 normal with St Gallen 2013 were G1 and consequently reclassified as Luminal A-like with St Gallen 2013+G. None of these twenty-five patients developed metastases during the follow-up period. Thirty-eight patients showed the opposite pattern (G3 Luminal A-like with St Gallen but Luminal B-like according to St Gallen 2013+G). Seventeen of these patients developed distant metastases already during the first five years.

Conclusion: Our findings strongly suggest that ER-positive, HER-2 normal, and G1 BC is a good prognosis group, independent of Ki-67 and PR, and should be treated as Luminal A-like BC, whereas ER-positive, HER-2 normal, and G3 BC should be considered as a worse prognosis group, independent of Ki-67 and PR, and should be treated as Luminal B-like BC. Based on our findings the importance of Ki-67 and PR is restricted to G2 BC for the discrimination between Luminal A-like and Luminal B-like HER-2 normal subtypes of BC.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-08-44
Average Grade: 4.00

Title: Impact of the immunohistochemical subtypes of breast cancer on prediction of axillary metastasis: Experience of one breast center in Argentina

Maria F Calvo¹, Carola Allemand¹, Francisco H Corrao¹, Roberto Orti¹, Liliana B Zamora¹, Maria C Riggi¹, Maria F Ilzarbe¹, Jorge Piccolini¹, Alejandra Wernicke¹, Sebastian Gogorza¹, Gustavo Izbizky¹ and Claudio Lorusso¹. ¹Hospital Italiano, Buenos Aires, Argentina.

Body:
INTRODUCTION
Axillary node metastasis is one of the most important prognostic factors to be considered in the treatment of Breast Cancer. Although the association between axillary metastasis and pathologic tumor size has been extensively studied, the correlation between the immunohistochemical (IHC) subtype and axillary compromise has not. The aim of this study was to evaluate the correlation between the immunohistochemical subtype of Breast Cancer (BC) and axillary extension. As secondary outcomes, we assessed disease-free (DFS) and overall survival (OS).

MATERIALS AND METHODS
1413 consecutive patients who underwent surgery for invasive primary breast cancer at the Hospital Italiano de Buenos Aires between the years of 2007 and 2012 were included. Patients presenting with stage IV disease were excluded. We analyzed the clinical and pathologic data of patients who were submitted to either sentinel node biopsy (SNB) or axillary lymph node dissection (ALND). Based on IHC, tumors were classified into four groups: Luminal A (RE+ RP+ HER2-; KI 67 <14%), Luminal B (RE+ RP+, HER2+; KI 67 >15%), HER2 (RE- RP- HER+) and Triple Negative (TNBC) breast cancer (RE- RP- HER-).

RESULTS
We evaluated 1413 patients, among which 1248 patients were eligible for inclusion and analysis. In this population, 386 patients (31%) had axillary metastasis. By considering the Luminal A subtype population as our control group, we found that axillary metastasis was significantly increased in the Luminal B and HER2 positive subtypes (p<0.0001), but not in the TNBC subtype (p=0.4468). When adjusted by tumor size and IHC, in tumors smaller than 2 cm (pT1), the Luminal B and TN subtypes significantly increase the risk of node metastasis with an OR 2.73 (CI95% 1.73 - 4.31, P > 0.000), and OR 2.05 (CI95% 1.13 - 3.70, P=0.017) respectively. In the case of HER2 positive tumors, the odds ratio for axillary extension was 6.62 (CI95% 3.02 - 14.50, P > 0.000). The median follow-up was 29 months (17- 44 months), and the overall survival estimated by Kaplan-Meier was 91% (CI95% 87-94), with a disease-free survival of 62% (CI95% 28-83).

DISCUSSION
In this cohort, immunohistochemical subtype was an important independent predictor of axillary metastasis. Tumors smaller than 2 cm, that overexpress HER2 in absence of estrogen and progesterone receptor, have up to six times greater incidence of axillary extension than those belonging to the Luminal A subtype. Luminal B and Triple Negative cancers on the other hand appear to present twice the risk when compared to the Luminal A subtype.
Body: Background
Staging of axillary lymph nodes in invasive breast cancer is an important prognostic indicator. Various prediction models have been developed to predict the risk of not having additional axillary metastases in patients with a positive sentinel node, thereby disregarding patients with a positive ultrasound. However, it is important to identify all patients with extensive nodal involvement, defined as 3 or more positive axillary lymph nodes, in whom an axillary lymph node dissection cannot be omitted.

Aim
This study aims to identify factors predicting extensive nodal involvement in the axilla, with the emphasis on the method of axillary staging; ultrasound guided lymph node biopsy versus sentinel node procedure.

Methods
All patients diagnosed with invasive breast cancer in the period between January 2006 and December 2011 at the breast center of the Máxima Medical Center were studied. Univariate and multivariate regression analyses were performed. Variables with a p-value of ≤0.10 in univariate analyses were entered in the multivariate model where a p-value of ≤0.05 was considered statistically significant.

Results
We included 307 cases, representing 306 node positive patients, of whom 178 cases had 1 or 2 positive lymph nodes and 129 cases had 3 or more positive lymph nodes. Multivariate analyses showed that factors as a positive axillary ultrasound (OR=4.513; 95%CI=2.30-8.86), palpability of axillary lymph nodes (OR=2.143; 95%CI=1.04-4.42) and lymphovascular invasion (OR=3.622; 95%CI=1.63-7.81) are significantly associated with extensive nodal involvement in patients with invasive breast cancer.

Conclusion
This study has identified clinically important factors predicting extensive nodal involvement in patients with a positive lymph node biopsy by either a sentinel lymph node procedure or an ultrasound guided lymph node biopsy. Hence, the role of axillary staging by ultrasound should be redefined since it might play an important role in selecting patients with extensive nodal involvement who, in our opinion, may still benefit from axillary treatment.
**Title:** Clinical outcomes of breast cancer patients with intermediate oncotype DX recurrence scores (RS): A review of the Cleveland Clinic Experience

Lindsey M Goodman, Alberto J Montero, Lisa Rybicki, Karen Mrazeck, Benjamin Calhoun, Raymond Tubbs and Halle Moore. 'Cleveland Clinic Foundation, Cleveland, OH.

**Body:**

**Background:**

The 21 gene RT-PCR assay (Oncotype Dx, Genomic Health, Inc, Redwood City, CA) performed on formalin-fixed paraffin-embedded tissue quantifies the likelihood of distant recurrence in patients with estrogen receptor (ER)-positive, axillary lymph node-negative breast cancer. The 21 gene recurrence score (RS) also quantifies the magnitude of clinical benefit of adjuvant cytotoxic chemotherapy to endocrine therapy. Patients with a high risk RS clearly benefit from the addition of adjuvant chemotherapy, while patients with a low RS do not. However, approximately one-third of patients have an intermediate RS (18-31) and the incremental benefit from adjuvant chemotherapy is unclear. The primary objective of this study was to evaluate the clinical outcomes of early stage breast cancer patients with intermediate RS.

**Methods:**

We identified 262 patients with intermediate RS (18-31) in a prospectively maintained registry of patients with Oncotype Dx testing at the Cleveland Clinic from 2004-2013. ER status, progesterone receptor (PR) status, HER2 gene amplification, treatment with chemotherapy, follow-up for distant recurrences and other clinical and pathological variables were collected for all patients in the registry.

**Results:**

Patient characteristics are listed in Table 1. One hundred patients (38.2%) were treated with chemotherapy plus endocrine therapy, while 156 (59.5%) were treated with endocrine therapy alone. Treatment data was unavailable for 6 patients (2%). Distant recurrence status is unknown for 2 patients. The overall rate of distant plus local recurrence for all 262 patients was 3.8% (3 local and 7 distant recurrences). There were 6 and 4 recurrences respectively, for patients received chemotherapy plus endocrine therapy (6%), and in patients treated with endocrine therapy alone (2.5%), which was not significantly different (p=.21).

**Table 1. Patient Characteristics**

<table>
<thead>
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<th>Characteristic</th>
<th>Value</th>
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<tbody>
<tr>
<td>Median age at diagnosis (Range)</td>
<td>58 (27-88)</td>
</tr>
<tr>
<td>Median RS (Range)</td>
<td>22 (18-31)</td>
</tr>
<tr>
<td>ER+ (%)</td>
<td>262 (99.6)</td>
</tr>
<tr>
<td>PR+ (%)</td>
<td>234 (89)</td>
</tr>
<tr>
<td>HER-2 amplified (%)</td>
<td>10 (3.8)</td>
</tr>
<tr>
<td>Grade (%)</td>
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<tr>
<td>1</td>
<td>84 (32.8)</td>
</tr>
<tr>
<td>2</td>
<td>136 (53.1)</td>
</tr>
<tr>
<td>3</td>
<td>36 (14.1)</td>
</tr>
<tr>
<td>Histology (%)</td>
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<tr>
<td>Ductal</td>
<td>187 (71.1%)</td>
</tr>
<tr>
<td>Lobular</td>
<td>45 (17.1%)</td>
</tr>
<tr>
<td>Mixed</td>
<td>31 (11.8%)</td>
</tr>
<tr>
<td>Median Tumor size (range)</td>
<td>1.6 cm (0.2-6.5)</td>
</tr>
<tr>
<td>Lymph Node Status (%)</td>
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<tr>
<td>pN0</td>
<td>238 (90.8)</td>
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<tr>
<td>1-3 positive nodes</td>
<td>24 (9.2)</td>
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</table>
Conclusions:
With a median follow-up of 45 months, our data indicate that the realized distant recurrence rate for patients with an intermediate range RS was less than 5%. These data do not exclude the possibility that some patients with an intermediate RS may derive a small incremental benefit from the addition of adjuvant chemotherapy. Larger, randomized prospective trials like TAILORx should provide additional guidance for the management of this patient population.
Androgen receptor expression in pre-menopausal early breast cancer patients treated with endocrine therapy within the ABCSG-12 trial - a single center pilot analysis

Gabriel Rinnerthaler¹, Anna M Knopp², Cornelia Hauser-Kronberger², Simon P Gampenrieder¹, Patrick Morre¹, Brigitte Mlineritsch¹, Christian Fesl³, Michael Gnant⁴ and Richard Greil¹. ¹Salzburg Cancer Research Institute with Laboratory of Immunological and Molecular Cancer Research and Center for Clinical Cancer and Immunology Trials, Paracelsus Medical University, Salzburg, Austria; ²Paracelsus Medical University, Salzburg, Austria; ³Austrian Breast & Colorectal Cancer Study Group, Vienna, Austria and ⁴Breast Health Center, Comprehensive Cancer Center, Medical University of Vienna, Vienna, Austria.

Body: Background: Estrogen-receptor (ER) positive breast cancer co-expresses androgen receptor (AR) in 75% to 95% of cases. The Austrian Breast & Colorectal Cancer Study Group (ABCSG) trial 12 compared anastrozole plus goserelin with tamoxifen plus goserelin in premenopausal patients with hormone receptor positive early breast cancer. In addition, patients were randomized to receive zoledronic acid or not. The aim of this analysis was to investigate the effect of AR expression on outcome in dependency of the treatment arm.

Patients and methods: AR expression was analyzed by immunohistochemistry using a mouse monoclonal antibody (Novocastra) in formalin-fixed paraffin-embedded specimens from 194 early breast cancer patients treated within the ABCSG 12 trial at our institution. As there is no generally accepted cut-off level defining AR positivity, we used two definitions for this analysis: A) positive staining of ≥ 10% of tumor cells B) an immune reactive score (IRS; intensity of staining X percentage of stained cells) ≥ 3 according to the Remmele score used for ER/PR evaluation.

Results: A total of 194 patients were included in this analysis. In tissue samples collected before 2001 AR staining was very weak, interpreted as a loss of antigenicity due to archival time and conditions. Therefore, 40 patients with tissue samples achieved earlier than 2001 were excluded from further analysis. Seventy-nine percent (122 of 154) and 63% (97 of 154) of tumors were AR positive by definition A and B, respectively. All except one of the AR positive tumors according to definition B, were also positive according to definition A.

Disease free survival (DFS) and overall survival (OS) data

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<th>N (%)</th>
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<th>OS</th>
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<td><strong>Definition A (≥ 10%)</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>AR positive</td>
<td>122 (79)</td>
<td>HR 0.61 (0.21-1.72) P=0.3428</td>
<td>HR 0.17 (0.03-0.99) P=0.025</td>
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<td>AR negative</td>
<td>32 (21)</td>
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<tr>
<td><strong>Definition B (IRS ≥ 3)</strong></td>
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<tr>
<td>AR positive</td>
<td>97 (63)</td>
<td>HR 0.88 (0.33-2.35) P=0.7994</td>
<td>HR 0.39 (0.07-2.34) P=0.2846</td>
</tr>
<tr>
<td>AR negative</td>
<td>57 (37)</td>
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</table>

DFS and OS did not differ between both endocrine treatment arms in a Cox regression model tested for interaction between AR expression and endocrine treatment.

Conclusion: In this pilot study patients with AR positive disease had a numerically better DFS and OS compared to AR negative patients, but only prolongation of OS in patients with ≥ 10% AR positive tumor cells was statistically significant. AR expression did not influence outcome between tamoxifen and anastrozole treated patients, but based on the small number of events, this results have to be interpreted with caution. This data will be confirmed in a larger proportion of patients treated within the ABCSG-12 trial.
Neutrophil/lymphocyte ratio (NLR) can be one of the useful predictive prognostic markers for the metastatic breast cancer (MBC)

Takeshi Miyamoto¹, Tomomi Fujisawa¹, Akiko Morishita¹ and Yasuhiro Yanagita¹. ¹Gunma Prefectural Cancer Center, Ota, Gunma, Japan.

Body: Introduction: The possibility of tumor infiltrating lymphocytes as a predictive marker of the neoadjuvant chemotherapy for breast cancer was discussed at SABCS 2013. Cancer microenvironment formed by the immune and inflammatory cells is noticed to be one of the factors for tumor growth, invasion or metastasis. To figure out the macro inflammatory environment as an extension of the microenvironment, the neutrophil / lymphocyte ratio (NLR) is a useful method and a simple indicator of systemic inflammatory state. We have some reports that NLR can predict the prognosis in gastric cancer and colorectal cancer. But in breast cancer, few reports can be seen. We examined the relationship of NLR and the risk of recurrence in curable breast cancer in our hospital, and could not detect the specific relations. We hypothesized one of the reasons that resectable breast cancers are not in systemic phase in the view of inflammatory or immune reaction. Therefore, the next, in metastatic breast cancer (MBC), absolutely systemic disease, we tried to reveal the relationship of the NLR at the recurrence and the prognosis.

Purpose: To evaluate the NLR affects the Overall Survival (OS) of the patients of MBC or not.

Patients: From 2003 to 2013, we have 300 MBC patients in our hospital included 53 Stage4 patients at the first visit. Median Disease free survival (DFS) is 911-day, the median OS after the recurrence is 1196-day. Average value of the NLR is 2.85. The reasons of MBC are bone metastases, pleural and pulmonary metastases, liver metastasis, lymph node metastasis, central nervous system (CNS) metastasis, unresectable metastatic chest wall recurrence, or other.

Result: By univariate analysis, NLR $\geq$ 3.7(p<0.01), DFS >1000 days, (p<0.01), liver metastases, (p<0.05), CNS metastasis (p<0.01), and 2 or more organs metastasis (p<0.05) made a contribution to poor OS. Between these 5 factors, special relations were not seen. Other factors, stage4, organ metastasis except for liver and CNS, tumor subtype and the age at recurrence had no significant effects for OS. Out of these 5, not only DFS >1000(p<0.01) or CNS metastasis (p<0.01) but also NLR $\geq$3.7 (p<0.05) were the independent prognostic factors by using multivariate analysis.

Discussion: The NLR was one of the prognostic factors which we can easily and simply examine by blood sample. The reason of this fact, we suggest, is that the whole body micro-environment caused by the immune or inflammatory cells at MBC occurred contributes to tumor growth. For hormone receptor positive MBC without life threading organ metastasis, we select hormonal therapy first, usually. But, high NLR MBC patient have a possibility of selection for up-front chemotherapy even if without life threading metastasis. High Ki67-index as well as high NLR may be useful for the prognostic biomarker, however, make no decision for the chemotherapy agents. First, we need to accumulate further retrospective cases and plan the prospective study to make sure of the adequate treatment divided by NLR.

Conclusion: The NLR $\geq$3.7 is one of the independent predictive prognostic factors for MBC as well as DFS >1000 days and CNS metastasis.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P6-08-49  
**Average Grade:** 4.67

**Title:** Do traditional prognostic factors in early breast cancer still have a role in the molecular era?

Valentina Rossi¹, Paola Berchialla², Ivana Sarotto¹, Furio Maggiorotto¹, Nicoletta Tomasi Cont¹, Riccardo Ponzone¹, Massimo Aglietta¹ and Filippo Montemurro¹. ¹Candiolo Cancer Institute â–“ FPO, IRCCS, Italy and ²University of Turin, Italy.

**Body:**

**Background**

The combined immunohistochemical (IHC) analysis of the oestrogen receptor (ER), progesterone receptor (PgR), human epidermal growth factor receptor 2 (HER2) and proliferation index (Ki67) allows the approximation of the molecularly-defined breast cancer subtypes. As this classification is now increasingly being used to determine the adjuvant therapy choice in early breast cancer patients, the additional role of traditional prognostic factors in each distinct tumour subtype deserves further investigation.

**Methods**

A total of 1,202 women undergoing surgery for Early Breast Cancer (EBC) were selected for this retrospective analysis. Breast cancer subtypes were defined as luminal A, luminal B, HER2 luminal, HER2 enriched and Triple Negative [TN] by combining IHC. Kaplan-Meier survival analysis and log rank tests were used to determine Event Free Survival (EFS). Cox proportional hazard models were used to estimate hazard ratios, adjusted for potential confounders.

**Results**

A total of 345 tumours (29%) were luminal A, 558 (46%) luminal B, 107 (9%) HER2 luminal, 76 (6%) HER2 enriched and 116 (10%) TN. At a median follow-up of 58 months (4-137 months) relapse events occurred in 38 (11%) patients with luminal A, 102 (18%) with luminal B, 33 (31%) with HER2 luminal, 32 (42%) with HER2 enriched and 40 (35%) with TN tumours. Cox proportional hazard multivariable analysis identified the following independent associations with EFS in each subtype: \( \geq 4 \) positive axillary nodes (HR 4.96, 95% CI 1.97-12.48, \( p<0.001 \)), tumour grading=3 (HR 2.97, 95% CI 1.00-8.79, \( p<0.049 \)), adjuvant radiotherapy (HR 0.46, 95% CI 0.22-0.95, \( p<0.035 \)) in luminal A tumours; \( \geq 4 \) positive axillary nodes (HR 3.97, 95% CI 2.34-6.74, \( p<0.001 \)), PgR<20% (HR 1.55, 95% CI 1.04-2.29, \( p<0.03 \)), age at first diagnosis >35 years (HR 0.22, 95% CI 0.10-0.48, \( p<0.001 \)), adjuvant hormonotherapy (HR 0.43, 95% CI 0.22-0.85, \( p<0.01 \)), adjuvant radiotherapy (HR 0.57, 95% CI 0.36-0.91, \( p<0.02 \)) in luminal B tumours; \( \geq 4 \) positive axillary nodes (HR 5.22, 95% CI 1.91-14.28, \( p<0.001 \)) and adjuvant trastuzumab (HR 0.41, 95% CI 0.17-0.95, \( p<0.039 \)) in HER2 enriched, \( \geq 4 \) positive axillary nodes in HER2 luminal (HR 4.78, 95% CI 1.84-12.39, \( p<0.001 \)) and TN (HR 4.12, 95% CI 1.96-8.65, \( p<0.001 \)) tumours.

**Conclusion**

Conventional risk factors retain their independent value in a subtype-specific fashion in early breast cancer. Although growing importance is being given to stratification based on biological characteristics, the integration of traditional factors facilitates a better definition of risk categories in early breast cancer patients.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-08-50
Average Grade: 5.40

Title: Clinical predictors of long-term survival in Her2-positive (HER2+) metastatic breast cancer (MBC)

Pooja Murthy¹, Kelley M Kidwell¹, Dafydd G Thomas¹, Jennifer J Griggs¹, Sofia D Merajver¹, Anne F Schott¹, Jeffrey B Smerage¹, Catherine H Van Poznak¹, Max Wicha¹, Daniel F Hayes¹ and N Lynn Henry¹. ¹University of Michigan Medical School, Ann Arbor, MI.

Body: Introduction: Clinical observation suggests that a subset of patients with HER2+ MBC survive for prolonged periods when treated with Her2-targeting regimens. We hypothesized that we could identify clinical and pathological factors associated with prolonged survival.

Methods: An IRB approved, retrospective, single institution review of patients diagnosed with HER2+ MBC was performed. Patients treated with pertuzumab or ado-trastuzumab emtansine were not included because recent FDA approval did not permit long-term follow-up. Clinical and pathologic characteristics were abstracted from the medical record. Kaplan Meier curves were constructed to evaluate time to progression after first metastasis, and overall survival from time of first metastasis. Cox proportional hazards analysis was used to assess for factors associated with long-term survival. A p value of <0.05 is statistically significant.

Results: Review identified 181 patients with HER2+ MBC. Median age was 47 (range 35-80). More than half (N=107) had hormone receptor positive disease; 21% had received adjuvant trastuzumab; and 25% had stage IV disease at diagnosis. Median overall survival from the time of MBC diagnosis was 4.2 yrs (range 0.1-15.5). Since the diagnosis of MBC, 70 (38%) survived for 5 or more yrs and 15 (8%) survived more than 10 yrs. One third (N=59) of patients had brain metastases. These patients had a median survival of 1.5 yrs (range 0-12.5 yrs) with 14% (N=8) living for more than 5 yrs following diagnosis of brain metastasis. Factors associated with decreased survival are listed in Table 1.

Factors Associated with Decreased Survival in Her2+ MBC

<table>
<thead>
<tr>
<th>Factor</th>
<th>Hazard Ratio (95% C.I.)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (continuous)</td>
<td>1.03 (1.01-1.04)</td>
<td>0.0004</td>
</tr>
<tr>
<td>Time to recurrence (continuous)</td>
<td>0.99 (0.94-1.04)</td>
<td>0.63</td>
</tr>
<tr>
<td>Hormone receptor positive</td>
<td>0.70 (0.49-0.99)</td>
<td>0.042</td>
</tr>
<tr>
<td>Brain metastasis</td>
<td>1.09 (0.77-1.56)</td>
<td>0.042</td>
</tr>
<tr>
<td>Adjuvant anti-Her2 therapy</td>
<td>0.98 (0.61-1.58)</td>
<td>0.63</td>
</tr>
<tr>
<td>Multiple (vs single) sites of disease at initial diagnosis of MBC</td>
<td>1.66 (1.17-2.35)</td>
<td>0.005</td>
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Conclusions: In the treatment era of trastuzumab and lapatinib, 8% of patients within this cohort with HER2+ MBC lived more than 10 yrs. Analysis of current standard clinical and pathologic characteristics are not predictive of survival duration. Identifying factors associated with prolonged survival may provide insights for individualizing treatment selection.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-08-51
Average Grade: 7.20

**Title:** αβ-crystallin and laminin-5 are prognostic markers for triple negative breast cancers in a Brazilian series

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**Body:** AIMS. Recently, an association between increased αβ-crystallin (ABC) and abnormal laminin 5 (LN5) expression in triple negative and basal-like breast cancers has been suggested. This study aims to evaluate ABC and LN5 immunohistochemical expression in a cohort of triple negative infiltrating ductal breast carcinomas (TNBC), as well as its correlation with clinical-pathological features and prognostic significance. METHODS. We examined the expression of ABC, LN5, CK5/6 and EGFR by immunohistochemistry (IHC) in tissue microarrays of 69 cases of TNBC cases with linked clinical (median follow-up 71.4 months; range 2-305) and pathological data (age, tumor size, lymph node metastases, histological grade and subtype, lymphovascular invasion). Tumors were scored as strong, moderate and weakly positive for the biomarkers. RESULTS. ABC was expressed in 72.7% (48/66) and LN5 in 63.8% (44/69) of cases. ABC immunostaining was predominantly cytoplasmic (73.5%) but definitive nuclear staining was observed in 14.7% of the cases. LN5 immunostaining was exclusively cytoplasmic in all 44 positive cases. Using an IHC surrogate to identify basal-like tumors (negative for ER, PR and HER-2 and positive for EGFR/HER-1 and/or CK5/6), we observed that ABC was expressed in 83% of basal-like breast carcinomas in the TMAs, followed by the penta negative profile (negative for all 5 biomarkers) (17%). Kaplan-Meier analyses revealed that isolated expression of ABC and LN5 in TNBC were respectively associated with a 67% and 75% cumulative survival probability in this cohort at 5 years. The basal-like group showed a 67% probability of survival (equal to ABC) and the survival for the penta negative group was only 37%. When combined profiles of ABC/LN5 immunostaining were compared against other profiles, the ABC-negative/LN5-positive profile was associated with a statistically significant longer probability of non-recurrence (10-year disease-specific survival, 85.7% for ABC-negative/LN5-positive tumors versus 35.9% of other profiles; P=0.021). The ABC-negative/LN5-positive profile was also associated with longer cumulative survival (10-year disease-specific survival, 100% for ABC-negative/LN5-positive tumors versus 53.3% of other profiles; P=0.031). There was no association between the ABC-negative/LN5-positive profile and basal-like subtype. CONCLUSIONS. These results indicate that ABC and LN5 are commonly expressed in TNBC of the basal-like phenotype and the ABC-negative/LN5-positive profile suggests a better clinical outcome in this aggressive cohort of breast carcinomas.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P6-08-52  
**Average Grade:** 6.20

**Title:** Predicting the likelihood of additional non sentinel lymph node metastasis in early breast cancer: Novel sentinel nodal station status versus Singapore General Hospital nomogram

Sue Zann Lim¹, Puay Hoon Tan², Gay Hui Ho³, Preetha Madhukumar³, Yirong Sim¹, Shaun Shi Yan Tan¹, Cindy Lim⁴, Veronique Kiak Mien Tan³ and Kong Wee Ong³. ¹Singapore General Hospital, Singapore; ²Singapore General Hospital, Singapore; ³National Cancer Centre Singapore, Singapore and ⁴National Cancer Centre, Singapore.

**Body:** Background: Sentinel lymph node biopsy (SLNB) has been widely used in early breast cancer patients for the detection of axillary nodal metastasis. We were the first to describe 2 novel sentinel nodal stations (SNS) in relation to the intercostobrachial nerve (ICB) and the medial pectoral neurovascular bundle (MP) at which sentinel lymph nodes (SLN) were consistently identified, even only with the use of blue dye. In a pilot study involving 176 cases, we have shown that the ICB and MP SNS represent sequential echelons of SLN draining the breast. It was observed that the status of the MP SNS can be used in predicting the likelihood of additional non sentinel lymph node metastasis in early breast cancer. Thus, we aim to compare this against the Singapore General Hospital (SGH) nomogram, the existing standard predictive model in the local population. The SGH nomogram was developed from predictors in the Memorial Sloan-Kettering Cancer Centre (MSKCC) nomogram. It uses only 3 pathological parameters: lymphovascular invasion, number of positive and negative SLN. This has been shown to be at least equal if not better than the MSKCC nomogram as a predictive model in the Singapore population.

**Methods:** All patients who underwent oncologic breast surgery and SLNB (using the SNS identification technique) at the Department of Surgical Oncology, National Cancer Centre Singapore from February 2012 to December 2013 inclusive were reviewed. Patients who fulfilled the following selection criteria were included in the study: [1] invasive ductal or lobular carcinoma, [2] SLN identified in both ICB and MP SNS, [3] axillary clearance done with total lymph nodes ≥ 10, based on a positive SLNB. The performance of the MP SNS status and SGH nomogram in predicting the likelihood of additional non sentinel lymph node metastasis was compared with the calculation of the area under the receiver-operating characteristic curve (AUC).

**Results:** A total of 49 patients were identified. Majority of the patients had early breast cancers: 94% had tumour size ≤ 5cm and 71% had N1 disease. The median number of total SLN, ICB and MP nodes identified were 3 (range 2-14), 2 (range 1-7) and 1 (range 1-12) respectively. The median number of positive and negative SLN were both 1 (range 1-5 and 0-9 respectively). The positive predictive value of MP SNS status for additional non sentinel lymph node metastasis was 76.5% (95% CI: 50.1-93.2). The strong association was proven by an odds ratio of 7.15 (95% CI 1.86-27.50, p-value: 0.002). The negative predictive value of MP SNS for eventual N stage was 93.8% (95% CI: 79.2-99.2). In most of the cases, the nodal stage remained at N1 in the presence of negative MP node. The model with MP SNS status yielded an AUC of 0.706 (95% CI: 0.579-0.832) which was higher than that of the SGH nomogram, 0.658 (95% CI: 0.503-0.813).

**Conclusions:** The novel MP SNS proved to be a single parameter which predicts the likelihood of additional non sentinel lymph nodes metastasis better than the SGH nomogram. More importantly, from the clinical point of view, the MP SNS status can be made available intra-operatively and hence guide the decision for further axillary dissection.
Title: Her2 FISH amplification in ER/PR/Her2 IHC negative breast cancer

Sunati Sahoo1 and Helena Hwang1. 1University of Texas Southwestern, Dallas, TX.

Body: Background:
Triple negative (ER negative, PR negative, Her2 negative) breast cancer is an aggressive cancer that is not likely to respond to endocrine or anti-Her2 therapy. While Her2 positivity is a poor prognostic factor, patients with Her2 positive tumors are eligible for anti-Her2 therapy. Her2 positivity is based on either immunohistochemistry (IHC) or fluorescence in situ hybridization (FISH) result. Her2 IHC scores of 0+ and 1+ are negative, 2+ is equivocal, and 3+ is positive. The Her2 gene is amplified when the Her2/CEP17 ratio is ≥2.0. While most tumors will show IHC/FISH concordance, some tumors may show discordant findings between IHC staining and FISH. Previous studies have shown benefit from anti-Her2 therapy in tumors with discordant IHC/FISH results. The purpose of this study was to determine the frequency of Her2 amplification in triple IHC negative breast cancer to determine whether routine Her2 FISH testing is necessary in this subset of patients.

Methods:
After Institutional Review Board approval, the pathology databases were searched for cases of triple IHC negative breast cancer from 2003-August 2013. All cases of triple IHC negative with corresponding Her2 FISH testing were included in this study. The results of Her2 FISH were correlated with Her2 IHC. The clinical history and IHC slides for all discordant Her2 cases were reviewed when available.

Results:
A total of 659 triple IHC negative breast cancer cases from 516 patients were found. The patients’ age at diagnosis ranged from 26-92. The cases were classified using the ratio of Her2/CEP17 per the 2013ASCO/CAP guidelines: non-amplified < 2.0, amplified ≥ 2.0. As the copy number for Her2 was not always available, the 2013 ASCO/CAP criterion for amplification based on the copy number was not used in reclassifying the cases. Based on the ratio, 20 tumors were amplified, 631 tumors were not amplified, and 8 tumors had insufficient tissue for Her2 FISH testing. The Her2/CEP17 ratio for amplified cases ranged from 2.0 to 11.1 and for non-amplified cases from 0.5 to 1.9. Of these 20 patients, 10 received some trastuzumab treatment, 6 did not receive any trastuzumab, and in 4 patients, there was no additional information available regarding treatment with trastuzumab. Of the 10 who received trastuzumab treatment, 2 died of disease (DOD), 26 months and 45 months after their diagnosis. The overall survival of the other patients in this group was 23-106 months. Of the 6 who did not receive trastuzumab, one DOD after 76 months. The other 5 patients have overall survival ranging from 15-93 months. Of the patients with unknown Her2 targeted therapy status, one is deceased, the status of 2 is unknown, and one patient is alive at 110 months.

Discussion:
Our study showed 3% of triple IHC negative breast cancers to be Her2 FISH amplified. Of the 20 tumors, 19 showed Her2/CEP17 between 2.0 to 4.2 with one case showing Her2/CEP17 of 11.1. Firm conclusions on the efficacy of trastuzumab treatment in this small subset of patients with Her2 amplification but no protein overexpression cannot be drawn. However, the overall survival of those treated and not treated is similar. Based on the small number of cases with Her2 IHC/FISH discordance, a compelling case for routine testing of all Her2 IHC negative tumors cannot be made.
2014 San Antonio Breast Cancer Symposium

Publication Number:  P6-08-54
Average Grade:  4.50

Title: TIMP-4 is a prognostic and predictive marker in triple-negative breast cancers

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Body: BACKGROUND – Tissue inhibitor of metalloprotease-4 (TIMP-4) is a secreted multi-functional protein associated with poor survival prognosis among early-stage triple-negative breast cancers (TNBC). TNBC represent a highly aggressive form of this disease with an unmet need for effective predictive markers and targeted therapy. Extracellular TIMP-4 binds to the membrane bound tetraspanin CD63 and induces the activation of the tumor promoting PI3K/AKT/mTOR pathway.

Here we report that TIMP-4 induced aggressive tumor growth and metastasis can be averted by directly targeting TIMP-4 using a newly developed monoclonal antibody (mAb) to sequester TIMP-4 and the varied responses to common chemotherapy (CTX) regimen.

METHODS – The role of elevated TIMP-4 in TNBC cell behavior was tested in cell culture and animal experiments using the human breast cancer line MDA-MB-468. Cells with or without TIMP-4 added to the medium were used to determine the effects on growth, clonogenic survival and response to chemotherapeutic agents such as adriamycin, Taxol, and the new TIMP-4 mAb. The same cell-line was used to induce tumor growth in nude mice with or without TIMP-4 containing slow-release pellets implanted into the mammary fatpad (mfp). Tumor growth and response to therapy was followed over a six-week period. Lungs, liver, spleen and mfp were collected and analyzed for presence of human cells using a specific anti-human MHC I mAb.

Prospectively collected patient samples, in accordance with the IRB approved protocol, were tested for circulating levels of TIMP-4 using a commercially available ELISA assay in samples collected prior to chemotherapy and at each treatment cycle. The medical oncology staff recommended therapy without knowledge of TIMP-4 status.

RESULTS – Augmentation of TIMP-4 levels in cell culture medium or the mfp of mice resulted in similar tumor phenotype as in the clinic; fast growing tumors with accelerated disease progression.

Elevated TIMP-4 levels in the tumor environment resulted in a 1.5-fold increased growth rate with liver and/or lung metastasis in 25% of animals (N=16). No metastases were found in animals with normal TIMP-4 levels. Treating cell cultures or tumor-bearing mice (i.p. injections) with our TIMP-4 mAb resulted in decelerated growth rate and no detectable metastatic disease in the animals.

Results from patient samples demonstrated that circulating TIMP-4 levels in breast cancer patients remain elevated after definitive surgery, indicating that TIMP-4 might continuously stimulate any remaining disseminated tumor cells. Adriamycin containing regiments was the only CTX to suppress the TIMP-4 levels independent of primary tumor size and nodal status.

CONCLUSIONS – Based on these clinical and experimental data we suggest that TIMP-4 may represent a prognostic and predictive marker, and a therapeutic target for TNBC patients at highest risk. The presence of TIMP-4 identifies a patient population likely to recur quickly due to continuous activation of the PI3K/AKT/mTOR pathway. Though adriamycin therapy can reduce the TIMP-4 levels, the toxicity of this agent suggests that targeted therapy of the PI3K/AKT pathway and/or a biological therapeutic approach directed against TIMP-4 may be of benefit in this subset of pts and should be further explored.
Title: High triple-negative breast cancer prevalence and poor outcome of hormone receptor positive breast cancer among young Mexican women

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1Instituto Nacional de Cancerologia, Mexico, DF, Mexico and 2Tecnologico de Monterrey, Monterrey, NL, Mexico.

Body: Background: Breast cancer (BC) among young women is an emerging public health issue in Mexico, as the proportion of incident cases and deaths has been reported to be greater in women aged <40 years compared to high-income regions. However, there is scarce information in Hispanic populations regarding the presentation and outcome of this subgroup of patients.

The purpose of our study was to compare the distribution of BC subtypes between age subgroups and to determine the prognostic significance of young age stratified by molecular subtype based on ER, PR, and HER2 status.

Methods: This study included all consecutive patients with BC diagnosed at the National Cancer Institute in Mexico in the year 2007. A panel of ER, PR, and HER2 was used as a means of classifying BC into three molecular subtypes: (a) Hormone-receptor (HR) positive and HER2-negative, (b) Triple-negative, and (c) HER2-positive. The Chi-square test was used to compare the distribution of baseline characteristics among groups according to age (≤40 years vs. >40 years). Cox proportional hazard analysis was applied to assess the association of clinical and pathological factors with recurrence free survival with follow-up through 2013.

Results: Of the 696 analyzed patients, 134 (19.3%) were ≤40 years. For the whole group, locally advanced BC accounted for the majority of the cases (61%), and there was no difference regarding clinical stage of presentation between age subgroups. The BC subtype distribution differed among age subgroups (HR-pos/HER2-neg: 42% vs. 52%, triple-negative: 30% vs. 21%, HER2-pos: 24% vs. 23%, for young vs. non-young respectively). After a median follow-up of 74.6 months, 48% of young patients experienced a recurrence compared to 39% of women >40 years. After subtype and clinical stage stratification, young age remained a significant independent predictor of recurrence in patients with HR-pos/HER2-neg tumors (hazard ratio 1.71; 95% CI:1.09-2.69; P = 0.019), but was not found to be a predictor of recurrence for patients with HER2-pos and triple negative subtypes.

Conclusions: The results of our present study suggest that the poor survival associated with young age is not only driven by a high proportion of triple-negative BC, but also by poor prognosis in the most prevalent HR-pos/HER2-neg subgroups. Possible explanations for this finding are a greater proportion of Luminal B BC subtype and/or tamoxifen resistance in the young age group. Further characterization of HR-pos BC should be pursued and aggressive therapeutic strategies must be considered for treatment of young women with high-risk features.

It is critical to note the extremely high prevalence of BC among young women in our Institution, which comprised 19% of the total population. To address this unmet, growing burden, we adopt the model of a specialized program for the care of young women with BC, the first in Latin America, at the National Cancer Institute in Mexico. The goals of our program include optimizing complex clinical care and supporting needs for young women and their families; promoting medical, biomedical, and social research; and educating women and health professionals to promote early cancer detection and improved multidisciplinary management.
Title: Recent weight gain and increased breast cancer risk varies by receptor classification among pre and postmenopausal women

Graham A Colditz\textsuperscript{1}, Heather Eliassen\textsuperscript{2}, Adetunji T Toriola\textsuperscript{1}, Susan E Hankinson\textsuperscript{3}, Walter C Willett\textsuperscript{4}, Loki Natarajan\textsuperscript{5} and Bernard Rosner\textsuperscript{2}. \textsuperscript{1}Washington University School of Medicine, St Louis, MO; \textsuperscript{2}Harvard Medical School, Boston, MA; \textsuperscript{3}University of Massachusetts, Amherst, MA; \textsuperscript{4}Harvard School of Public Health, Boston, MA and \textsuperscript{5}Mores UCSD, Cancer Center, La Jolla, CA.

Body: Obesity is well established as a cause of postmenopausal breast cancer incidence and mortality. Furthermore, adiposity in early life – through premenopausal years reduces breast cancer incidence. However, studies using measures of adiposity at age 18 also report inverse relations with premenopausal breast cancer and for some but not all subtypes of breast cancer defined by molecular status. To assess the relations of adiposity in childhood, in late adolescence, and in adult years as well as change in weight in relation to total invasive breast cancer and subtype defined by receptor status we fit models based on the Rosner-Colditz log-incidence model of breast cancer.

The Nurses' Health Study cohort was established in 1976 when 121,701 female US registered nurses ages 30-55 responded to a mail questionnaire inquiring about risk factors for breast cancer including reproductive factors, hormone use, anthropometric variables, benign breast disease (BBD), and family history of breast cancer. The risk factors have been updated by repeat questionnaires every 2 years. We followed a cohort of 77,232 women from 1980 to 2006 (1,445,578 person-years) documenting 4,196 incident cases of invasive breast cancer. ER and PR status were obtained from pathology reports and medical records. A total of 2,033 ER+/PR+ tumors, 595 ER-/PR- tumors, 512 ER+/PR- tumors were identified among women with complete information on breast cancer risk factors.

Overall, weight at age 18 was inversely related to incidence. The relative risk per 25 lb weight difference was 0.89 (0.85, 0.93). After controlling for weight at age 18, long-term weight change was positively related to total incident breast cancer risk among both premenopausal (RR per 25 lb weight gain since age 18=1.08; 95% CI=1.05-1.12) and postmenopausal women (RR per 25 lb weight gain since menopause=1.16; 95% CI=1.09-1.23).

In addition, we focus on the effect of short-term weight gain (over past 4 years) on breast cancer risk while controlling for weight at age 18 and long-term change in weight during premenopause and postmenopause. We found a significant effect of short-term weight change and breast cancer risk (RR=1.20; 95% CI=1.09-1.33) for a 4-year weight gain of ≥ 15 lbs vs no change (≤ 5lbs) (RR per 25 lb weight gain=1.13; 95% CI=1.06-1.21, p<0.001). However, the effect was stronger for premenopausal women (≥ 15 lb weight gain vs no change, RR=1.38; 95% CI=1.13-1.69) (RR per 25 lb weight gain=1.26; 95% CI=1.08-1.48, p=0.004) than for postmenopausal women (≥ 15 lb weight gain vs no change, RR=1.10; 95% CI=0.97-1.25) (RR per 25 lb weight gain=1.08; 95% CI=1.00-1.16, p=0.063).

The effect of short-term weight gain during premenopause was stronger for ER+/PR- (RR per 25 lb weight gain=2.19; 95% CI=1.33-3.61, p=0.002) and ER/-PR- breast cancer (RR per 25 lb weight gain=1.61; 95% CI=1.09-2.38, p=0.016) than for ER+/PR+ breast cancer (RR per 25 lb weight gain=1.13; 95% CI=0.89-1.43, p=0.32).

In conclusion, adiposity in childhood has a protective lifelong relation to breast cancer risk, though mechanisms remain largely unexplained. There are long-term deleterious effects of weight change both pre- and post-menopause and deleterious effects of short-term weight gain during premenopausal years.
Title: Development of a cardiac toxicity prediction tool for HER2 (+) breast cancer patients receiving trastuzumab

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Body: Background: In HER2 positive breast cancer, the addition of trastuzumab prolongs overall survival in both early stage and metastatic disease. However, moderate to severe cardiac toxicity is a potentially serious complication which can lead to dose reductions, delays, hospitalizations, and premature discontinuation of treatment. Patient care could be substantially improved if such cardiac events are accurately predicted through the use of validated and easy-to-use mathematical models. In this study, the development of a model to predict the risk of cardiac toxicity prior to initiation of trastuzumab therapy is described.

Methods: Medical records of 498 HER2 positive breast cancer patients who received trastuzumab at the Ottawa Hospital Cancer Centre were identified. Charts were reviewed for potential cardiac toxicity risk factors and cardiac events. Potential cardiac toxicity variables included: cardiac risk factors, medications, previous chemotherapy/radiation, baseline left ventricular ejection fraction (LVEF), and exposure to anthracyclines. Cardiac toxicity was defined as: decreased LVEF greater or equal to 10% and to less than 50%, referral to the cardiac oncology service, or clinical symptoms of heart failure. General linear modeling for a discrete bivariate outcome was used to identify risk factors for cardiac toxicity using a backwards elimination process. Internal validation of the final regression coefficients was done using nonparametric bootstrapping. A risk scoring algorithm (range 0-100) was then derived from the final model coefficients. A receiver operating characteristic (ROC) curve analysis was then undertaken to measure the predictive accuracy of the final scoring algorithm.

Results: Baseline LVEF, concomitant use of any cardiac medication or lipid lower drugs and doxorubicin based chemotherapy were identified as being important predictors for cardiac toxicity. The ROC curve analysis indicated good predictive accuracy with an area under the curve of 0.69 (95%CI: 0.64 to 0.74). Prior to the initiation of trastuzumab, patients with risk scores greater than 45 units would be considered at high risk for developing cardiac toxicity (likelihood ratio = 2.9).

Conclusions: Risk of cardiac toxicity with trastuzumab is increased in patients with a low baseline LVEF, history of coronary artery disease and hyperlipidemia, as well as those receiving doxorubicin. The planned external validation and eventual clinical application of this prediction tool will be an important source of risk information for the practicing oncologist, and may enhance patient care by optimizing preventative therapies and selecting high risk patients for cardiac imaging and cardiology follow-up.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-09-04
Average Grade: 4.60

Title: Volumetric breast density improves breast cancer risk prediction

Jennifer A Harvey¹, George J Stukenborg¹, Wendy F Cohn¹, Kathy L Repich¹, Olivier Alonzo², Wendy M Novicoff¹, Martin D Yaffe², and William A Knaus³. ¹University of Virginia, Charlottesville, VA; ²Sunnybrook Health Sciences Centre, Toronto, ON, Canada and ³Northshore University Research Institute, Evanston, IL.

Body: Introduction: There is increasing interest in implementing personalized breast cancer screening strategies rather than relying on population based guidelines. Most risk models do not include breast density and two models that do rely on subjective BI-RADS categories; all have limited discriminatory ability (C-statistics ranging from 0.60-0.74). Our aim was to develop a model that includes an automated objective and numeric volumetric measurement of breast density with other known risk factors to improve risk prediction.

Methods: A case-control study design was used to evaluate the association between risk factors and breast cancer diagnosis. All women diagnosed with breast cancer during 2003-13 with a digital contralateral mammogram at the University of Virginia at the time of diagnosis were eligible as cases. All women without a breast cancer diagnosis but with a digital mammogram at UVA during 2003-2008 were eligible as controls. Risk factor information was collected using a self-reported electronic questionnaire. Mean automated volumetric breast density (Volpara, NZ) was calculated for each patient as a percentage. Controls were matched to cases in a 2:1 ratio based on age group, race, and education, using the GREEDY algorithm. Case-control selections were made using the weighted sum of the absolute differences between the case and control matching factors. Conditional logistic regression using the partial likelihood function from Cox proportional hazard’s regression was used to fit risk prediction equations to the matched case-control study dataset, with stratification for each case matched set. A full model was estimated including all available covariates for use as a model performance reference standard. A reduced model was then estimated including covariates in the full model that had a Wald Chi-Square/degrees of freedom ratio > 1.0. The performance of the full and reduced models was measured using the C index and the maximum R-Square statistic.

Results: The study enrolled 3,445 women; 839 cases and 2,606 controls. Multivariable analysis was conducted using 825 cases and 1,628 controls with 1 or more breast studies reported for the surveyed population. The matching process yielded balanced matching factor values between cases and controls, with no significant differences in age group (p = 0.95), race (p = 0.13), or education (p = 0.86). The full prediction model (with 97 df) yielded a C index of 0.88, and an R-Square of 0.53. The reduced model (with 15 df) had a C index of 0.83 and an R-Square of 0.54. Variables in the reduced model included: mean breast density; biopsy showing ADH, ALH/LCIS; BMI; use of HRT, contraceptives, NSAIDS; smoking; exercise; parity; diabetes; family history of breast cancer, HBOC, Li-Fraumeni, or Cowden Syndromes and/or BRCA mutation. Mean volumetric breast density was a leading independent predictor of case status in both the full (p<0.0001) and reduced models (p=0.0212).

Conclusion: Volumetric breast density with other known risk factors may provide more accurate individual risk assessment, enabling clinicians to develop patient centered risk based screening protocols that better inform decision making while including patient preferences. The next steps require independent validation of the risk model in other populations.
Title: Value of adding single-nucleotide polymorphism panel markers to phenotypic algorithms of breast cancer risk

Gillian Dite¹, Richard Allman² and John L Hopper¹. ¹Centre for Epidemiology and Biostatistics Melbourne School of Population and Global Health, University of Melbourne, Melbourne, VIC, Australia; ²Genetic Technologies Ltd, Fitzroy, VIC, Australia and ³Centre for Epidemiology and Biostatistics Melbourne School of Population and Global Health, University of Melbourne, Melbourne, VIC, Australia.

Body: Currently, breast cancer risk prediction algorithms incorporate phenotypic risk factors, including age, family history, reproductive history and benign breast disease. Since 2007, genome-wide association studies have identified a growing number of single-nucleotide polymorphisms (SNPs) that are associated with an increased risk of breast cancer. While no single SNP is very informative on its own, a polygenic approach to genetic screening could improve estimates of individual risk, creating the possibility of screening strategies individually tailored to each woman.

We studied cases and controls recruited to the Australian Breast Cancer Family Registry, to investigate whether a SNP risk score derived from a panel of 77 SNPs can improve risk estimates obtained from the commonly used breast cancer risk prediction models (BCRAT, IBIS, BRCAPRO and BOADICEA). SNP risk scores were calculated using previously published Odds Ratios (ORs) and risk-allele frequencies based on the assumption of independence of additive risks on the log OR scale. SNP risk scores were calculated by multiplying adjusted risk scores for each of the SNPs. Combined risk scores were calculated by multiplying the SNP risk score and the algorithm derived risk scores, under the assumption of independence.

Overall, the inclusion of data on 77 SNPs improved the area under the receiver operating characteristic curve (AUC) for all of the breast cancer risk prediction models: Preliminary data analysis provided AUC values as follows: BCRAT+SNP = 0.656, IBIS+SNP= 0.628, BRCAPRO+SNP= 0.689, BOADICEA+SNP= 0.698.

The breast cancer risk prediction models examined place different weighting on the phenotypic components. For example, the IBIS model is weighted towards uncommon high-risk phenotypes, while the BCRAT is weighted towards lower risks in the general population. A combination of the 77 SNP score and the most appropriate model might substantially improve the ability to identify high-risk women in different screening populations.
Title: Balancing the harms and benefits of radiation therapy for DCIS: A decision analysis examining the risk of radiation-associated sarcoma

Marquita R Decker¹, Joseph F Levy², Lee G Wilke¹, David J Vanness² and Heather B Neuman¹. ¹University of Wisconsin, Madison, WI and ²University of Wisconsin, Madison, WI.

Body: INTRODUCTION:
More than 60,000 women are diagnosed with ductal carcinoma in situ (DCIS) annually and offered the option of breast conserving surgery (BCS), often including radiation (RT) to reduce local recurrence. Although the incidence of radiation-associated sarcoma (RAS) is low (0.05-0.25% at 10 years), the low mortality associated with DCIS and large number of DCIS diagnoses means that an increasingly large number of women are at risk of RAS. This study sought to weigh the risk of RAS with the benefits of BCS+RT for DCIS.

METHODS: A second-order Monte Carlo micro-simulation model of women ages 35 and older with DCIS was constructed. The decision analysis compared harm-benefit ratios of sarcoma-related deaths per breast cancer deaths averted within 20 years of treatment with BCS+RT versus BCS alone. Stratified analyses were performed by age group to account for differential life expectancy. To generate parameter estimates for model inputs, Bayesian network meta-analysis was used to synthesize rates of DCIS and invasive recurrence from clinical trials of BCS+RT and BCS alone using a Weibull specification. Sarcoma incidence was estimated non-parametrically using SEER. Constant hazard rates for breast cancer mortality after invasive recurrence and RAS mortality were estimated from clinical trials. To account for uncertainty, probabilistic sensitivity analysis was conducted using 10,000 Monte Carlo samples and 95% credible intervals (CrI) were constructed for event rates and harm-benefit ratios.

RESULTS: The micro-simulation model of an age-distributed cohort demonstrated that 1 in 840 women with DCIS (95%CrI 1:648 to 1:3522) would develop RAS within 20 years after treatment with BCS+RT. Overall, there would be 1 RAS-related death for every 12 breast cancer deaths averted (95%CrI 1:7 to 1:19) by the addition of RT to BCS. Stratified analysis demonstrated that the harm-benefit ratio was higher in women <75 years of age, with more RAS-related deaths caused per breast cancer deaths averted. The model was most impacted by parameter estimates for rates of invasive recurrence, breast cancer mortality after invasive recurrence, and RAS incidence rates.

CONCLUSIONS:
The risk of developing a RAS following BCS+RT for DCIS should not be overlooked. This may be especially true for women at low risk of recurrence and younger women (<75 years in our model). These findings contribute to the ongoing conversation about consequences of overtreatment of DCIS, and should be incorporated into shared-decision making discussions regarding the optimal management of DCIS for a given patient.

Age-Stratified Incremental Harm-Benefit Ratios for BCS+RT versus BCS Alone

<table>
<thead>
<tr>
<th>Age Group</th>
<th>RAS Deaths: Breast Cancer Deaths Averted*</th>
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<tbody>
<tr>
<td></td>
<td>Posterior Median Ratio (95% CrI)</td>
</tr>
<tr>
<td>Overall</td>
<td>1:12 (1:7 to 1:19)</td>
</tr>
<tr>
<td>35 to 54</td>
<td>1:10 (1:6 to 1:14)</td>
</tr>
<tr>
<td>55 to 74</td>
<td>1:11 (1:7 to 1:15)</td>
</tr>
<tr>
<td>75+</td>
<td>1:17 (1:9 to 1:24)</td>
</tr>
</tbody>
</table>

*Probabilistic sensitivity analysis using 10,000 second-order parameter samples with a 20 year time horizon
The development of a prediction tool for moderate to severe diarrhea in HER-2/hormone positive metastatic breast cancer (MBC) patients receiving lapatinib in combination with letrozole (L-L)

George Dranitsaris¹ and Mario E Locouture². ¹Augmentium Pharma Consulting and ²Memorial Sloan Kettering Cancer Center.

Background: For patients with HER-2/hormone positive MBC, the addition of lapatinib to letrozole is associated improvements in tumour response rates and a prolongation of progression free survival. However, moderate to severe diarrhea (≥ grade 2) is a potentially serious toxicity which can lead to dose reductions, delays, hospitalizations and even the premature discontinuation of treatment. Patient care could substantially be improved if these diarrhea events could be accurately predicted through the use of validated and easy-to-use mathematical models. In this study, the development of a repeated measures model to predict the risk of ≥ grade 2 diarrhea prior to each month of L-L therapy is described.

Methods Data from 111 patients who received the L-L combination as part of a clinical trial were reviewed [Johnston, 2009]. Generalized estimating equations (GEE) were used to develop the final risk model using a backwards elimination process. Internal validation of the final regression coefficients was done using nonparametric bootstrapping. A risk scoring algorithm (range 0-250) was then derived from the final model coefficients. A receiver operating characteristic curve (ROC) analysis was then undertaken to measure the predictive accuracy of the final scoring algorithm.

Results: Presence of skin and lung metastases at baseline, cumulative lapatinib dose and Hg level (nadir) were identified as being important predictors for ≥ grade 2 diarrhea. There was also a negative association between time on therapy and risk of diarrhea where a higher frequency was observed in the first few months. The ROC analysis indicated good predictive accuracy with an area under the curve of 0.80 (95%CI: 0.72 – 0.88). Prior to each new month of therapy, patients with risk scores > 125 units would be considered at high risk for developing ≥ grade 2 diarrhea.

Conclusions: Risk of ≥ grade 2 diarrhea is associated with cumulative lapatinib exposure, disease related factors as well as Hg level. The planned external validation and eventual clinical application of this prediction tool will be an important source of risk information for the practicing oncologist and can enhance patient care by optimizing preventative therapies earlier in a proactive manner.
Title: Can proton therapy for localized mediastinal Hodgkin's lymphoma reduce second breast cancer incidence?

Samy R Horn¹, Victor Pernin¹, Nathalie Fournier-Bidoz¹ and Youlia Kirova¹. ¹Institut Curie, Paris, France.

Body: Introduction
Secondary breast cancer (SBC) is a recognized late complication of radiation therapy, after treatment for Hodgkin lymphoma (HL), with reported relative risks as high as 50 with older techniques (Mantle Field). Within the past, it has been estimated that reduction in dose and volume will reduce the incidence of late complications such as secondary neoplasms and cardiovascular disease. We evaluate the impact of mediastinal proton therapy on potential limitation of SBC risk.

Material & methods
For 14 young female patients with early-stage, mediastinal HL, treated with chemotherapy and involved-field radiation therapy (IFRT), we simulated similar treatment plans with conformal radiotherapy (CRT), helical tomotherapy (HT) and proton therapy at the dose of 30 Gy. We report the respective doses to the breasts. Treatment plans were not specifically designed for breast sparing.

Results
Proton therapy significantly lowered the dose to the breasts, with mean doses of 2.76/1.53 Gy to the left and right breast respectively with proton therapy (vs. 4.95/3.88 Gy with HT and vs. 5.56/3.58 Gy with CRT). Proton therapy best limited lower doses (V4Gy and V10Gy) compared to CRT, while HT could limit the higher doses (V20Gy) at the expense of larger volume irradiated at low doses (V4Gy).

Conclusion
Relative reduction in mean doses to the left and right breasts was of 50 and 57% respectively with proton therapy compared to CRT. Relation between radiation dose and SBC seems to be linear, and reduction in SBC should be in such proportion with proton therapy. Proton therapy seems highly interesting for breast sparing after curative treatment for HL, but these results need to be confirmed by individualized risk estimations and prospective trials.
Title: Body mass index and prognosis after breast cancer diagnosis in Japanese women

Toshinari Yamashita¹, Tomoyuki Aruga¹, Hiromi Miyamoto¹, Kazumi Horiguchi¹, Yayoi Honda¹, Nami Idera¹, Risa Goto¹ and Katsumasa Kuroi¹.

¹Tokyo Metropolitan Komagome Hospital, Tokyo, Japan.

Body: Background: Studies conducted mainly in Western countries have reported a relationship between body mass index (BMI) and prognosis among women with breast cancer. Only a few studies have been conducted in Japan so far because the percentage of high BMI is low. In the present retrospective study, we investigated the associations between BMI and the clinical characteristics and prognosis among breast cancer patients.

Methods: We analyzed 1,744 breast cancer patients who started treatment between 2004 and 2012 at a single hospital in Japan. All patients with ductal carcinoma in situ, male breast cancer as well as metachronous and synchronous bilateral breast cancer were excluded. Median age was 57 years (range 23–91). The number of patients less than 50 years old was 496. World Health Organization BMI classifications were used: Underweight, less than 18.5 kg/m², n=157; Normal, 18.5–24.9 kg/m², n=1181; Overweight, 25–29.9 kg/m², n=316; and Obese, more than or equal to 30kg/m², n=90. The Cox proportional hazards model was used to estimate hazard ratios for recurrence free survival (RFS) in relation to BMI classifications.

Results: Median follow up interval was 4.2 years. During the follow-up period, 126 breast cancer recurrences were observed. BMI classification correlated with clinical tumor size (cT) significantly and BMI classification tended to correlate with lymph node metastases and estrogen receptor (ER) status.

Among patients less than 50 years old, the RFS of those with BMI ≥25.0 kg/m² was compared to that of patients with BMI <25.0 kg/m². In multivariate analyses, BMI classification was one of the significant factors (p=0.02) along with lymph node metastases (p=0.0001) and ER status (p=0.04). However in patients aged 50 years or over BMI category was not a significant factor (p=0.12).

Conclusions: It has been reported that higher BMI is a risk factor for breast cancer recurrence among postmenopausal patients. Our results suggest that higher BMI is also associated with an increased risk of breast cancer recurrence among premenopausal patients. It raises the possibility that maintaining an appropriate body weight improves the prognosis in premenopausal patients after they have been diagnosed.
Title: The comparison of the distribution of breast cancer risk factors between Chinese women, non-Chinese Asian and Caucasian women in the screening cohort of Athena Breast Health Network

Bo Pan¹,², Jeffrey Tice¹, Qiang Sun², Celia Kaplan¹, Zhou Yidong², Yali Xu², Songjie Shen², Changjun Wang², Alexandra Solomon¹, Lauren Ryan¹, Paige Kendall¹, Timothy Henderson¹, Laura Esserman¹, Beth Crawford¹, Athena Breast Health Network¹ and Laura van 't Veer¹. ¹Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA and ²Peking Union Medical College Hospital, Beijing, China.

Body: Background: A major area of innovation in breast cancer (BC) is improving risk models for screening efforts. The University of California Athena Breast Health Network uses several risk models, including the Gail model [PMID: 10491430]. The Breast Cancer Surveillance Consortium (BCSC) model [PMID: 18316752] which integrated information on breast density was developed and validated by UCSF investigators and reports Asian-specific risk. The Peking Union Medical College (PUMC) model [PMID: 22662004] is developed from a case-control study in China and reports BC risk in Chinese women. Risk factors and their respective weights included in these models are different.

Methods: From December 2012 to April 2014, 2,305 women without previous history of BC and consented to research were enrolled in the UCSF Athena screening cohort. Questions asked included risk factors used in Gail, BCSC and PUMC model, such as age, age of menarche (AOM), age at first live birth, body mass index (BMI), breast biopsy, breast density, hormone replacement therapy (HRT), oral contraceptives (OCP). Women were considered high risk when: Gail > 1.67% 5-year risk, BCSC > 1.67% 5-year risk, PUMC score > 30 (equals >0.20% 1-year risk) respectively. The distribution of the risk factors and the high risk population percentage were compared between Chinese women versus non-Chinese Asian and Caucasian women.

Results: 402 Asian women comprise 17.4% of all 2,305 Athena screening cases with 234 Chinese, and 168 non-Chinese Asian (NCA). Differences in risk factor distribution were observed for the following: positive family history was observed 23% for Caucasian, 15% for Chinese and 13% for NCA (p=0.001), and previous breast biopsy was 27%, 17% and 22% respectively (p=0.002). Ever use of HRT was 36% in Caucasian, 17% in Chinese and 11% in NCA (p<0.001). Ever give birth was 68% in Chinese, 65% in NCA, and 57% in Caucasian (p=0.001), while the age at first live birth <30 was 35%, 33%, and 26% correspondingly (p=0.001). Breast density appeared to be higher in Asian women (p=0.095). The high risk proportions by each model are given in Table 1.

Conclusion: Chinese and NCA women have a lower proportion of high risk by the BCSC and PUMC model compared to the Caucasian women, whereas by Gail model these proportions appear to be similar.

Table 1 Percentage of high risk population by ethnicity for different risk models

<table>
<thead>
<tr>
<th>Risk models</th>
<th>Chinese (n=234)</th>
<th>non-Chinese Asian (n=168)</th>
<th>Caucasian (n=1,492)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>low</td>
<td>high</td>
<td>low</td>
<td>high</td>
</tr>
<tr>
<td>Gail</td>
<td>137 (58.6%)</td>
<td>97 (41.4%)</td>
<td>107 (63.7%)</td>
<td>61 (36.3%)</td>
</tr>
<tr>
<td>BCSC</td>
<td>211 (90.2%)</td>
<td>23 (9.8%)</td>
<td>152 (90.5%)</td>
<td>16 (9.5%)</td>
</tr>
<tr>
<td>PUMC</td>
<td>167 (71.7%)</td>
<td>66 (28.3%)</td>
<td>108 (64.7%)</td>
<td>59 (35.3%)</td>
</tr>
</tbody>
</table>
Risk of chemotherapy-induced febrile neutropenia (FN) in patients (pts) with non-metastatic breast cancer (BC) and documented risk factors for FN

Derek Weycker, Xiaoyan Li, Richard Barron, Hongsheng Wu, Phuong Khanh Morrow, Hairong Xu, Maureen Reiner, Jacob Garcia, Shivani Mhatre and Gary Lyman. 1Policy Analysis Inc (PAI), Brookline, MA; 2Amgen Inc, Thousand Oaks, CA; 3University of Houston, Houston, TX and 4Hutchinson Institute for Cancer Outcomes Research, Seattle, WA.

Body: Background: Clinical practice guidelines recommend primary prophylactic colony-stimulating factor (CSF) for pts with cancer receiving myelosuppressive chemotherapy when their risk of FN is high (≥20%). Evaluating FN risk in pts who receive regimens that are not documented as high risk in guidelines can be challenging; these pts may be at a high risk of developing FN based on a combination of regimen and pt risk factors. This retrospective study estimated FN risk among subgroups of pts with non-metastatic BC receiving 1 of 3 commonly used, non-high risk chemotherapy regimens.

Methods: Pt-level data from 2 US healthcare claims databases comprising medical and outpatient pharmacy claims from commercial and Medicare supplemental plans were pooled. Eligible pts were ≥18 years old and had initiated a course of TC, TCH, or non-dose-dense AC/AC-T for non-metastatic BC between July 1, 2003 and June 30, 2012. Occurrence of FN during any cycle of the first chemotherapy course was identified using diagnosis codes for neutropenia, fever, or infection. Risk factors, documented in guidelines and the published literature, were evaluated during the 12 months before chemotherapy initiation. The percentage of pts, FN risk, and relative risk (RR, compared to pts with no risk factors) for subgroups of pts with specific risk factors are presented.

Results: 50,893 pts were included in the analysis. FN risks by chemotherapy regimen and subgroup are shown in the table.

<table>
<thead>
<tr>
<th></th>
<th>TC N=26,266</th>
<th>TCH N=9,105</th>
<th>AC/AC-T N=15,522</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any risk factor</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Pts FN Risk (RR, 95% CI)</td>
<td>78.1</td>
<td>74.5</td>
<td>73.6</td>
</tr>
<tr>
<td>Age ≥65</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Pts FN Risk (RR, 95% CI)</td>
<td>15.1</td>
<td>11.5</td>
<td>11.4</td>
</tr>
<tr>
<td>Chronic comorbidities</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>16.8</td>
<td>14.6</td>
<td>16.6</td>
</tr>
<tr>
<td>Diabetes</td>
<td>11.8</td>
<td>10.2</td>
<td>11.1</td>
</tr>
<tr>
<td>Liver disease</td>
<td>1.9</td>
<td>2.2</td>
<td>2.0</td>
</tr>
<tr>
<td>Lung disease</td>
<td>2.0</td>
<td>1.5</td>
<td>1.9</td>
</tr>
<tr>
<td>Renal disease</td>
<td>1.3</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Osteoarthritis</td>
<td>7.0</td>
<td>6.1</td>
<td>6.0</td>
</tr>
<tr>
<td>Thyroid disorder</td>
<td>13.0</td>
<td>11.9</td>
<td>10.6</td>
</tr>
<tr>
<td>Number of risk factors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>33.2</td>
<td>33.9</td>
<td>33.7</td>
</tr>
<tr>
<td>2</td>
<td>24.6</td>
<td>23.2</td>
<td>22.9</td>
</tr>
<tr>
<td>3</td>
<td>12.5</td>
<td>11.2</td>
<td>11.1</td>
</tr>
<tr>
<td>4+</td>
<td>7.8</td>
<td>6.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Reference group (0 risk factor)</td>
<td>21.9</td>
<td>25.5</td>
<td>26.4</td>
</tr>
</tbody>
</table>

Selected risk factors are presented.

A high proportion of pts (74%-78%) had ≥1 risk factor for FN, and these pts had a higher FN risk than pts with no risk factors. 55.4%, 56.7%, and 42.2% of all pts who received TC, TCH, or AC/AC-T, respectively, received CSF prophylaxis in cycle 1.
Conclusions: FN risk assessments are needed for pts who are receiving non-high risk regimens.
Title: The KRAS-variant, multiple breast cancer risk, and estrogen withdrawal

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Body: Background: The KRAS-variant is a germ-line, functional microRNA binding site mutation in KRAS that leads to increased risk of premenopausal triple negative breast cancer (TNBC) (1), as well as double primary breast/ovarian cancer in women (2). Evidence of an association with post-menopausal (PM) cancer onset (3), as well as altered BC biology in hormone replacement therapy (HRT) users (4), led us to investigate the hypothesis that estrogen exposure alters BC risk and biology in KRAS-variant individuals.

Methods: Women with a confirmed diagnosis of BC were invited to join a study of the KRAS-variant and hormones, and asked to complete a questionnaire, send in a DNA sample and submit pathologic information. For the study 1712 BC survivors were eligible for inclusion. An additional cohort of unaffected women, positive for the KRAS-variant (n=80), were included from Ohio State University as the control population. Differences were evaluated through 2 tailed t-test and Chi squared analysis. Additionally, isogenic, perfectly matched cell lines +/- or -/- for the KRAS-variant were created to study transformation in vitro.

Results: Of the 1712 study participants, 17.4% (298) had the KRAS-variant. Compared to controls, KRAS-variant BC patients had significantly lower Body Mass Index (BMI) (p<0.0001), were significantly likely to have fewer live births (p=0.0028), and to be older at their first birth (p=0.0070). There was no difference in age of testing between the BC and control KRAS-variant cohorts. Excluding 68 (3.9%) women with other known BC associated mutations (BRCA1, BRCA2, p53, and PTEN), KRAS-variant BC patients were significantly more likely to be diagnosed with a second, independent BC compared to non-variant BC patients (p=0.0046). No differences were found between the variant and non-variant groups in synchronous versus metachronous presentation, time between first and second breast cancer diagnoses, time between diagnosis and enrollment, or lobular histology.

For women diagnosed when PM in our cohort (n=765), tumor biology in KRAS-variant women was significantly impacted by HRT history. In KRAS-variant PM BC patients that stopped HRT before their BC diagnosis (ex-HRT users), the proportion developing TNBC was significantly higher than for non-variant ex-HRT users (35.5% versus 7.3%, p<0.0001)). The proportion of KRAS-variant ex-HRT users developing TNBC was also significantly higher than for KRAS-variant women who were never on HRT (11.4%, p=0.020) or for those who were on HRT when diagnosed with BC (3.6%, p<0.0001). In addition, KRAS-variant ex-HRT users had significantly higher-grade tumors than non-variant ex-HRT users (p=0.0286).

Finally, cell line studies with isogenic KRAS-variant cell lines demonstrated that estrogen withdrawal led to significantly enhanced cell line growth, and apparent transformation for the KRAS-variant+/- line compared to the non-variant -/- line.

Conclusions: Based on our studies, it appears that estrogen withdrawal may be a risk for breast cancer development and aggressive breast tumor biology for women with the KRAS-variant.

The contribution of common genetic variation to breast cancer risk among women receiving tamoxifen or raloxifene within the National Surgical Adjuvant Breast and Bowel Project (NSABP) P-1 and P-2 trials

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¹Mayo Clinic, Rochester, MN; ²Section of Cancer Genetics and Prevention, Allegheny General Hospital, Pittsburgh, PA; ³National Surgical Adjuvant Breast and Bowel Project, Pittsburgh, PA; ⁴RIKEN Center for Genomic Medicine, Tokyo, Japan; ⁵Allegheny General Hospital, Pittsburgh, PA and ⁶University of Chicago Medical Center, Chicago, IL.

Body: Purpose: Tamoxifen and raloxifene, are primary prevention strategies for women at high risk of breast cancer (Visvanathan, 2013). Recent advances in genetic studies of breast cancer risk have identified common susceptibility loci that explain 14% of familial risk for breast cancer in the general population (Michailidou, 2013). However, it is not known if these loci are risk factors for breast cancer among high-risk women treated with SERMs for breast cancer prevention. We hypothesized that the large risk reduction associated with SERMs, coupled with the fact that several breast cancer loci correlate with family history, may limit the contribution of these common genetic loci to breast cancer in this high risk population. We present the first report to evaluate 75 established breast cancer susceptibility loci, in the context of a polygenic risk score (PRS), as a risk factor for breast cancer among high risk women taking raloxifene and tamoxifen for breast cancer prevention.

Methods: We conducted a matched case-control study of 594 cases (i.e., participants who developed breast cancer while on SERM therapy) and 1,171 matched controls selected from the 33,000 participants enrolled in the NSABP P-1 and P-2 breast cancer prevention trials. Genotypes of 75 single nucleotide polymorphisms (SNPs) were available from a genome-wide association study conducted at the RIKEN Center for Genomic Medicine. We formed a quantitative PRS from reported per-SNP odds ratios (OR) for the 75 susceptibility loci. Conditional logistic regression was used to examine the PRS as a risk factor for breast cancer and to assess whether the PRS and breast cancer association differed by treatment type, family history, or other clinical characteristics. Analyses also examined associations of PRS with invasive vs. in situ cancer and ER-positive vs. ER-negative cancer.

Results: The PRS ranged from 3.98 to 7.74, and a one unit change in PRS was associated with a 42% increase in breast cancer (OR=1.42; 95% CI: 1.18-1.70; P = 0.0002). There was evidence of a stronger association of PRS with breast cancer among women with no first-degree family history (OR=1.62, 95% CI: 1.18-2.21) compared to those with a positive family history (OR=1.32, 95% CI: 1.06-1.66) (P<sub>int</sub>=0.05). The PRS also appeared a stronger risk factor for ER-positive (OR=1.59, 95% CI: 1.25-2.02, P < 0.0002) vs. ER-negative (OR=1.05, 95% CI: 0.68-1.62, P=0.84) breast cancer, although differences did not reach statistical significance (P<sub>int</sub>=0.10). PRS and breast cancer associations were similar across tamoxifen and raloxifene treatments, age at trial entry, 5-year predicted Gail model risk, hysterectomy status, BMI, presence of atypical hyperplasia and invasive vs. in situ cancer.

Conclusion: A polygenic risk score composed of 75 loci was a risk factor for ER-positive breast cancer, especially in the absence of a first-degree family history of breast cancer. Further, the PRS associations with breast cancer were similar for women taking tamoxifen or raloxifene for prevention. These data suggest that common genetic variation adds information on risk of ER-positive breast cancer in a high-risk population receiving SERMs.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-10-04
Average Grade: 5.60

Title: Statins and breast cancer stage and mortality in the Women’s Health Initiative cohort

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¹Boston Medical Center, Boston, MA; ²Weill Cornell Medical College, New York, NY; ³Ohio State University, Columbus, OH; ⁴Kaiser Permanente of Northern California, Oakland, CA; ⁵Brigham and Women's Hospital, Boston, MA; ⁶Karmanos Cancer Institute, Detroit, MI; ⁷Albert Einstein College of Medicine, Bronx, NY; ⁸University of Wisconsin, Madison, WI; ⁹Brigham and Women's Hospital, Boston, MA; ¹⁰University of Pittsburgh, Pittsburgh, PA; ¹¹Mayo Clinic, Rochester, MN and ¹²David Geffen School of Medicine at UCLA, Torrance, CA.

Body: Background: Statins are the most widely prescribed cholesterol-lowering drugs in the United States, with approximately 25% of US adults using statins by 2008. The anti-carcinogenic effect of statins may reduce the metastatic potential of cancer cells leading to ‘stage migration’ with statin users more likely diagnosed with early rather than late stage cancer. We evaluated the effects of statins on breast cancer stage migration and breast cancer-specific mortality in the Women’s Health Initiative (WHI) Clinical Trial and Observational Study. Methods: The study population included 128,675 postmenopausal women aged 50 to 79 years, with 7,883 newly-diagnosed pathologically-confirmed cases of in situ (19%), local stage (61%), regional stage (19%) and distant stage (1%) breast cancer and 401 deaths due to breast cancer after an average of 11.5 (SD=3.7) years of follow-up. To reduce the possibility of detection bias, we excluded women who did not report a mammogram within 5 years of study entry, and who had no health insurance or medical care provider (n=28,237). Stage was coded using criteria implemented in the Surveillance, Epidemiology and End Results Program and stratified into early stage (in situ and local) vs. late stage (regional and distant). Information on statin use prior to breast cancer diagnosis was collected at baseline and years one, three, six, and nine years post-baseline. Self- and interviewer-administered questionnaires were used to collect risk factor information. Cause of death was based on medical record review by physician adjudicators. Cox proportional hazards regression models were used to calculate hazard ratios (HR) with 95% confidence intervals (CI) to evaluate the relationship between statin use as a time-dependent exposure and diagnosis of late stage breast cancer and breast cancer-specific mortality adjusting for important confounders. For these models, participants who had early stage breast cancer or who died during follow-up were censored at time of diagnosis or death, respectively. We also evaluated the effect of statins stratified by estrogen receptor (ER) status. Statistical tests were two-sided. Results: Statins were used by 10,474 women (8%) at baseline. In the multivariable-adjusted time-dependent model, statin use was associated with a marginal reduction in risk of late stage breast cancer (HR 0.84, 95% CI: 0.70-1.02, p=0.082) and a reduction in risk of late stage estrogen receptor positive breast cancer (HR 0.79, 95% CI: 0.63-0.99, p=0.044). Statin use was also associated with a marginal reduction in breast cancer-specific mortality although it did not reach statistical significance (HR 0.59, 95% CI: 0.32-1.06, p=0.075). Conclusions: In the WHI, statin use was associated with a modest reduction in risk of late stage estrogen positive breast cancer and a non-significant decrease in breast cancer mortality. We are currently performing sensitivity analyses on these models. Studies using other large data sets with longer follow-up and information on cancer-directed therapy are needed to further evaluate this possible association.
Title: Histologic features of benign breast biopsy tissue and association with ER positive and ER negative breast cancer in the Mayo BBD cohort study

Amy C Degnim¹, Derek C Radisky¹, Robert A Vierkant¹, Ryan D Frank¹, Marlene H Frost¹, Vernon S Pankratz¹, Celine M Vachon¹, Tanya L Hoskin¹, Julie M Cunningham¹, Chen Wang¹, Jean-Pierre Kocher¹, Teresa M Allers¹, Joanne L Johnson¹, Tina J Hieken¹, Karthik Ghosh¹, Lynn C Hartmann¹ and Daniel W Visscher¹. ¹Mayo Clinic, Rochester, MN.

Body: Introduction: Current models to predict breast cancer risk do not differentiate risk for estrogen receptor (ER) positive and negative breast cancer (BC), despite growing evidence that these tumors are biologically very different. We hypothesized that women with ER+ BC cancers have different clinical risk factors and histologic findings on prior benign breast biopsies than those with ER- BC.

Methods: After IRB approval, we examined associations of age at benign biopsy and histologic features of the benign biopsy with ER status of incident BCs within the Mayo Benign Breast Disease Cohort. Benign biopsy slides were reviewed for extent of lobular involution and degree of epithelial proliferation by a single breast pathologist blinded to BC events. Invasive BCs occurring within 15 years after benign biopsy were classified as ER+ if ER staining was >1%. BC case-only associations with ER status were evaluated using multivariate logistic regression. Full-cohort hazard ratios (HR) and 95% confidence intervals (CI) for risk of ER-specific subtypes were estimated using Cox proportion hazards regression.

Results: Among 13,410 women undergoing a benign breast biopsy from 1967-2001, 656 invasive BCs (459 ER+, 106 ER, 106 unknown) occurred within 15 years. Women who developed ER+ and ER- BCs were similar in age at the time of their prior benign breast biopsy (p=0.34). Although benign biopsies in women who later developed ER+ BC were more likely to show complete involution (23% vs 15% for ER- BC), this was not statistically significant (p=0.06). However, the degree of epithelial proliferation was significantly associated with ER status of later BCs (p=0.001), with ER+ BCs more likely than ER- BCs to have had a prior biopsy with atypical hyperplasia (16% vs 8%), and ER+ BCs less likely than ER- BCs to have had a prior biopsy with proliferative disease without atypia (33% vs 52%); this association remained after multivariate adjustment (p=0.003). We further pursued the association of epithelial proliferation with differential risk of ER+ and ER- BC in our overall cohort of 13,410 women (Table 1).

Conclusion: ER+ and ER- breast cancers appear to have different features on prior benign breast biopsy, with atypical hyperplasia showing increased risk for both types of breast cancer, but a greater risk for ER+ tumors.
Title: The birth outcomes of pre-menopausal breast cancer survivors: Do they have a greater prevalence of delivering a preterm infant?

Kristin Z Black and Diane L Rowley. 1University of North Carolina, Gillings School of Global Public Health, Chapel Hill, NC.

Body: BACKGROUND: Due to the advancement of screening and treatment options for cancer, more people are able to live fruitful lives after a cancer diagnosis, yet for pre-menopausal breast cancer survivors the effects of disease and treatment on birth outcomes is not well documented. POPULATION: Linked North Carolina birth record-cancer registry data were used to examine the birth outcomes of pre-menopausal breast cancer survivors. Out of the 2,213,464 eligible live births that occurred between 1990 and 2009 in North Carolina, 539 of the mothers are breast cancer survivors and 10.6% (n=235,262) of the mothers experienced a preterm birth (which is below the national average of 12%). A vast majority of the women have a high school diploma or are college educated (81.4%; n=1,796,594), 14.0% (n=309,208) of the women reported that they smoked during pregnancy, and about two-thirds of the women were not married at the time of the birth of their child (67.7%; n=1,499,053). A majority of the study population is non-Hispanic White (62.6%; n=1,385,393) followed by non-Hispanic Blacks (24.0%; n=531,584), Hispanics/Latinos (9.7%; 215,224), and non-Hispanics of other races (3.7%; n=81,250). METHODS: The aim of this study was to determine if breast cancer survivors of reproductive age (ages 18-49) who had a live birth after their diagnosis have a greater prevalence of preterm birth than women who were not diagnosed with breast cancer. Binomial regression was used to estimate the exposure-outcome association in this case-cohort study. FINDINGS: The crude prevalence of preterm birth for pre-menopausal breast cancer survivors is 2.01 (95% CI: 1.71-2.36) times the crude prevalence of preterm birth for women who were not diagnosed with breast cancer. When the data were stratified by race/ethnicity, the prevalence of preterm birth for pre-menopausal breast cancer survivors compared to women not diagnosed with breast cancer within each racial/ethnic group is 2.27 (1.85-2.79) for Whites, 1.45 (1.10-1.91) for Blacks, 2.23 (0.64-7.81) for Hispanics/Latinos, and 1.83 (0.52-6.50) for other races. Controlling for the mother’s education level, marital status, and smoking status during pregnancy, the prevalence of preterm birth for pre-menopausal breast cancer survivors compared to women not diagnosed with breast cancer within each racial/ethnic group is 2.37 (1.93-2.91) for Whites, 1.50 (1.14-1.98) for Blacks, 2.28 (0.65-7.97) for Hispanics/Latinos, and 1.79 (0.51-6.31) for other races. CONCLUSION: Women diagnosed with breast cancer during their reproductive years are potentially at greater risk of experiencing a preterm birth and may benefit from targeted preconception health interventions.
2014 San Antonio Breast Cancer Symposium

**Title:** Background risk of breast cancer influences the association between physical activity and mammographic density

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**Body:**

**Background:** High physical activity has been shown to decrease the risk of breast cancer, partly by reducing mammographic density. We tested the hypothesis that the background risk of breast cancer influences the association between physical activity and mammographic density.

**Methods:** A total of 38,948 Swedish women aged 40-74 years reported their recent physical activity through the validated web-interactive self-administrated questionnaire Active-Q. Mammographic density was measured using the fully automated volumetric method Volpara™. Background risk of breast cancer was estimated using the Tyrer-Cuzick model. Linear regression analyses were performed to assess a potential association between physical activity and mammographic density adjusted for confounders, as well as effect measure modification by background breast cancer risk and menopausal status.

**Results:** We observed a statistically significant decline in absolute mammographic dense volume ($P$ for trend $<0.001$) with increasing levels of any type of physical activity among women at low risk of breast cancer. High levels of total and vigorous activities were associated with lower absolute dense volume (both $P$ for trend $<0.001$) among women with a moderate breast cancer risk. For women at high risk of breast cancer only vigorous activity was associated with lower absolute dense volume ($P$ for trend $= 0.01$). An inverse association with absolute dense volume was found between any type of physical activity among premenopausal women ($P$ for trend $<0.001$), and with total and vigorous activities among postmenopausal women ($P$ for trend $= 0.03$ and $0.01$, respectively). As anticipated, high physical activity was also associated with lower non-dense, that is fatty, volume ($P$ for trend $<0.001$). No consistent association was found for percent dense volume.

**Conclusion:** High physical activity was associated with lower absolute mammographic density. Our results suggest that physical activity may decrease breast cancer risk through reducing mammographic density and that high intensity activity may be required to reduce mammographic density among women at an increased risk of breast cancer.
History of chemoprevention in patients with newly diagnosed breast cancers

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Background: Chemoprevention (including tamoxifen, raloxifene and exemestane) is a strategy to reduce breast cancer incidence in high risk women. Studies have shown at least a 50% decrease in the incidence of breast cancer in users of these drugs. The benefit is limited to a reduction in the incidence of ER/PR positive breast cancer. However, there is a growing population of women who have used these agents for primary breast cancer prevention, and a larger population of breast cancer survivors who have used these drugs as part of their systemic treatment. The purpose of this study was to identify a contemporary cohort of women with newly diagnosed breast cancers who had utilized chemoprevention strategies and describe their patterns of disease.

Methods: The Breast Cancer Database at our institution was queried for patients who reported use of chemopreventive drugs and developed breast cancer between 1/2010-4/2014. Patients were divided into primary and secondary chemoprevention groups (no previous history of breast cancer and previous history of breast cancer, respectively). Descriptive statistics were utilized.

Results: Out of a total of 1782 patients with newly diagnosed breast cancer, there were 106 (6%) patients who had used a chemopreventive agent. Out of these 106 patients, 91 (86%), had used the drug as part of their systemic therapy for prior breast cancer, with a median of 11 years from the initial diagnosis to the diagnosis of a second breast cancer. The primary chemoprevention group included women with risk based on family history and atypical hyperplasias. The majority of patients (81%) were diagnosed with early stage disease (stage 0, 1). In both groups, the majority of cancers were ductal in origin (84%). Eight of the 15 patients (53%) in the primary chemoprevention group were on treatment at the time of their cancer diagnosis; while 29% of patients in the secondary group were on treatment at the time of diagnosis. In the secondary group, 49% were contralateral second primary breast cancers and 44% were ipsilateral recurrences. Interestingly, the majority of cancers in both groups were ER/PR positive.

Conclusions: Our cohort of women who used chemoprevention drugs were overwhelmingly diagnosed with early stage breast cancer, likely reflecting their commitment to screening. The majority of cancers were ER/PR positive. In this group, the choice of cancer treatment will need to be modified in light of prior hormonal treatment. Many of the patients in the secondary group were past users of prevention agents and further work is needed to clarify the duration of benefit of these drugs. In a similar vein, we look forward to research efforts to define the optimal age to initiate primary chemoprevention in high risk women.
Title: Longitudinal comparison of weight change in breast cancer survivors to cancer-free women: a prospective study in women with a familial risk of breast cancer

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Body: Background: Although postdiagnosis weight gain has been reported in breast cancer (BC) survivors from the general population, weight gain has not been studied in survivors with a familial BC risk who tend to be younger, treated with chemotherapy and more frequently diagnosed with an estrogen receptor (ER) negative BC. In addition, most studies of weight change in survivors have not included a comparison group of cancer-free women and therefore are unable to distinguish the impact of cancer or related treatment on weight gain versus increasing age. In this study we examine the change in weight among survivors compared to cancer-free women from the same high-risk cohort.

Methods: In an ongoing prospective cohort of women at Johns Hopkins with a family history of BC, ovarian cancer, and/or a BRCA1/2 mutation, we identified 303 survivors of incident stage 0-III BC and 552 cancer-free women who were ≥ 20 years at enrollment, completed a baseline questionnaire and at least one follow-up questionnaire within 4 years. Linear and logistic regression was used to estimate change in self-reported weight from baseline to follow-up between survivors and cancer-free women. Survivors were categorized based on time between BC diagnosis and baseline, as well as BC treatment categories.

Results: Greater than 60 percent of BC survivors were diagnosed under 50 years, and 22.3% had ER negative tumors. Neither weight nor body mass index (BMI) at baseline differed between survivors and cancer-free women; this was irrespective of tumor subtype. Survivors were more likely than cancer-free women to have ever used statins (19.5% in survivors vs. 14.9% in cancer-free women). Average change in weight during follow-up was 2.92 (95% CI 0.92, 4.91) pounds greater in survivors compared to cancer-free women after adjustment for age, baseline BMI, menopausal status, physical activity, statin use, and enrollment year. The greatest weight gain was observed in survivors diagnosed within 5 years prior to baseline, and treated with chemotherapy and not hormone therapy compared to cancer-free women. This group of survivors was 2.12 (95% CI 1.16, 3.90) times more likely than cancer-free women to have gained at least 11 lbs during follow-up. Further those survivors who had used statins gained significantly more weight compared to cancer-free statin users and non-users with and without cancer (p for interaction = 0.012); this increase in weight gain was largely driven by survivors who had received chemotherapy and statins (p for interaction = 0.008).

Conclusion: In this study, BC survivors recently diagnosed and treated with chemotherapy were twice as likely to gain at least 11 lbs, compared to cancer-free women. This amount of weight gain has been linked to an increased risk of heart disease and diabetes in women. Therefore survivors in these categories may benefit from early interventions aimed to reduce weight gain. Intriguingly, we observed a statistically significant interaction for statin use on weight gain in chemotherapy-treated survivors, which may reflect an underlying biological interaction between these agents in BC survivors. Given the prevalence of statin use in this population, this needs to be explored further.
Title: Metformin increases survival in hormone receptor-positive, Her2-positive breast cancer patients with diabetes

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Body: Purpose
Metformin use has recently been observed to decrease both the rate and mortality of breast cancer. Our study was aim to determine whether metformin use is associated with survival in diabetic breast cancer patients by breast cancer subtype and systemic treatment.

Patients and Methods
Data from the Asan Medical Center Breast Cancer Database from 1997 to 2007 were analyzed. The study cohort comprised 6,967 nondiabetic patients, 202 diabetic patients treated with metformin, and 184 diabetic patients that did not receive metformin. Patients who were divided into three groups by diabetes status and metformin use were also divided into four subgroups by hormone receptor and HER2-neu status.

Results
In Kaplan-Meier analysis, the metformin group had a significantly better overall and cancer specific survival outcome compared with non metformin diabetic group (\(P <0.005\) for both). There was no difference in survival between the nondiabetic and metformin groups. In multivariate analysis, Compared with metformin group, patients who did not receive metformin tended to have a higher risk of metastasis with HR 5.37 (95%CI, 1.88 to 15.28) and breast cancer death with HR 6.51 (95% CI, 1.88 to 15.28) on the hormone receptor-positive and Her2-negative breast cancer. The significant survival benefit of metformin observed in diabetic patients who received chemotherapy and endocrine therapy (HR for DFS 2.14; 95% CI 1.14 to 4.04) was not seen in diabetic patients who did not receive these treatments.

Conclusion
Patients receiving metformin treatment when breast cancer diagnosis show a better prognosis only if they have hormone receptor-positive, HER2-positive tumors. Metformin treatment might provide a survival benefit when added to systemic therapy in diabetic patients.
Title: Diabetes medications and risk of incident breast cancer

Denise M Boudreau¹, Onchee Yu¹ and Joann G Elmore². ¹Group Health Research Institute, Seattle, WA and ²University of Washington, Seattle, WA.

BACKGROUND: Studies indicate a 20% increased risk of breast cancer (BC) among women with diabetes vs without. This is concerning given that type 2 diabetes is becoming the most common chronic disease in the US. Medications used to treat diabetes have the potential to further alter risk with metformin holding promise for reducing BC risk while sulfonylureas and insulin are hypothesized to increase risk. The objective of this study was to evaluate the association between diabetes medications and risk of invasive BC among a unique US population based cohort of women with diabetes and complete data on screening, BMI, and standardized risk factors usually lacking in other populations.

METHODS: Retrospective cohort study of women ≥40 years of age with diabetes, no prior BC, and enrolled in a large health plan in WA State during 1996-2011. Data sources were electronic health records including diagnoses, medication fills, lab values, and mammography screening; WA state SEER tumor registry; state death tapes; and a GH survey on risk factors for breast cancer. Women were followed to the earliest of death, disenrollment, prophylactic mastectomy, invasive BC diagnosis, or end of study (12/31/2012). Multivariable Cox proportional hazards models were used to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for "ever" use and duration of metformin, sulfonylurea, insulin, and other oral diabetes medication use compared to respective non-user groups while accounting for potential confounders including those not commonly available in prior studies (i.e., BMI and mammography screening).

RESULTS: 10,050 diabetic women (mean age, 62 yrs) were followed for a median of 7 yrs. 57% used metformin, 43% sulfonylureas, 32% insulin, and 1.5% other medications (non-mutually exclusive) during the 2 years prior to cohort entry through end of follow-up. During follow-up, 301 BC cases (85% stage 1-2, 9% stage 3, 2% stage 4, 4% unknown) were diagnosed. Results suggested a potential but non-significant decrease in BC with metformin use (HR=0.86; 95% CI: 0.65-1.12). Insulin use was associated with a decreased risk of BC (HR=0.67; 95% CI: 0.50-0.91) vs. non-users. Long acting insulin, taken by 24% of insulin users, was not associated with risk of BC (HR=0.95; 95% CI: 0.51-1.77) but other types of insulin were associated with a decreased risk (HR=0.69; 95% CI: 0.50-0.94). The point estimate for sulfonylureas (HR=1.18; 95% CI: 0.90-1.53) suggested a potential increase in BC but CIs included 1.0. Linear trends supported a reduced risk of BC with increasing duration of insulin use and an increased risk of BC with increasing duration of sulfonylurea use. Subgroup analyses adjusted for BMI yielded similar results but adjustment for screening altered some results (i.e., insulin no longer associated with a reduced risk of BC - HR=0.94; 95% CI: 0.54-1.65; point estimate for sulfonylureas not suggestive of an increased risk– HR=0.96; 95% CI: 0.62-1.48).

CONCLUSION: Diabetes medications appear safe with respect to BC risk but metformin’s potential as a preventive agent is questionable. Further research is warranted to understand potential differences in risk by insulin type as well as the degree to which any observed differences in BC risk by medication use are attributable to differences in mammography screening.
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Average Grade: 5.25

Title: Association between mammographic breast density and histologic features of benign breast disease

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Body: Background: Mammographic breast density (MBD) reflects the proportion of fibroglandular tissue in the breast, and is an established risk factor for breast cancer. Benign breast disease (BBD) is also a known risk factor for breast cancer. We previously showed an association between lobular involution (physiologic atrophy) and MBD in a large cohort study of women with BBD. We performed a comprehensive evaluation of the association of histologic features of BBD with MBD. Methods: The Mayo BBD cohort includes women, 18 to 85 years, who were diagnosed with BBD at Mayo Clinic in Rochester, MN between 01/01/1967 and 12/31/1991. For this study, we included women who had a mammogram within 6 months of BBD diagnosis, and diagnosed with BBD between 1985 and 1991 when parenchymal pattern of breast density was clinically recorded. Risk factor information such as age, and body mass index (BMI) was collected from medical records. Parenchymal pattern, assessed clinically and previously used in multiple studies of MBD and risk, classified breast density based on extent and type of density, into four categories: N1 (non-dense, no ducts visible); P1 (ductal prominence occupying <25% breast); P2 (prominent ductal pattern occupying >25% of the breast; DY (homogenous plaque-like areas of density). All assessments of benign breast tissue were performed by expert breast pathologists blinded to both MBD and BBD reports. Histologic characteristics included overall impression (non-proliferative disease (NPD), proliferative disease without atypia (PDWA), and atypical hyperplasia (AH)), proportion of normal lobules that were involuted (no (0%), partial (1 to 74%), or complete (≥75%)), type of AH (ADH or ALH), ductal and lobular hyperplasia, calcifications, sclerosing adenosis, columnar alteration, cyst, fibroadenoma, marked fibrosis, intra-ductal papilloma, radial scar (number and size), duct ectasia, and mucocele like tumors. Associations of parenchymal pattern with BBD characteristics were examined using multicategorical nominal logistic regression models. We first examined associations after adjustment for age and BMI. All variables statistically significant (p<0.05) in these models were then simultaneously included in a fully-adjusted model. Results: Of 2,257 women in the study, 14% were <40 years old, 52.7% between 40- 59 years, and 32.9% were ≥60 years. In this sample, 55.2% of women had NPD, 36% had PDWA, and AH in 8.8%. MBD was classified as N1 in 21.8%, P1 in 12.6%, P2 in 23.7% and DY in 41.9%. Age- and BMI-adjusted analyses showed that there was an association of parenchymal pattern with overall impression, lobular involution, ductal hyperplasia, sclerosing adenosis, columnar alteration, cyst, duct ectasia and fibrosis. Multivariate analyses found that women of younger age (p<0.001) and with lower BMI (p<0.001), presence of fibrosis (p<0.001) and no involution (p=0.016) were more likely to have P2 or DY parenchymal pattern than women without those characteristics. Conclusion: Among women with benign breast disease, higher MBD is associated with younger age, lower BMI, fibrosis, and lack of lobular involution. These findings, concordant with other studies of MBD, provide insight into underlying mechanisms by which MBD and BBD contribute to breast cancer risk.
Association between vitamin D supplementation and mammographic density change over time in women at high risk for breast cancer

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Body: Background: Vitamin D deficiency has been linked to breast cancer risk, but less is known about vitamin D and changes over time in mammographic density (MD), a strong predictor of breast cancer risk. Studies that have evaluated the association between MD and vitamin D have primarily been cross-sectional designs and focused on average-risk postmenopausal women.

Methods: Using data from a prospective cohort study (1991-2013), we examined whether vitamin D supplementation was associated with MD at baseline and changes in MD over time. High-risk women had a first-degree family history of breast cancer, atypical hyperplasia, lobular or ductal carcinoma in situ. They completed baseline questionnaires with self-reported vitamin D supplement use (Y/N) and had serial mammograms with qualitative assessment of MD (BIRADS categories: 1=0-24%, 2=25-50%, 3=51-75%, 4=76-100%). GEE logistic regression and unordered polytomous regression models were used to assess the association between change in MD in the short-term (<3 years) and long-term (≥3 years) with vitamin D use (stayed dense: BIRADS 3/4 for both exams; stayed nondense: BIRADS 1/2; increased: BIRADS 1/2 to 3/4; decreased: BIRADS 3/4 to 1/2). Primary confounders were included in every model (age, race, body mass index [BMI], menopausal status) and other additional confounders were selected based on 10% change-of-coefficient rule.

Results: Of 1171 women who had vitamin D supplement information and a baseline mammogram, 615 had two mammograms within 3 years from baseline and 461 had a long-term follow-up mammogram. Median age was 49 (range, 17-88), median BMI 23.6 kg/m\textsuperscript{2} (range, 14.9-53.4), and mean follow-up time 6 years (range, 9 months-18 years). Among women with a BMI<25, no vitamin D supplementation was associated with dense baseline MD (BIRADS 3/4) after adjusting for age, race, menopausal status, and annual household income (OR=1.61, 95% CI=1.12-2.33). Those who reported vitamin D use were about 50% less likely to demonstrate long-term increases in MD (see table below).

Vitamin D and short-term and long-term mammographic density changes

<table>
<thead>
<tr>
<th>Vitamin D</th>
<th>Stay dense</th>
<th>Increase</th>
<th>Decrease</th>
<th>Stay nondense</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n; OR (95% CI)</td>
<td>n; OR (95% CI)</td>
<td>n; OR (95% CI)</td>
<td>n; OR (95% CI)</td>
</tr>
<tr>
<td>Vitamin D*</td>
<td>291</td>
<td>36; 0.80 (0.38-1.66)</td>
<td>60; 1.00 (0.53-1.89)</td>
<td>209; 1.41 (0.91-2.17)</td>
</tr>
<tr>
<td>Vitamin D**</td>
<td>291</td>
<td>36; 0.82 (0.37-1.80)</td>
<td>60; 0.74 (0.37-1.47)</td>
<td>209; 1.37 (0.87-2.16)</td>
</tr>
<tr>
<td>Vitamin D***</td>
<td>211</td>
<td>32; 0.46 (0.20-1.04)</td>
<td>69; 1.08 (0.58-1.99)</td>
<td>134; 1.32 (0.78-2.23)</td>
</tr>
<tr>
<td>Vitamin D****</td>
<td>211</td>
<td>32; 0.49 (0.21-1.14)</td>
<td>69; 1.12 (0.60-2.11)</td>
<td>134; 1.34 (0.78-2.30)</td>
</tr>
</tbody>
</table>

*Short-term: adjusted for age, BMI, race and menopausal status; **Short-term: additional adjustment for highest education level, annual household income; ***Long-term: adjusted for age, BMI, race and menopausal status; ****Long-term: additional adjustment for highest education level, age of first childbirth, time intervals from the first to the last mammogram

Discussion: Although vitamin D supplementation was not associated with short-term changes in MD, we did observe a trend toward an association with long-term change among high-risk women. If replicated in larger studies, our study gives added evidence that MD changes may need longer observation time.
Title: Characterization of the tumor and risk factors in Japanese young breast cancer patients

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Body: BACKGROUND AND OBJECTIVES: Breast cancer in young patients (<= 40 years) (YBC) is an uncommon disease and is the leading cause of cancer death in women in their age. The objective of this study was to characterize and identify potential differences in the molecular and clinical features of breast carcinomas from patients <=40 years versus a cohort of older premenopausal counterparts as a control matched by breast cancer subtype. METHODS: We performed a retrospective analysis of a prospectively maintained database that included 427 premenopausal patients diagnosed with an invasive breast carcinoma from 2005 to 2013 at University of Tsukuba Hospital. We selected 286 patients separated in two groups, the study group with young women <=40 years old and with no or unknown BRCA mutation, and a second group with women over 40 years. Data related to clinical and pathological features from both groups such as tumor size, nodal status, histological grade, Ki 67 labeling index, estrogen and progesterone receptor, HER2 overexpression, and pregnancy related information were obtained from medical records and we used the statistic model of chi-squared to compare the two groups. Survival analysis was performed using the Kaplan Meier method. RESULTS: The median age of the 93 patients <= 40 year was 34.6 years, and 193 patients >40 years were included in the control group (median age 46.4 years). There were 22 pregnancy associated breast cancer (PABC) cases in YBC cohort. Ductal carcinoma was the most common histological subtype in both groups. By subtype, 65% of YBC presented an immunohistochemical luminal subtype, compared to 41% in PABC and 77% in older patients (p=0.01). Triple negative and HER2 profiles were 19% and 16% in YBC versus 36% and 23% in PABC and 12% and 11% in control respectively (p=0.01). The YBC cohort had a larger tumor (24% of YBC had size tumor > 5 cm, versus 9% in control, p=0.01), and more frequent nodal involvement (48% in YBC vs 39% in control, p<0.05), the higher proportion of Ki67 >30% (35% in YBC vs 24% in control, p<0.05). Limiting to YBC, subtype nor PABC were not identified as an independent risk factor for disease free survival, but luminal subtype in YBC demonstrated worse survival compared with those in control. CONCLUSIONS: We observed more aggressive tumor characteristics in patients diagnosed with breast cancer at <=40 years, or PABC patients compared to older premenopausal patients. Clinical implications of age on tumor behavior were different according to subtypes. Further research to overcome worse survival for luminal YBC is warranted.
Title: Psychosocial adjustment, cancer worry and perceived risk in 6-13 year old girls from breast cancer families

Angela R Bradbury¹, Linda Patrick-Miller⁴, Brian Egleston⁷, Lisa Schwartz⁵, Colleen B Sands¹, Wendy Chung⁵, Gord Glendon¹⁰, Lisa Tuchman⁶, Cindy Moore⁷, Paula Rauch⁷, Irene Andrilis¹⁰, Saundra Buys⁸, Caren J Frost⁸, Esther M John⁹, Theresa Keegan⁹, Julia Knight¹⁰, Mary Beth Terry⁴ and Mary Daly³. ¹University of Pennsylvania, Philadelphia, PA; ²Children’s Hospital of Philadelphia, Philadelphia, PA; ³Fox Chase Cancer Center, Philadelphia, PA; ⁴University of Chicago, Chicago, IL; ⁵Columbia University, New York, NY; ⁶Children’s National Medical Center, Washington, DC; ⁷Partners Healthcare; ⁸University of Utah; ⁹Cancer Prevention Institute of California, Fremont, CA and ¹⁰Lunenfeld-Tanenbaum Research Institute.

Body: Background: Many parents discuss familial and genetic risk of breast cancer (BC) with offspring. What girls know and perceive of BC risk and its psychosocial impact is unknown.

Methods: In the multisite LEGACY Girls Study, 6-13 YO daughters and their parents/guardians from BC families (FH+) and families without BC (FH-) were recruited to examine early determinants of, and responses to BC risk. Parents/guardians completed surveys reporting on their daughters’ psychosocial adjustment (PSA). Mothers and daughters 10-13 YO completed surveys reporting their PSA and perceptions of breast cancer risk. We used linear and logistic regressions with variable selection.

Results: 731 parents/guardians reported on their own and the PSA of 898 daughters (450 FH+, 448 FH-), and 447 girls (10-13 YO) completed surveys (227 FH+, 220 FH-). FH+ girls have lower somatization and internalizing behaviors by parent/guardian report than FH- girls (Table 1). Intrusive BC (IBC) worry, and avoidant BC (ABC) worry were significantly higher in FH+ girls. IBC worry (coef=0.8, p=0.04) and ABC worry (coef 1.8, p=0.007) were higher in daughters whose mother had BC. Daughter perceived stress (PS), anxiety, depression, somatization, and internalizing did not differ by mother BC history. In multivariable models, daughter-anxiety was associated with mother-anxiety (Coef 1.4, p<0.0001) and BRCA+ family (coef -13.5, p=0.040). Daughter-depression was associated with mother-depression (coef 1.0, p=0.009). Daughter-IBC worry was associated with mother-IBC worry (coef 0.2, p<0.001). Daughter-ABC worry was associated with mother-IBC worry (coef 0.2, p=0.002) and being FH+ (coef 1.3, p=0.02). Daughter-PS was associated with mother-depression (coef 0.2, p=0.003). Somatization was not associated with any variables in multivariable models. FH+ girls were more likely to report themselves at higher risk for BC, although many reported uncertainty about their own BC risk.

Table 1

<table>
<thead>
<tr>
<th>Daughter Psychosocial Adjustment</th>
<th>FH+ Mean(SD)</th>
<th>FH-Mean(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>By parent/guardian report</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>46.7 (30.3)</td>
<td>49.1 (29.1)</td>
</tr>
<tr>
<td>Depression</td>
<td>51.2 (27.2)</td>
<td>52.8 (27.3)</td>
</tr>
<tr>
<td>Somatization</td>
<td>43.9 (30.7)**</td>
<td>50.3 (30.7)**</td>
</tr>
<tr>
<td>Internalizing</td>
<td>46.1 (29.6)*</td>
<td>50.8 (29.1)*</td>
</tr>
<tr>
<td><strong>Daughter reported</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>40.2 (28.5)</td>
<td>42.4 (29.5)</td>
</tr>
<tr>
<td>Depression</td>
<td>32.4 (26.2)</td>
<td>32.4 (27.7)</td>
</tr>
<tr>
<td>Somatization</td>
<td>36.5 (25.5)</td>
<td>44.0 (27.7)</td>
</tr>
<tr>
<td>Internalizing</td>
<td>33.1 (25.5)</td>
<td>34.0 (26.7)</td>
</tr>
<tr>
<td>IBC Worry</td>
<td>2.0 (3.4)**</td>
<td>1.2 (2.4)**</td>
</tr>
<tr>
<td>ABC Worry</td>
<td>3.8 (5.8)**</td>
<td>2.0 (4.3)**</td>
</tr>
<tr>
<td>Perceived Stress</td>
<td>4.8 (2.8)</td>
<td>4.6 (2.8)</td>
</tr>
<tr>
<td><strong>Daughter Perceived BC Risk</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n=219</td>
<td>n=209</td>
<td></td>
</tr>
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</table>
**Conclusions:** Pre-adolescent girls from BC families have lower somatization and internalizing behaviors by parent report, but higher self-reported BC worry. Daughter general anxiety, depression and BC worry are associated with corresponding mother affect. Some girls from BC families are aware of their increased risk and related research suggests this may increase with age. Understanding how PSA and BC worry changes over time and the impact on health and risk behaviors can inform interventions to optimize responses to growing up in families at familial and genetic risk for breast cancer.
Bone mineral density (BMD) monitoring in postmenopausal women with early stage estrogen and/or progesterone receptor positive breast cancer on aromastase inhibitor (AI) therapy at University of Toledo medical center. A quality improvement project

Asma Taj1, Sadik Khuder1 and Iman Mohamed1. 1University of Toledo, Toledo, OH.

Body: Background
More than 70% of breast cancer patients develop endocrine-responsive disease with estrogen receptor (ER)-positive or progesterone receptor-positive tumors or both [1] and require endocrine treatment with either estrogen blockage or ablation. The aromatase inhibitors are highly effective well-tolerated treatment for postmenopausal endocrine-responsive breast cancer. However, their use is associated with accelerated bone loss and an increase in fracture risk (2, 3).

Method
Post menopausal patients with early stage breast cancer (stage I and stage II) which was ER and/or PR positive and who were on AI therapy were included. Total patients 243. Stage I (101) and stage II (142). Agents used were Anastrazole, letrozole, exemestane. BMD (bone mineral density monitoring) was done by DEXA (Dual-energy X-ray absorptiometry) scan, and compared with national guidelines.

Results
243 patients were included. At 0 years (at start of AI therapy) 150 (62%) did not get a DEXA scan, 40 (16.4%) had normal DEXA scan, 48 (19.8%) had osteopenia and 5 (2%) had osteoporosis. At 2 years from AI therapy 126 (51.8%) did not get a DEXA scan, 35 (14.4%) had normal DEXA scan, 73 (30%) had osteopenia and 9 (3.7%) had osteoporosis. At 5 years from AI therapy 180 (74%) did not get a DEXA scan, 21 (8.6%) had normal DEXA scan, 35 (14.4%) had osteopenia and 7 (2.8%) had osteoporosis.

Anastrazole and letrozole were equally associated with osteopenia/osteoporosis. Only 50% of patients with osteoporosis (10/21) received treatment for osteoporosis.

Conclusions
Although there is evidence of negative impact of the aromatase inhibitors on bone, our data still show a poor application of the recommendations in order to prevent osteoporosis related to the administration of these drugs. Part of the problem is the mixed literature on diagnosis and management of osteoporosis. Also medicare covers for a DEXA scan only once very two years. The national compliance for managing AI induced bone loss is very low which points out that this is a global problem. Our suggestion is a more active implementation of the guidelines, also by means of a greater collaboration between the oncologist and the specialist in osteoporosis, and the offer of a diagnostic and therapeutic pathway.
Title: A broad spectrum therapeutic strategy for TNBC revealed by a new pathway that coordinates oncogenic RTKs

Body: Triple-negative breast cancer (TNBC) is a collection of diseases with distinct clinical behaviors and heterogeneous molecular features. Such clinical and genetic heterogeneity has called into question whether there are common pathogenic mechanisms (and potential therapeutic targets) driving the TNBC subtype of breast cancer. Herein, we present evidence of a novel tumor suppressor network that is frequently compromised in TNBC, and a broadly-effective strategy to target this pathway for TNBC therapeutic intervention. Using an unbiased genetic screen, we identified a tumor suppressor network governing tumor survival of TNBCs \textit{in vitro} and \textit{in vivo}. We define the tyrosine phosphatase PTPN12 as a core component in this network. PTPN12 is a potent suppressor of mammary epithelial cell survival and transformation, and PTPN12 function is compromised in more than 70% of human TNBCs. Notably, the tumorigenic and metastatic potential of PTPN12-deficient TNBCs is severely impaired by restoring PTPN12, suggesting that strategies to mimic PTPN12 function have substantive therapeutic potential. Using integrative proteomic, genetic, and pharmacologic approaches, we demonstrate that PTPN12 suppresses TNBC survival by inhibiting multiple oncogenic receptor tyrosine kinases (TKs) including MET, PDGFR\(\beta\), and others. Frequent inactivation of PTPN12 in human TNBC unleashes these oncogenic TKs in a concerted manner. Importantly, combination inhibitors targeting these PTPN12-regulated TKs significantly impair TNBC cell survival and confer robust tumor regression across a panel of 18 patient-derived xenograft ("PDX") models of human TNBC. This suggests that TNBCs are broadly dependent on a distinct combination of proto-oncogenic tyrosine kinases constrained by PTPN12. Collectively, these data identify PTPN12 as a commonly inactivated tumor suppressor in TNBC and provide a rationale for combinatorially targeting select receptor tyrosine kinases in TNBC and other cancers based on their defects in tyrosine phosphatase activity.
Body: Background: Veliparib, an inhibitor of Poly(ADP-ribose) polymerase (PARPi), in combination with DNA-damaging agents showed significant efficacy, especially in triple negative breast cancer (TNBC) patients. However, in I-SPY 2, approximately 42% of TNBC did not optimally respond to the veliparib based treatment. To exert therapeutic effect, drugs such as veliparib or the DNA damaging agent carboplatin must reach cancer cells at an adequate concentration. Differences within the microenvironment of the tumor (e.g. reduced vascular density and leaky endothelium) are responsible for variability in the uptake and distribution of the drug in the tumor. We hypothesize that heterogeneous or insufficient drug concentrations in the solid tumor lead to inadequate response to PARPi in some TNBC patients. First we performed a mouse xenograft study to determine the penetration and distribution of veliparib and carboplatin in TNBC tumors in mice.

Methods: 1*10^6 MDA.MB.231, HCC70 or MDA.MB.436 cells were injected bilaterally into mammary fat pad of a total of 36 Beige SCID mice. When the tumors exceeded 200 mm3, veliparib (20mg/kg or 60mg/kg) or placebo was orally administered 3 times daily for 3 days + Carboplatin 60mg/kg on day 1+2. These doses and dosing schedules were estimated to result equivalent plasma and tumor concentrations in mice compared to the doses currently used in clinical trials. Mice were euthanized 3 hours after the last dose of veliparib and tumors, and muscle tissues were obtained. In addition, control tissues were spiked with a broad range of concentrations of veliparib. The spatial distribution of the drugs in the tumor tissue was measured using Matrix-assisted laser desorption/ ionization mass spectrometric imaging (MALDI). The protonated molecular ion at m/z 245.1 was used to construct 2D ion maps of the tissue showing relative quantitation of drug levels. H&E staining was performed to characterize the tumors.

Results: Veliparib in control tissues was detected at very low concentrations with a range of detection between 100 fmol-1nmol. After dosing, veliparib penetrates into the tumors and was detectable at both dose levels. However, drug distribution within the tumors was observed to be inhomogeneous. In spots where the drug accumulated, necrosis was observed. Veliparib accumulated in spots in the tumor and near the rim of the tumor. Differences between cell lines were observed, with the largest accumulation at the rim in BRCA mutated cells.

Conclusions: Veliparib can be measured using MALDI with good specificity and sensitivity and using concentrations equivalent to patients. TNBC tumors show largely heterogeneous distribution of PARPi, with accumulation in the pushing margings of the tumor. Future analyses will determine whether this heterogeneity in drug distribution explains variability in response to PARPi therapies.
**Title:** HACE1 loss results in hyperactive Rac signaling conferring resistance to HER2 targeted therapies

Erik T Goka¹, Dana S Senderoff¹, Gustavo Munguba¹ and Marc E Lippman¹. ¹University of Miami Miller School of Medicine, Miami, FL.

**Body:** HER2-targeted therapies have been instrumental in improving the treatment of HER2+ breast cancer. However, drug resistance usually emerges and remains a big challenge in using anti-HER2 therapies. Previous studies have implicated activation of the Rho GTPase Rac1 by PTEN loss or IGF-1R overexpression as a mechanism of resistance to HER2-targeted therapies. We identified HACE1, an E3 ubiquitin ligase for Rac1, as a tumor suppressor capable of cooperating with HER2 to transform normal mammary epithelial cells. While HACE1 loss alone resulted in enhanced Rac activation, HER2 activation of Rac1 combined with HACE1 loss resulted in even higher levels of activated Rac resulting in the ability to form tumors in immunocompromised mice. In this study, we show that loss of HACE1 confers resistance to the HER2 tyrosine kinase inhibitor (TKI) Lapatinib due to sustained Rac signaling irrespective of EGFR/HER2 signaling. While Lapatinib inhibition alone is capable of attenuating proliferation of HER2 overexpressing mammary epithelial cells, HACE1 loss continues to drive proliferation of in vitro tumor formation (clonogenicity) as well in vivo tumor growth. We demonstrate that Lapatinib sensitivity can be restored using a Rac inhibitor in HACE1 knockdown cells. Moreover, while the Rac inhibitor alone was capable of attenuating the effects of HER2 overexpression and HACE1 loss, simultaneous inhibition of both HER2 and Rac signaling was superior to either monotherapy alone. These results support Rac activation as a mechanism of resistance to HER2-targeted therapies and highlight the use of a Rac inhibitor to treat HER2+ refractory breast cancers.
Title: OOTR-N007: A phase II neoadjuvant study of letrozole plus palbociclib in postmenopausal patients with ER positive, HER2 negative breast cancer

Louis WC Chow\textsuperscript{1,2,3}, Chi-Kei Lam\textsuperscript{2} and Wings TY Loo\textsuperscript{2}. \textsuperscript{1}Organisation for Oncology and Translational Research, Hong Kong; \textsuperscript{2}UNIMED Medical Institute, Hong Kong and \textsuperscript{3}Institute for Applied Research in Medicine and Health, Macau University of Science and Technology, Macau.

Body: Background: CDK4-associated kinase activity is required to maintain breast tumorigenesis. Virtually all ER-positive cell lines harbour loss of p16\textsuperscript{ink4a}. Low expression of CDK inhibitors p21 and p27 and high expression level of cyclin E and D1 have all been associated with resistance to anti-estrogen therapy. In preclinical models, Rb deficiency is associated with resistance to antiestrogen therapy. Palbociclib (PD 0332991, Pfizer Inc.) is an oral, potent, highly selective reversible inhibitor of CDK4/6 that prevents cellular DNA synthesis by prohibiting progression from G1 into the S phase. Palbociclib in combination with letrozole has shown promising activity in metastatic setting.

Methods: In an open-label, multi-center, single arm pilot study efficacy and safety of neo-adjuvant palbociclib 100 mg QD for 3 weeks plus letrozole 2.5 mg QD in 4 week cycles for 4 months was studied. Postmenopausal patients of mostly Chinese ancestry, with histologically confirmed ER+, HER2- invasive breast cancer and tumor greater than 2 cm were registered. Patients with T3N1, T4 or any N2, N3 were excluded.

Aim: The primary endpoint is Objective Response Rate (ORR). The secondary end-point include Pathologic Response Rate (PRR), Disease-Free Survival (DFS), safety. Exploratory analysis of gene and protein expression in tumors and serial whole blood is planned.

Results: As of June 2014, 11 patients were recruited. 9 patients completed treatment and 2 are still on study. Of the 9 patients that completed study at time of this abstract, 1 patient had a complete pathological response (pCR) and 7 had a partial response (ORR of 89%). Baseline Ki67 levels were low (median 18%), consistent with luminal type A disease. On average, patients underwent surgery 24 days after completion of the study. Ki67 was measured at baseline, cycle 1 day 15, and at the time of surgery. Median Ki67 at time of surgery was 10%. There were no significant changes in Ki67 that could be related to treatment outcome based on this preliminary data analysis in surgical samples only. In the first 8 patients, whom all started at a dose of 125mg palbociclib, 4 patients developed grade 3/4 neutropenia in the absence of fever. Neutropenia was well manageable with G-CSF and/or dose modification. Protocol amendment allowed starting dose of 100 mg with dose titration to 125mg after the first cycle. 3 patients started at 100mg and grade 4 neutropenia without fever occurred in 1 subject. Another common side effect was low grade mucositis (n=5).

Conclusion: Based on these initial results, the addition of palbociclib to neo-adjuvant letrozole appears to be safe and effective. Historically, ORR in this patient population with letrozole alone is below 55%. The addition of palbociclib increased the preliminary ORR to 89%. One patient had a pCR, uncommon for patients on neo-adjuvant endocrine therapy. Complete safety and efficacy data will be included for at least 11 patients, including initial biomarker results. The study continues enrolment up to 45 patients.
Title: Tumor priming with cyclophosphamide for enhanced tumor delivery, penetration and anti-tumor activity of MM-302, HER2-targeted liposomal doxorubicin

Elena Geretti\textsuperscript{1}, Shannon C Leonard\textsuperscript{1}, Nancy Dumont\textsuperscript{1}, Christopher W Espelin\textsuperscript{1}, Daniel F Gaddy\textsuperscript{1}, Thomas J Wickham\textsuperscript{1} and Bart S Hendriks\textsuperscript{1}. \textsuperscript{1}Merrimack Pharmaceuticals, Inc, Cambridge, MA.

Body: MM-302, HER2-targeted PEGylated liposomal doxorubicin, is a liposomal antibody drug conjugate designed to target doxorubicin to HER2-overexpressing cancer cells, while limiting uptake into non-target cells. Effective tumor delivery and penetration are critical barriers to the clinical activity of nanomedicines, including liposomes. Cyclophosphamide has been successfully combined with both doxorubicin and liposomal doxorubicin in breast cancer therapy, with the two drugs being administered on the same day to patients. We have evaluated a novel sequential combination regimen of cyclophosphamide with MM-302 with the goal of improving tumor delivery and penetration of MM-302.

Methods: Biodistribution studies were carried out in multiple tumor xenograft models to assess the delivery of MM-302 and free doxorubicin, either as single agents or in combination with cyclophosphamide at different dosing schedules. The total doxorubicin within tumors was quantified by HPLC and microscopically by determining the number of doxorubicin-positive nuclei within frozen tumor sections. Induction of DNA damage/repair, tumor cell apoptosis, and changes in the tumor architecture in response to drug treatment (tumor cell density and vascular parameters) were quantified by automated image analysis. Interstitial fluid pressure measurements were carried out to evaluate changes in the tumor physiology upon cyclophosphamide treatment. Anti-tumor activity studies in BT474-M3 tumor-bearing mice were performed to evaluate the ability of the different dosing regimens to inhibit tumor growth.

Results: Pre-dosing of tumors with cyclophosphamide enhanced subsequent MM-302 delivery to tumor xenografts (2-3-fold) without affecting delivery to non-target tissues, such as the heart and skin. We demonstrate that this effect is critically dependent on the timing of cyclophosphamide administration. Analysis of cyclophosphamide-treated tumors suggests that the mechanism for improved MM-302 delivery involves the induction of tumor cell apoptosis, reduction of overall tumor cell density, substantial lowering of interstitial fluid pressure and increase in vascular perfusion. Finally, treatment of tumors xenografts with cyclophosphamide followed by MM-302 resulted in a significantly greater tumor growth inhibition compared to either single agent alone.

Conclusions: Rational combination of MM-302 with cyclophosphamide results in an active and tolerable regimen in preclinical models that enhances the tumor delivery and activity of MM-302, without affecting doxorubicin exposure to non-target organs. This novel sequential dosing strategy represents an advance in addressing the critical challenge for tumor delivery of nanomedicines. This work provided data supporting the initiation of a clinical evaluation of the effect of cyclophosphamide on MM-302 delivery as part of an on-going Phase 1 clinical trial of MM-302 in HER2-positive metastatic breast cancer (http://clinicaltrials.gov/show/NCT01304797).
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-11-06
Average Grade: 0

Title: VS-6063 (defactinib) and VS-4718 reduce cancer stem cells in models of breast cancer: Implications for clinical trials in the neoadjuvant setting

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Body: Cancer stem cells (CSCs) are an underlying cause of tumor progression and metastasis. In breast cancer, CSCs can be identified by Aldehyde Dehydrogenase 1 (ALDH) or CD44-high/CD24-low expression. Neoadjuvant chemotherapy has been shown to lead to an increase in CSCs in locally advanced breast cancer (Alamgeer et al., 2014, Br. Can. Res. R14). In addition, the presence of CSCs in residual axillary disease is associated with a significantly worse prognosis following neoadjuvant chemotherapy and surgery (Sakakibara et al. 2011, Cancer 3899, 2011). Currently, there are no approved therapies that effectively target and kill CSCs. VS-6063 and VS-4718 are orally bioavailable small molecules that kill cancer stem cells through the inhibition of Focal Adhesion Kinase (FAK). Both VS-6063 and VS-4718 have demonstrated preferential targeting of CSCs in preclinical models and are currently in clinical development.

Here we report that VS-6063 and VS-4718 effectively kill CSCs in multiple models of breast cancer. In an ex vivo model, biopsies from human breast tumors were obtained and cultured as primary explants within 24 hours of surgery. The primary explants were incubated with VS-6063, VS-4718 or paclitaxel for 4 days. Treatment with either VS-6063 or VS-4718 decreased the proportion of CSCs in contrast to paclitaxel. VS-6063 and VS-4718 diminished the self-renewal capacity of primary cultures from established TNBC patient-derived xenografts as measured by tumorsphere assays. In a MDA-MB-231 mouse xenograft model, treatment with VS-6063 decreased CSC more than 6-fold in an in vivo limiting dilutions assay. Similarly, using an imaging-based 4T1-luciferase TNBC orthotopic model, both VS-6063 and VS-4718 diminished the size of metastatic nodules within two weeks.

CSCs are readily detectable in primary breast cancers at surgery, yet methods to detect these populations are still developing. A multiplex assay for the CSC markers, ALDH1, CD44 and CD24, was explored with biopsies of primary tumor and matched lymph node, and primary tumors taken pre- and post-neoadjuvant chemotherapy. Consistent with previously reported data, elevated levels of ALDH were observed at higher levels post-treatment, and in lymph nodes. In addition, zones of ALDH+ and CD44-high/CD24-low tumor cells can be mapped with the multiplex assay for the potential detection of CSCs in breast cancer biopsies.

In summary, VS-6063 and VS-4718 diminish the CSC subpopulation in vitro, ex vivo and in xenograft models using a number of functional and biomarker assays. This critical subpopulation of CSCs is detectable in residual tumor following neoadjuvant therapy. Potentially, multiplex assays of CSC markers will be an improved means to monitor CSCs in clinical specimens. CSC-targeted agents such as VS-6063 or VS-4718 should be clinically tested in the neoadjuvant setting to potentially delay time to relapse and improve patient outcome.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-11-07
Average Grade: 4.25

Title: Perioperative administration of desmopressin (dDAVP) in breast cancer patients: A phase II dose-escalation study

Ruth S Weinberg¹, Marcelo O Grecco¹, Gimena S Ferro¹, Debora Seigelshifer¹, Nancy V Perroni¹, Francisco J Terrier², Analia Sanchez Luceros³, Enzo Domenichini⁴, Marcelo D Guthmann⁵, Daniela Di Leo⁵, Eduardo Spitzer⁶, Graciela N Ciccia⁶, Ana V Torbidoni⁷, Juan Garona⁷, Marina Pifano⁷, Giselle V Ripoll⁷, Roberto E Gomez⁵ and Daniel F Alonso⁷. ¹Eva Peron' Hospital, San Martin; ²Italian Hospital, La Plata; ³National Academy of Medicine, Buenos Aires, Argentina; ⁴Alexander Fleming' Institute, Buenos Aires, Argentina; ⁵Elea Laboratories, Buenos Aires, Argentina; ⁶Chemo-Romikin, Buenos Aires, Argentina and ⁷National University of Quilmes, Buenos Aires, Argentina.

Body: Background: dDAVP is a well known peptide analog of the antidiuretic hormone vasopressin, that has been used to prevent bleeding during surgical procedures in patients with hemostatic disorders. It induces a rapid increase of hemostatic mediators by stimulating their release from microvascular endothelial cells. In preclinical studies, dDAVP inhibited lymph node and early blood-borne metastasis from aggressive mouse mammary tumors. Besides, perioperative administration of dDAVP significantly prolonged disease-free and overall survival in a veterinary clinical trial enrolling dogs with locally advanced mammary cancer. The compound is a selective agonist of V2 vasopressin receptors present on both endothelial and breast cancer cells. Recent evidence indicated that dDAVP promotes tumor-mediated production of angiostatin and also activates endothelial release of von Willebrand factor (vWF), which may cause apoptosis of micrometastatic cells. Considering its hemostatic and antimetastatic properties, a phase II dose-escalation trial was performed in patients with breast cancer, administering a lyophilized formulation of dDAVP by IV infusion in saline, before and after surgical resection of primary tumor.

Methods: Eligibility included otherwise healthy female patients between 18 and 65 years of age, histological/cytological diagnosis of breast carcinoma (Stage 0, I, II), and mastectomy or lumpectomy with sentinel node biopsy, either requiring or not further axillary dissection. dDAVP was administered in two IV infusions, the first 30-60 minutes before surgery and the second 24 hours later. Five groups of at least 4 patients each received increasing total dDAVP doses of 0.5, 1.0, 1.25, 1.5 and 2.0 µg/kg. Primary endpoints were safety and tolerability in breast cancer patients undergoing surgery as first treatment, as well as selection of the best dose for clinical use. Secondary endpoints included surgical bleeding, plasma levels of vWF, and circulating tumor cells (CTC) as measured by quantitative PCR detection of cytokeratin 19 (CK-19) mRNA in whole blood.

Results: The trial accrued 21 patients from April 2012 to February 2014. Adverse events were reversible and observed from the third dose level (1.25 µg/kg), including nausea, hot flushing, skin rash, dyspnea and palpitations. Reactions were adequately managed by slowing the infusion rate of dDAVP (over 30 minutes). A reduced intraoperative bleeding of up to 50% was noted with increasing doses of dDAVP. Both vWF antigen and activity showed a rise after each dDAVP infusion, and maximum plasma levels were obtained at the higher dose level of 2 µg/kg. Interestingly, a preliminary analysis indicated a drop in CTC counts 24-48 hours after dDAVP treatment in patients with detectable CK-19 mRNA preoperative levels.

Conclusions: At the highest dose level evaluated (2 µg/kg) perioperative dDAVP appeared safe when administered in two slow IV infusions of 1 µg/kg, before and after the surgical procedure. The available data suggest that treatment is associated with reduction of intraoperative bleeding, higher circulating vWF levels and postoperative drop in CTC counts. Final results will be available at the time of the meeting.

Clinical trial number: NCT01606072.
Title: Exploring the best therapeutic partner of triple negative breast cancers: Using different characteristics/dependent pathways of triple negative breast cancer cell lines based on subgroups

Bora Lim¹, David T Dicker¹, Leah C Kline¹ and Wafik S El-Deiry¹. ¹Penn State Hershey Cancer Institute, Hershey, PA.

Body: Triple negative breast cancer (TNBC) still remains as a difficult disease given the absence of effective treatment modality, despite some success in PARP inhibition in BRCA gene mutation patients. Recently, TNBC was elucidated to be a large group of heterogenic subgroups based on their genetic/proteomic characters. Two main subgroups that consists of greater than 90% of whole TNBC include mesenchymal like (TNMBC) and basal (TNBBC) subgroups. Mesenchymal like subgroup harbor myoepithelial, epithelial-to-mesenchymal transition related phenotype, and genetic dependency on growth hormone pathway, higher sensitivity to death receptor inducing apoptosis pathway, as well as more dependency on PI3K-Akt-mTOR pathway. In contrast, basal like subgroup harbor the characteristics that is related to a defective DNA repair system, cell cycles regulation, and interestingly - dependency on RAS-MEK pathway for their survival and growth. Given explosive enrichment of understanding such pathways and enumeration of pathway targeting inhibitors, as well as the ability of timely recognition of biomarkers in patients sample which can differentiate subgroups of TNBC- offer an attractive strategy of novel therapeutics development in this difficult to treat disease. We tested targeting both dependent pathway and core characteristics of TNBC using representative panels of each subgroups: MDA-MB-468, BRCA1KD MCF10A, HCC1937 as in TNBBC panel showed effective killing when various combination of pAkt and PI3K, mTOR inhibitors, CDK1/2 inhibitors, death receptor mediated apoptosis inducing agents were introduced in combination. In contrast TNMBC MDA-MB-231, SUM159, BT549 showed effective killing when pERK and MEK inhibitors, in combination with BMI-1 inhibitors were given. Further elucidation of mechanisms of action in each combinations will further offer the ground of proper combination in subgroups of TNBC that could be further studied in animal model, and finally in clinical development.
Title: A preclinical study to demonstrate the utility of fulvestrant and MLN0128 combination against HR+ and HER2+ breast cancer

Shang Victoria Wu¹, Hannah Lu¹, Masaya Kai¹, Noriko Kanaya¹, Thenhang Luu¹, Courtney Vito¹, Laura Kruper¹, Joanne Mortimer¹ and Shiuan Chen¹. ¹Beckman Research Institute of the City of Hope, Duarte, CA.

Body: **Background:** Up to 10% of total breast cancers are positive for both hormone receptor [HR: estrogen receptor (ER) and/or progesterone receptor (PR)] and HER2. These patients belong mainly to the luminal B subtype, which exhibits resistance to endocrine therapy. Currently, systemic treatment for HR+/HER2+ breast cancer patients involves a combination of chemotherapy and HER2-directed therapy. While these therapies improve outcomes, they are associated with pernicious side effects.

**Results and Discussion:** As demonstrated by studies from a number of laboratories, in HR+/HER2+ cancer, ER is constitutively activated. In other words, the estrogen ligand is not needed for ER activation. The constitutively activated ER, through its non-genomic pathways, can stimulate both HER2 (a feed forward loop) and associated kinases. In patients with HR+/HER2+ tumors, genes in the PI3K and ER pathways have been altered. Activation of PI3K/Akt/mTOR by HER2 overexpression predicts tumor progression in breast cancer [Zhou et al., Clin Cancer Res. 10: 6779 (2004)]. Therefore, PI3k/AKT/mTOR is considered as an attractive therapeutic target for HR+/HER2+ breast cancer. Allosteric inhibitors of mTOR, such as RAD001 (Everlimus), only target mTORC1 but not mTORC2, relieving the negative feedback loop in this pathway, and leading to the activation of AKT. Our results suggest that to effectively treat HR+/HER2+ cancers, both mTORC1 and mTORC2 signaling must be suppressed. In these contexts; MLN0128 (i.e., INK128 or "MLN") is a new ATP-competitive inhibitor of mTOR. It targets both mTORC1 and mTORC2; and it does not interact with FKBP12 (an immunoregulatory protein). Consequently, MLN produces weaker immune-suppressing effects than everolimus. Results from our cell culture experiments reveal that MLN is twenty times more potent than everolimus against ER+/HER2+ cells. Furthermore, while MLN alone has been demonstrated to inhibit the proliferation of ER+/HER2+ cells, we have observed more benefits when it is used as part of a combination. Fulvestrant (ICI) by itself only suppresses the proliferation of ER+/HER2+ cells partially. However, when MLN and ICI are used together, our studies revealed a synergistic effect.

In addition, HR+/HER2+ PDX models have been generated to identify novel molecular networks and to examine the in vivo action of new targeted therapies against HR+/HER2+ cancer. We not only have verified the synergistic effect of MLN and ICI combination in vivo, we also identified a set of important genes playing roles in the growth of HR+/HER2+ tumors through RNA-Seq analysis.

**Conclusion.** Due to their synergistic and targeted action, this MLN and ICI combination could provide better clinical outcome and less side-effects to HR+/HER2+ breast cancer patients, compared to the currently available options in the clinics.
Title: A phase I study evaluating AZD2014 in combination with fulvestrant in patients with ER+ advanced metastatic breast cancer

Howard Burris III¹, Patricia LoRusso², W Larry Gluck³, Suzanne Jones⁴, Muaid Kittaneh², Erika Hamilton⁵, Stephen Green⁶, Wendy Burke⁶, Donald Strickland⁴, Elisabeth Oelmann⁶ and Manish Patel⁷. ¹Tennessee Oncology, PLLC/Sarah Cannon Research Institute, Nashville, TN; ²Karmanos Cancer Institute, Detroit, MI; ³Greenville Health System Institute for Translational Oncology Research, Greenville, SC; ⁴Sarah Cannon Research Institute, Nashville, TN; ⁵Tennessee Oncology, PLLC, Nashville, TN; ⁶AstraZeneca, Macclesfield, United Kingdom and ⁷Florida Cancer Specialists/Sarah Cannon Research Institute, Ft. Myers, FL.

Body: Background: Preclinical data suggest that patients with ER+ Breast Cancer become less sensitive to hormonal therapy by upregulation of the mTOR pathway. BOLERO-2 demonstrated that the combination of an allosteric mTOR inhibitor and an aromatase inhibitor improves progression-free survival in postmenopausal women with hormone resistant advanced breast cancer (NEJM 2012; 366:520-529). AZD2014 is a selective dual mTORC1 and mTORC2 inhibitor whilst fulvestrant is an estrogen receptor antagonist that is approved for the treatment of postmenopausal women with disease progression following antiestrogen therapy. This phase I trial assessed the safety, tolerability, pharmacokinetics, and preliminary efficacy of AZD2014 administered in combination with fulvestrant.

Methods: Continuous BID or QD dosing of AZD2014 was administered in combination with fulvestrant 500 mg intramuscularly on day 1 of each 28 day cycle. An additional 500 mg dose of fulvestrant was administered on day 15 of cycle 1 as per the approved dosing schedule. Single and multiple dose AZD2014 and fulvestrant pharmacokinetic samples were obtained. Optional tumor biopsies were also obtained.

Results: 43 patients (median age 61, range 32-82 years; prior chemo in the metastatic setting = 25; prior hormonal therapy = 43) have been treated in 4 dosing cohorts: 50 mg BID = 13; 35 mg BID = 6; 100 mg QD = 10; and 75 mg QD = 14. Patients have received 302+ treatment cycles (median 5 cycles/patient, range 1-21) and 4 patients continue on treatment. Review of preliminary unvalidated safety and Pk data revealed that dose-limiting toxicities included rash/mucositis (1 pt) and hyperglycemia (1 pt) at 50 mg BID; fatigue (1 pt) and mucositis (1 pt) at 100 mg QD; and rash (1 pt) at 75 mg QD. Treatment-related toxicities (any grade) include: fatigue (51%), mucositis (60%), rash (60%), nausea (47%), hyperglycemia (12%), and neutropenia (2%). Pharmacokinetic data show that AZD2014 is rapidly absorbed with a median time to maximum concentration of 1-1.75 hours and an estimated mean half-life of approximately 3.3-5.6 hours across the dose range. There is no evidence that co-administration of fulvestrant has a clinically relevant impact on exposure to AZD2014. In the 26 patients with measureable disease, 5 confirmed PRs have been observed (duration 4-18+ months) with an additional 14 patients experiencing stable disease for at least 6 months of treatment.

Conclusion: Continuous QD (75mg) and BID (35 and 50 mg) administration of AZD2014 in combination with fulvestrant is tolerable with clinical benefit observed in nearly half of the patients. Toxicities observed are broadly consistent with AEs observed in other trials with antihormonal agents and other mTOR inhibitors, but seem to be well manageable. There were no new or other additive toxicities, when the two drugs were combined and AZD2014 pharmacokinetic data are broadly consistent with what has been previously observed for AZD2014 single agent. A randomized phase II trial of the combination is ongoing and an intermittent weekly dosing schedule is being explored in additional patient cohorts in the current study.
Title: Phase Ib dose-escalation study of an Akt inhibitor ipatasertib (Ipat) in combination with docetaxel (Doc) or paclitaxel (Pac) in patients (pts) with metastatic breast cancer (MBC)

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Body: Background: The Akt pathway is frequently aberrantly activated in MBC (e.g. via PTEN loss, and/or alterations of PIK3CA, AKT1, or AKT3); additionally, Akt activation may occur in response to chemotherapy, leading to cell survival and chemoresistance. Ipat (GDC-0068) is a potent oral, ATP-competitive inhibitor of all Akt isoforms. In preclinical models, Ipat synergistically combined with taxanes. In the Phase I dose-escalation single agent study, Ipat was given to pts including MBC, and downregulated Akt signaling at doses ≥ 100 mg.

Methods: Eligible pts with MBC, treated with up to 3 prior systemic chemotherapy regimens, received Doc 75 mg/m² intravenously (IV) on Day 1 with escalating doses of Ipat PO QD on Days 2-15 every 21 days or received Pac 90 mg/m² IV on Days 1, 8, and 15 with escalating doses of Ipat PO QD on Days 1-21 every 28 days. A standard 3+3 does-escalation design was used, with an expansion cohort of HER2-negative/hormone receptor positive [HER2-/HR+] MBC patents (including triple-negative MBC [TNBC]) in the Pac cohort at the recommended phase II dose. Pharmacokinetic (PK) and circulating tumor cell (CTC) samples were collected. Archival tumors were assessed for PTEN by immunohistochemistry and for pathway-relevant mutations.

Results: As of 1 Apr 2014, 54 patients with multiple solid tumors, including 19 pts with MBC (TNBC: n=13, HER2-/HR+: n=4, and HER2-positive: n=2) were enrolled, and pts with MBC received Ipat with Doc (n=5) or Pac (n=14). The common Grade ≥ 2 adverse events (AEs) related to Ipat in combination with Doc (≥ 10% and > 1 patient) were diarrhea (80%), nausea (60%) and vomiting (40%), versus in combination with Pac (≥ 10% of pts) were diarrhea (43%), fatigue (29%) and hyperglycemia (14%). The PKs of Ipat, Doc, or Pac were comparable to the single agents. Partial responses by RECIST v1.1 were seen in 5 pts, including pts with HER2-/HR+ (n=2) or TNBC (n=3) who had previously progressed on Pac (n=4) or PI3K inhibitors (n=2) or had tumors with PI3K/Akt alterations [PTEN loss (n=1), PIK3CA mut (n=2), or AKT1 mut (n=1)]. Time on study > 9 months occurred in 4 pts (HER2-/HR+ and TNBC) who had progressed on prior Pac (n=3) or PI3K inhibitors (n=3), and/or had tumors with PIK3CA mut (n=3).

Conclusions: Ipat in combination with Doc or Pac is well-tolerated and has a safety profile generally consistent with the single agent. Anti-tumor activity with Ipat in combination with taxanes was seen in MBC, including HER2-/HR+ or TNBC with baseline PI3K/Akt alterations. Updated safety, efficacy, and biomarker data will be presented.
Title: Manumycin A derived nanoparticle induced cytoplasmic vacuolation mediated cell death in triple negative breast cancer

Prajjal K Singha¹, Srilakshmi Pandeswara¹, Manjeri A Venkatachalam¹ and Pothana Saikumar¹. ¹University of Texas Health Science Center, San Antonio, TX; ²University of Texas Health Science Center, San Antonio, TX; ³University of Texas Health Science Center, San Antonio, TX and ⁴University of Texas Health Science Center, San Antonio, TX.

Body: Background: Major treatment modalities of breast cancer therapy are endocrine, radiation and chemotherapies, and can inflict apoptosis resistance effects in most clinically aggressive breast tumors. Among these tumors, triple negative breast cancers (TNBC) are the major aggressive and treatment resistant subtypes. Therefore, it is important to find alternative modes of treatment to eradicate these TNBC and reduce tumor burden. Earlier we have reported the novel role of Manumycin A (ManA), a natural antibiotic produced by Streptomyces parvulus, induced cytoplasmic vacuolation mediated cell death in TNBC. In the present study, nanoparticle based approach was used for delivering ManA to the tumor site to increase the efficacy and reduction of the drug dose.

Methods: All cell lines were cultured according to the recommended procedures. Lecithin based nanoparticles were made for both vehicle and ManA. Cell proliferation, transmission electron microscope (TEM), immunoblotting, tumor xenografts and lung metastasis study were performed by standard methods.

Results: TEM imaging revealed spherical shaped homogenous size distribution of nanoparticles entrapped with vehicle (Veh-NP) or ManA (ManA-NP). To determine the effect of ManA-NP induced cytoplasmic vacuolation mediated cell death in TNBC, results of ManA-NP were compared to Veh-NP and ManA drug alone. Two fold reduction in the dose of ManA in ManA-NP induced cytoplasmic vacuolation death in several TNBC were observed compared to Veh-NP and ManA drug groups. Indeed ManA-NP caused significantly higher cell death than other groups. ManA-NP induced cytoplasmic vacuolation death was also associated with increased endoplasmic reticulum (ER) stress markers, LC3, p62 proteins and accumulation of ubiquitinated proteins similar to that of ManA alone. Importantly, ManA-NP reduced pAkt and increased PTEN and p21 proteins in TNBC. Notably, apoptotic as well as autophagic inhibitors were unable to protect TNBC against ManA-NP induced cell death. Importantly, thiol antioxidant N-alpha-acetyl-L-cysteine inhibited the formation of cytoplasmic vacuolation mediated cell death along with the induction of ER stress, LC3, p62 and protein ubiquitination in TNBC. Interestingly, ManA-NP failed to induce cytoplasmic vacuolation mediated cell death in slowly dividing normal human mammary epithelial cells even after treating for 72 h at same concentration that is required for ManA drug alone to induce cytoplasmic vacuolation mediated cell death in rapidly proliferating TNBC. Most importantly, ManA-NP reduced breast tumor growth derived from MDA-MB-231 cells in mice at 1 mg/ kg of body weight compared to 5 mg/ kg of body weight of ManA and Veh-NP groups. Finally, ManA-NP completely impeded the lung metastasis of MDA-MB-231 cells compared to Veh-NP mice. Moreover, lung sections of ManA-NP treated mice showed normal thin-walled alveoli structure compared to Veh-NP treated lung sections where cancer cells invaded the lung tissue.

Conclusions: These results clearly indicate that ManA-NP enhances the therapeutic efficacy and induces cytoplasmic vacuolation which serves as a “Trojan-Horse” like mechanism of cell death in TNBC.
Title: Targeting STAT3 with novel small molecule inhibitors to sensitize breast cancer cells to radiation therapy

Lili Wang¹, Zhengduo Yang¹, Qing Xia¹, Haijun Chen², Guoshuai Cai¹, Christopher Wild², Jia Zhou² and Qiang Shen¹. ¹University of Texas MD Anderson Cancer Center, Houston, TX and ²University of Texas Medical Branch, Galveston, TX.

Body: Radiation therapy plays an important role in controlling the growth and progression of breast cancer. However, its efficacy is limited by the radiation-associated toxicity to normal tissue, and the intrinsic or acquired radioresistance developed in cancer cells. It was well documented that radiation onto cancer tissues cause complex changes in gene expression patterns. Thus, it may be possible to manipulate the expression of specific genes in cancer cells to increase radiosensitivity and reduce radioresistance. Accumulating studies strongly demonstrated that signal transducers and activators of transcription 3 (STAT3) is involved in cell survival, proliferation, inflammation, invasion, metastasis, angiogenesis and immune responses. Particularly, STAT3 is activated by ionizing radiation. We hypothesized that blocking STAT3 will increase sensitivity to irradiation in cancer cells. To this end, we synthesized a series of novel STAT3 inhibitors to address the challenge of radioresistance in breast cancer cells including metastatic and triple-negative lines. Among them, the compound HJC0152 and HJC0123 displayed significant inhibition of proliferation of MDA-MB-231, MCF-7 and MCF-7/Adr in dose and time dependent manners. HJC0152 and HJC0123 also induced apoptosis and necrosis in comparison to the control cells. We also found that these STAT3 inhibitors induce apoptosis of MDA-MB-231, MCF-7 and MCF-7/Adr cells by inhibiting anti-apoptotic protein Bcl-2 expression, increasing the expression of apoptotic effector protein caspase-3 and Bax. In addition, we found that HJC0152 and HJC0123 in combination with X-ray irradiation induce G2/M cell cycle arrest in MDA-MB-231, MCF-7 and MCF-7/Adr cells. The new STAT3 inhibitors increased the radiosensitivity of MDA-MB-231, MCF-7 and MCF-7/Adr cell lines, inhibited radiation-induced DNA damage repair, and promoted cells to enter mitosis, a phase more sensitive to irradiation. These changes were accompanied with decreased activation of STAT3 and decreased expression of the STAT3 downstream gene, Bcl-2. Our findings suggest that STAT3 blockade may represent an effective strategy to overcome radiation resistance, using STAT3 inhibitors as radiation sensitizers to restore the sensitivity of cancer cells to radiation therapy.
Title: Inducible suppression of insulin receptor substrate I inhibits IGF-I/insulin/estradiol dependent cell growth in MCF-7L breast cancer cells

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Body: Insulin receptor substrate (IRS) proteins are adaptor proteins downstream of insulin-like growth factor (IGF) and insulin receptors. IRS proteins play a role in cancer biology, with IRS1 function linked to mitogenesis and survival and IRS2 associated with metastasis. Moreover, this family of adaptor proteins is also involved in the signal transduction of many other transmembrane receptors. Therefore IRS proteins could be potential therapeutic targets for cancer therapy. To test the function of IRS-1, we created a doxycycline inducible IRS1 shRNA and stably transfected MCF-7L breast cancer cells. The level of IRS1 protein knockdown varied within different clones from 50 to 95% reduction compared to non-induced cells. IRS2 mRNA and protein levels were not significantly affected. We chose a high (3G5) and intermediate (3A7) IRS1 knockdown clones for further study. Doxycycline treated 3G5 and 3A7, showed partial inhibition of IGF-I, IGF-II and insulin stimulated phosphorylation of IRS1(Tyr1222) and AKT. pErk1,2 were slightly increased and pIGF-IR was unchanged compared to doxycycline non-treated cells. IRS-2 tyrosine phosphorylation was stimulated when IRS-1 was suppressed and may account for the persistent downstream cell signaling. Anti IGFIR antibody dalotuzumab (20µg/ml) effectively inhibited cell signaling stimulated by IGF-I, IGF-II and insulin in both doxycycline treated and non-treated cells. There was no obvious difference in cell signaling pattern between plus and minus doxycycline treated cells within 24 hour time course. In monolayer cell growth assays, IGF-I, IGF-II, insulin and estradiol growth stimulation were significantly inhibited when IRS-1 was suppressed in 3G5 cells. In 3A7 cells, which have a lower level of IRS-1 suppression, growth stimulation was moderately inhibited. In anchorage independent growth assays stimulated by IGF-I, colony number and size were inhibited by doxycycline-induced IRS-1 suppression with a reduction of 80% in 3G5 cells and 50% reduction in 3A7 cells. These results show that growth of MCF-7L cells are dependent on IRS-1 expression. Reduced IRS1 can hinder cancer cell growth and tumorigenicity even when signaling was not impaired potentially due to compensation by other adaptor proteins. We conclude that suppression of IRS-1 may inhibit several growth regulatory pathways in breast cancer and could serve as a potential drug target.
Title: Development of a monoclonal PITPNM3 antibody for breast cancer therapy

Shicheng Su¹, Chonghua He¹ and Erwei Song¹. ¹Breast Tumor Center, Sun Yat-sen Memorial Hospital, Sun Yat-Sen University, Guangzhou, China.

Body: In breast cancer, the overexpression of CCL18 promotes tumor metastasis and progression by interacting with its receptor PITPNM3. In addition, the abundant expression of PITPNM3 promotes hepatocellular carcinoma metastasis induced by CCL18 in vitro and in vivo, indicating that PITPNM3 may serve as an attractive therapeutic target for treatment of cancer metastasis. Monoclonal antibodies (mAbs) represent an attractive approach to anticancer treatment, which can be designed to selectively target tumor cell membrane proteins and block the interaction between ligands and receptors. Here, we report that we successfully develop a monoclonal antibody of PITPNM3 which exhibits an excellent therapeutic effect of cancer treatment by means of hybridoma. In vitro migration assay showed that PITPNM3 monoclonal antibody can inhibit 70% CCL18 induced migration in breast cancer cells indicating that the monoclonal antibody can neutralize the effect of CCL18. Radiolabelled competing binding assay showed that PITPNM3 antibody can specifically bind to breast cancer cells which abundantly expressed PITPNM3. Furthermore, the PITPNM3 monoclonal antibody exhibits an excellent therapeutic effect in breast cancer xenografts. In vivo assay showed that the PITPNM3 monoclonal antibody can remarkably reduce the primary tumor size, inhibit lung and liver metastasis and significantly prolong the survival. In addition, the PITPNM3 antibody showed good resolution in different temperature and pH value. Besides, PITPNM3 antibody has no toxic effect both in vitro and in vivo through MTT assay to check cells proliferation and by measuring a serial of Physiological index when PITPNM3 antibody was injected in the xenografts. Collectively, our results suggested that the PITPNM3 antibody may serve as targeting therapy for breast cancer metastasis.
Title: Discordance in cyclin D1 changes after metformin exposure by different protein expression methods: Results from a "Window of Opportunity" trial

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Body: Background: Laboratory and population studies demonstrate that metformin offers a beneficial breast cancer (BC) effect. In vitro, metformin has been shown to induce cycle cycle arrest. In a pre-surgical metformin trial of overweight/obese, multi-ethnic BC patients, we reported no difference in tumor proliferation, as measured by ki-67. Reverse Phase Protein Array (RPPA) is a high-throughput antibody-based technique to assess cellular protein activity in signaling networks. The goal of this study was to assess changes in cyclin D1 by RPPA as compared to immunohistochemistry (IHC) in patients treated in a pre-surgical metformin trial.

Methods: Metformin 1500mg PO daily (500mg am/1000 mg pm) was administered for 2-4 weeks prior to resection in 35 patients with stage 0-III operable BC, BMI ≥ 25 kg/m², and no history of diabetes. All tumor analysis was performed on paraffin-embedded tumor tissue. For RPPA, protein was extracted from pre- and post-metformin tissue, denatured by sodium dodecyl sulfate, and printed on nitrocellulose-coated slides. Samples were probed with 160 antibodies associated with various cellular activities, including cyclin D1. For RPPA, the cyclin D1 antibody used was by Santa Cruz (SC-718 rabbit polyclonal). For IHC, the cyclin D1 antibody used was by Ventana (SP4-R rabbit monoclonal). We analyzed changes in protein expression in tumor tissue of study patients with those of untreated historical controls, matched by age, BMI, and tumor characteristics. For RPPA and IHC, paired t-test was used to calculate within-group changes, and two-sample t-tests were used to compare between-group changes in cases and controls (significance: p ≤ 0.05). For RPPA, multiple comparisons were adjusted for by fixing the false discovery rate (FDR) at 25%. Pearson’s correlation coefficient was used for correlation between RPPA and IHC.

Results: Of the 35 metformin-treated patients, 32 were evaluable. The majority were Hispanic (80%). Metformin was administered for a median of 23 days (range: 8-64). Of the invasive BCs (n=21/35), 80% of patients had HR+/HER2- BC. The 33 historical controls were well-matched. Adjusting for multiple comparisons, there was a statistically significant increase in cyclin D1 by RPPA after metformin vs. control [mean change from baseline after metformin: +0.065 (0.118) vs. mean change from baseline in control: -0.044 (0.152), p=0.002]. There was no change in cyclin D1 by IHC after metformin vs. control [mean change from baseline after metformin: -5.64 (21.5) vs. mean change from baseline in control: -6.37 (14.28), p =0.88]. There was no correlation in cyclin D1 between IHC and RPPA (estimate = 0.09, p=0.31). In vitro assessment of cyclin D1 after metformin use in various cell lines is ongoing.

Conclusions: We report no correlation between cyclin D1 between IHC and RPPA. Pre-clinical assessment of changes in the cell cycle after metformin is ongoing. Interpreting results of proteomic findings based off of paraffin-embedded tissue should be done so with caution.
Title: The effects of the BKM120 in combination with herceptin on HER2+ BC cells and BCSCs

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Body: Introduction: Breast cancer stem cells (BCSCs) are suspected to be responsible for tumour recurrence, metastasis as well as chemo-resistance. Dysregulated PI3K/Akt signaling is implicated in the pathogenesis of a number of breast cancers, including the HER2+ breast cancer. This study evaluated the efficacy of BKM120 monotherapy and BKM120 plus Herceptin in treatment of normal cells and the BCSCs of the HER2+ breast cancer.

Methods: The BCSCs of SK-BR-3 cells were isolated by Serum free cells suspension culture. The impacts of BKM120 monotherapy and BKM120 combined with Herceptin on normal breast cancer cells and BCSCs were assayed by MTT assay, wound-healing assay and plate clone formation assay. The mammosphere-forming efficiency (MFE) of breast cancer cells treated with BKM120 and BKM120 plus Herceptin were also calculated. The nature of the drug interaction of BKM120 and Herceptin was evaluated by using the combination index (CI) according to the method of Chou and Talalay. The impacts of BKM120 monotherapy and BKM120 combined with Herceptin in PI3K/AKT pathway on the normal cancer cells and BCSCs of SK-BR-3 were assayed by Western blotting.

Results: BKM120 showed significant antiproliferative activity in the BCSCs, as well as in normal SK-BR-3 cells. After treated with BKM120, invasion abilities of SK-BR-3 cells was significantly weakened (P<0.01). The colony forming efficiency and the MFE was also decreased compared with the control (P<0.01). Additionally, the pAKT and pS6 expression levels were inhibited. The effects of BKM120 in combination with Herceptin were investigated, which was called Combination Index, showed synergism between the two agents in BCSCs as well as in the normal cells. Furthermore, BKM120 plus Herceptin showed more significantly suppression on following aspects in BCSCs and normal cancer cells: proliferation, invasion ability, colony forming as well as MFE. In addition, the levels of pAKT, pS6 in the BKM120 plus Herceptin group were decreased more obviously than those in control and monotherapy group respectively (P<0.05).

Conclusions: BKM120 can inhibit the growth of normal cancer cells and BCSCs of SK-BR-3, by inhibiting cell proliferation, impairing the invasion abilities and the MFE, and reducing the expression of pAKT(Thr308,S473) and pS6(Thr389). Moreover, these results suggest that the combination of PI3K Inhibitor BKM120 with Herceptin may provide a novel therapy strategy for HER2+ BC.
Title: Local excision without radiation for ductal carcinoma in situ: 12-year results from the ECOG E5194 study

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Body: Background: The ECOG E5194 study was a prospective trial designed to evaluate surgical excision (lumpectomy) without radiation for selected women with ductal carcinoma in situ (DCIS) of the breast with low risk clinical and pathologic features.

Methods: Eligible patients were enrolled on two study cohorts (not randomized): (1) low or intermediate grade DCIS, tumor size ≤ 2.5 cm; or (2) high grade DCIS, tumor size ≤ 1.0 cm. Cohort assignment was based on pathology assessment from the treating institution. Protocol specifications included surgical excision of the DCIS tumor with a minimum negative margin width of at least 3 mm or no tumor on re-excision. Radiation treatment was not allowed. From April 1997 to October 2002, 665 evaluable patients were enrolled through ECOG or NCCTG (561 in Cohort 1; 104 in Cohort 2). Tamoxifen was optional (not randomized) beginning in May 2000, and was given to 30% of the patients. The primary study endpoint was the rate of developing an ipsilateral breast event (IBE), defined as local recurrence of DCIS or invasive carcinoma in the treated breast. The median follow-up was 12.3 years. We have previously reported 7-year results (L. Hughes, J Clin Oncol 27:5319, 2009; median follow-up 6.3 years; 66 IBE’s), and we herein provide 12-year results.

Results: Median patient age was 60 years and 58 years for Cohort 1 and Cohort 2, respectively. Tumor size was < 10 mm for 79% and 80% of patients, respectively. The minimum negative margin width was ≥ 5 mm for 64% and 69% of patients, respectively. There were 99 IBE’s, of which 51 (52%) were an invasive IBE. The IBE and invasive IBE rates increased over time in both cohorts (see Table). The 12-year rates of an IBE were 14.4% for Cohort 1 and 24.6% for Cohort 2 (p = 0.003), and for an invasive IBE, 7.5% and 13.4%, respectively (p = 0.08). No difference was seen for the 12-year rates of overall survival (84.0% vs 82.8%; p = .96) or contralateral breast events (6.7% vs 12.0%; p = 0.16). On multivariate analysis, study cohort (hazard ratio = 1.81; p = 0.01) and tumor size (p = 0.01) were statistically significant for an IBE, and study cohort was borderline statistically significant for an invasive IBE (p = 0.08). On central pathology review (75% of cases), neither grade nor comedo necrosis was associated with the risk of an IBE or invasive IBE (all p > 0.15). Salvage treatment at the time of an IBE included mastectomy for 42% (31/74) and 64% (16/25) of the patients, respectively.

Conclusions: For these selected patients with favorable DCIS based on clinical and pathologic characteristics treated with surgical excision without radiation, the rates of an IBE and an invasive IBE continued to increase through at least 12 years of follow-up.

IBE Rates According To Study Cohort.

<table>
<thead>
<tr>
<th>Time</th>
<th>Cohort 1 (Low or Intermediate Grade)</th>
<th>Cohort 2 (High Grade)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IBE</td>
<td>Invasive IBE</td>
</tr>
<tr>
<td>At 5 years</td>
<td>6.0% (4.0%, 8.1%)</td>
<td>2.7% (1.3%, 4.1%)</td>
</tr>
<tr>
<td>At 7 years</td>
<td>9.5% (7.0%, 12.0%)</td>
<td>4.8% (2.9%, 6.6%)</td>
</tr>
<tr>
<td>At 10 years</td>
<td>12.5% (9.5%, 15.4%)</td>
<td>6.4% (4.2%, 8.6%)</td>
</tr>
<tr>
<td>At 12 years</td>
<td>14.4% (11.2%, 17.6%)</td>
<td>7.5% (5.1%, 10.0%)</td>
</tr>
</tbody>
</table>
Body: Introduction
LCIS is considered both a risk indicator and a non-obligate precursor of invasive breast cancer. A diagnosis of LCIS confers a risk that is 8-10x higher than that of the general population; yet we remain unable to predict risk of progression and hence lack the tools to make personalized treatment recommendations. Here we examine the relationship between pathologic features and extent of LCIS at surgical excision with risk of subsequent breast cancer.

Methods
From a prospective database of 1032 patients with LCIS diagnosed from 1980-2009, we identified patients with and without a subsequent cancer diagnosis and created a nested case-control cohort study. A case was defined as a breast cancer diagnosis ≥6 months after LCIS diagnosis. All cases were matched to ≥1 control (cancer free) based on age at diagnosis of LCIS +/- 5 yrs and length of follow up. All H&E slides from the first diagnosis of LCIS were requested for central pathology review. Comparisons between cases and controls were done using conditional logistic regression analysis.

Results
At median followup of 79 mos (2-368mos) 156 pts (15%) in the parent database have developed breast cancer. Case-control matching resulted in a study cohort of 72 cases and 274 controls for which the original LCIS diagnostic slides were available. There were no significant differences in clinical characteristics between the study cohort and the parent population. Median time to cancer among cases was 3.6 yrs (0.5-12.7yrs). Median cancer-free follow up for controls was 9.9 yrs (1.1-19.8yrs). No significant differences in any of the features examined were observed between cases and controls (Table). Although the median number of slides with LCIS did not differ, the ratio of the total number of slides with LCIS over the total number of slides reviewed was significantly associated with case-control status. Cases had a significantly higher ratio (median 0.5, (0.3-1.0)) than controls (median 0.3, (0-1); p=0.003). On conditional logistic regression analysis, a ratio of >0.5 was associated with a 2.7 (95%CI, 1.4-4.9) greater odds for cancer development when compared to a ratio of <0.25 (p=0.008).

Conclusion
In this nested case-control study, the quantity of LCIS at excision, as measured by the ratio of the number of slides with LCIS over the total number of slides reviewed, was found to be a significant predictor of subsequent breast cancer. Patients with LCIS in >50% of slides reviewed had a 2.7 increased odds for cancer; suggesting that the extent of LCIS in a biopsy specimen should be considered when counseling patients about future risk and risk reducing options.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Cases n=72</th>
<th>Controls n=274</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilateral LCIS at diagnosis</td>
<td>2 (3%)</td>
<td>6 (2%)</td>
<td>0.81</td>
</tr>
<tr>
<td>Multicentric LCIS at diagnosis</td>
<td>7 (10%)</td>
<td>25 (9%)</td>
<td>0.68</td>
</tr>
<tr>
<td>Recurrent LCIS over time</td>
<td>9 (13%)</td>
<td>24 (9%)</td>
<td>0.30</td>
</tr>
<tr>
<td>Median # slides with LCIS</td>
<td>4 (1-19)</td>
<td>3 (1-41)</td>
<td>0.42</td>
</tr>
<tr>
<td>Median # TDLU with LCIS</td>
<td>11 (1-129)</td>
<td>6 (1-342)</td>
<td>0.40</td>
</tr>
<tr>
<td>Cell Type</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>24 (33%)</td>
<td>99 (36%)</td>
<td>0.70</td>
</tr>
<tr>
<td>A/B</td>
<td>45 (63%)</td>
<td>145 (53%)</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>3 (4%)</td>
<td>30 (11%)</td>
<td></td>
</tr>
<tr>
<td>Nuclear Grade</td>
<td>14 (19%)</td>
<td>58 (21%)</td>
<td>0.54</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>2</td>
<td>3</td>
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<tr>
<td>-------</td>
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<tr>
<td></td>
<td>54 (75%)</td>
<td>189 (69%)</td>
<td>27 (10%)</td>
</tr>
<tr>
<td></td>
<td>4 (6%)</td>
<td>27 (10%)</td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>0</td>
<td>6 (2%)</td>
<td>NA</td>
</tr>
<tr>
<td>Calcifications</td>
<td>27 (38%)</td>
<td>114 (42%)</td>
<td>0.57</td>
</tr>
<tr>
<td>Ductal Extension</td>
<td>60 (83%)</td>
<td>249 (91%)</td>
<td>0.09</td>
</tr>
<tr>
<td>ALH</td>
<td>12 (17%)</td>
<td>31 (11%)</td>
<td>0.19</td>
</tr>
</tbody>
</table>
Title: The prognostic role of HER2 expression in ductal breast carcinoma in situ

Signe Borgquist¹, Wenjing Zhou², Karin Jirström³, Rose-Marie Amini⁴, Thomas Sollie⁵, Therese Sørlie⁶, Salma Butt¹, Carl Blomqvist¹ and Fredrik Wärnberg². ¹Lund University, Lund, Sweden; ²Uppsala University, Uppsala, Sweden; ³Lund University, Lund, Sweden; ⁴Uppsala University, Uppsala, Sweden; ⁵Örebro University, Örebro, Sweden; ⁶Institute for Cancer Research, Oslo University Hospital, Radium Hospital, Oslo, Norway and ⁷Helsinki University Central Hospital, Helsinki, Finland.

Body: Background: HER2 is a well established prognostic and predictive factor in invasive breast cancer. The role of HER2 in ductal breast carcinoma in situ (DCIS) is much debated and recent data have suggested that HER2 is mainly related to in situ recurrences. This contrasts the proposed role of HER2 in the progression from in situ to invasive cancer. Our aim was to study HER2 as a prognostic factor in a large population based cohort of DCIS.

Methods: All 458 women diagnosed with a primary DCIS 1986-2004 in two Swedish regions were included and tissue microarrays constructed. Silver-enhanced in situ hybridisation (SISH) and immunohistochemistry (IHC) were used for detection of HER2 amplification and IHC expression. HER2 status and its relation to invasive breast cancer recurrence (IBCR) (ipsilateral or contralateral invasive events and regional or distant metastasis) and ipsilateral events (IBE) were studied. Kaplan-Meier survival analyses and Cox proportional hazards regression models were used. Adjustments were made for age, size, radiotherapy and ER status.

Results: Mean follow up was 184 months. DCIS was screening detected in 75.5% of cases. Breast conserving surgery (BCS) was performed in 78.6% of whom 44.0% received postoperative radiotherapy. No women had hormonal or chemotherapy. A total of 106 IBCRs and 105 IBEs were identified. 54 IBEs were in situ and 51 invasive cancer. Eighteen women died from breast cancer and another 114 had died from other causes. 420 tumours could be classified using available SISH or IHC data; 132 were HER2 positive (31.4%) and 288 HER2 negative. SISH and IHC data were concordant in 296 of 332 (89.2%) available cases. HER2 positivity was related to size, grade and ER and PR negativity.

The risk of IBCR was statistically significantly lower subsequent to a HER2 positive DCIS, hazard ratio (HR) 0.53 (95% CI 0.31-0.90), log rank p=0.01. However, the curves did not separate until after almost ten years. HRs after adjustments were similar to the crude analyses. The risk of any IBE was not statistically differently changed by HER2 status. In women undergoing BCS, HR was 1.18 (0.77-1.82), log rank p=0.44. But interestingly, divided by type of IBE, HER2-positivity showed an increased risk of in situ IBEs, HR 1.64 (0.92-2.90), log rank p=0.09 and, a decreased risk of invasive IBEs, HR 0.76 (0.39-1.50), log rank p=0.43. These correlations were however not statistically significant.

Risk of invasive and local recurrence by HER2 status in a population based cohort of women with a primary DCIS

<table>
<thead>
<tr>
<th>Invasive Breast Cancer Recurrence</th>
<th>HER2 positive</th>
<th>HER2 negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients (n=420)(events=101)</td>
<td>0.53 (0.31-0.90)</td>
<td>Reference 1.0</td>
</tr>
<tr>
<td>Ipsilateral new Breast Events (IBE)</td>
<td>1.18 (0.77-1.82)</td>
<td>Ref</td>
</tr>
<tr>
<td>BCS (n=324) (events=94)</td>
<td>1.64 (0.92-2.90)</td>
<td>Ref</td>
</tr>
<tr>
<td>BCS, in situ IBEs (events=48)</td>
<td>0.76 (0.39-1.50)</td>
<td>Ref</td>
</tr>
<tr>
<td>BCS, invasive IBEs (events=46)</td>
<td>0.76 (0.39-1.50)</td>
<td>Ref</td>
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Conclusions: In this long term follow-up DCIS cohort positive HER2 status in the primary DCIS predicted a statistically lower risk of IBCR. This effect was seen from ten years after primary surgery and onwards. The risk of an in situ IBE was increased and the risk of an invasive IBE was decreased if the primary DCIS was HER2 positive. All together, this challenges the role of HER2 as a driving force of the progression from in situ to invasive cancer.
Title: Metformin for the treatment of ductal carcinoma in situ (DCIS)

Atreyi Dasgupta\textsuperscript{1}, David G Edwards\textsuperscript{1}, Frances S Kittrell\textsuperscript{1}, Susan G Hilsenbeck\textsuperscript{1}, Daniel Medina\textsuperscript{1} and Sao Jiralerspong\textsuperscript{1}. \textsuperscript{1}Baylor College of Medicine, Houston, TX.

**Body:** BACKGROUND: Each year about 60,000 new cases of breast ductal carcinoma in situ (DCIS) are diagnosed in the United States. DCIS significantly increases the risk of invasive breast cancer. To reduce this risk, tamoxifen is offered to patients with ER-positive DCIS in the adjuvant setting. In contrast, no adjuvant therapy exists for ER-negative DCIS. Metformin is a frontline therapy for type 2 diabetes and its safety and side effect profiles are well documented. Preclinical, epidemiologic, and retrospective data all support an antitumor effect of metformin in breast cancer. Metformin may act via activation of AMP kinase (AMPK), a sensor of cellular energy, which in turn inhibits mTOR activity and shuts down global protein synthesis. Previous data from our group showed that metformin treatment is associated with a higher rate of pathologic complete response in diabetic breast cancer patients receiving preoperative chemotherapy. In the current study, using preclinical models, we explored the effects of metformin in DCIS and its progression to invasive breast cancer.

**EXPERIMENTAL DESIGN AND METHODS:** We tested the anti-tumor activity of metformin in terms of proliferation and invasion using both DCIS cell lines and a mouse intraductal (MIND) transplantation model previously developed by us. The latter involves intraductal injection of human DCIS cells into mouse mammary glands, and importantly recapitulates the progression of DCIS to invasive cancer seen in human breast cancer. Western blot and immunofluorescent staining for AMP kinase, mTOR, and other relevant targets, as well as flow cytometry, were used to investigate mechanism.

**RESULTS:** Metformin significantly inhibited cell proliferation, migration, and invasion in all DCIS cell lines tested. In the mouse model, using metformin at a dose that is readily achievable in human patients, metformin significantly reduced the tumor burden in terms of median number of ducts filled, from 11 to 2 using DCIS.com cells (p < 0.001) and from 10 to 3 using SUM.225 cells (p = 0.004). In addition, importantly, metformin inhibited the progression of DCIS lesions to invasive breast cancer significantly, with a reduction in invasive lesions from 74 to 17\% (p = 0.003). In mechanistic studies, metformin showed activation of AMPK and subsequent inactivation of mTOR and other downstream targets. Treatment with metformin did not lead to significant cell cycle arrest but rather to increased cell death. We found that cell death by apoptosis increased only modestly with metformin treatment, while cell death by necrosis increased significantly.

**CONCLUSION:** Metformin inhibits the growth of DCIS and its progression to invasive breast cancer. To our knowledge, this is the first reported evidence of metformin’s anti-tumor activity in DCIS. Our data support the exciting prospect of developing metformin clinically for the treatment of DCIS.
Body: INTRODUCTION
The primary aim in the management of DCIS is the prevention of recurrence and contralateral tumor (CT). Previous studies reported that younger patients and African Americans (AAs) experience a higher risk of recurrence, second tumors and show worse overall survival. Nevertheless, risk factors for DCIS recurrence, treatments and outcome are still widely discussed. The aim of our analysis was to identify clinical-pathological features and treatment modalities associated with recurrence in DCIS and micro-invasive carcinoma (MIC).

METHODS
In the Thomas Jefferson University Tumor Registry, we identified 820 patients with DCIS and 61 with MIC treated between 2003 and 2013. Associations between recurrence and demographic factors, histopathological features and treatment were assessed.

RESULTS
The median age was 59 years with a racial distribution of 69.3% white, 19.5% AAs and 5.7% Asian. There was no significant difference in age at diagnosis by ethnicity and, 64.8% was ER/PR positive and 10.1% was HER2 positive. The associations of age and ethnicity with hormone status and HER2 status were not statistically significant. To date, 73 (8%) patients developed locoregional recurrence or CT, 50 (68.5%) of which were in situ lesions. Only one patient had MIC in primary lesion. There was no significant difference in age, while white women had higher recurrence rate than Asians and AAs (9% vs. 4%). ER/PR negative women were more likely to develop recurrence than ER/PR positive (16% vs. 8%). The hormonal status of primary DCIS and recurrences was concordant in 89% of cases. Moreover, 43.5% of recurrences were HER2 3+ and 23% were HER2 2+ (FISH unknown). Mastectomy was performed in 26.3% of patients and 73.1% had conservative surgery. Women who received conservative surgery were significantly older, while there were no significant ethnic differences. Among women who underwent conservative surgery, the 41.4% that received radiation therapy (RT) were significantly less likely to develop recurrence than the 53.2% that did not. However, there was no significant difference in recurrence between mastectomy and conservative surgery with RT. Among ER/PR positive patients, the 30% that received preventive hormone therapy was significantly less likely to develop recurrence than the 61.4% that did not.

CONCLUSION
Our study confirms that ER/PR negative DCIS is associated with significantly higher loco-regional recurrence and standard preventive modalities reduce the risk. White women appear to have a higher recurrence probably related to risk factors (e.g. obesity) while the distribution of histological subtypes of DCIS, age at diagnosis, MIC, and treatment modalities did not significantly differ among diverse ethnic groups. Interestingly, a high rate of HER2 positive recurrences has been reported in our sample, suggesting that HER2 may represent a potential biomarker for DCIS at high risk of recurrence and therefore HER-2 targeted therapeutic interventions (e.g. vaccines, trastuzumab) can contribute to prevent it. Further analyses are needed to confirm the correlation between age/ethnicity and DCIS outcome. Better define the subgroup at worse prognosis could help to identify biomarkers predictive of recurrence or second tumors.
Title: Tamoxifen acceptance by DCIS patients and effect on subsequent ipsilateral and contralateral breast events

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Body: Introduction: Existing data demonstrate that the use of endocrine therapy decreases the risk of in-breast recurrence and contralateral new primary cancers. However, the acceptance of tamoxifen is relatively low in women with DCIS, with reported acceptance rates ranging from 32% to 67%. We reported 67% tamoxifen acceptance in 2005 (Nakhlis F, JACS, p688); we now re-examine this question to assess the impact of patient and tumor characteristics, and the use of tamoxifen, on new breast events in women with DCIS undergoing breast conserving therapy (BCT) or mastectomy.

Methods: We conducted a retrospective review using a prospective database; 695 patients with DCIS were identified between 1998 and 2009. Patient and tumor characteristics included age, tumor grade, tumor size, margin status, re-excision status, use of BCT, adjuvant radiation therapy (RT) and tamoxifen therapy. Women undergoing bilateral mastectomy were excluded for analysis of in-breast events. The demographic data were examined for differences in continuous variables using the Wilcoxon rank sum test, and differences in categorical variables between groups were assessed using Fisher’s exact test. Multivariate odds ratios were obtained using logistic regression.

Results: The mean age of the patient population was 61.5 ± 11.4 (median and range: 61 (30-99). 44.9% of patients were over the age of 50. The median follow up time was 60 months. Among the BCT group, 58.5% of 348 women with ER+ DCIS accepted tamoxifen therapy, compared to 50.7% of 71 mastectomy patients with ER+ DCIS. 188 women (27.1%) underwent mastectomy (122 unilateral and 66 bilateral); 507 (72.9%) received BCT. Among all women with complete radiation and tamoxifen data, the number of new events was 88 (16.9%). Of these, 8 (1.5%) were in the mastectomy group, and 80 (17.9%) in the BCT group (p=0.0007). Ipsilateral events occurred in 1/99 (1%) woman undergoing mastectomy. In the BCT group complete radiation and tamoxifen data were available on 448 women, of whom 54 (12%) experienced ipsilateral events (p<0.0001). The multivariate odds ratio (OR) for ipsilateral breast events in the BCT group receiving RT+ surgery, was 0.66 (95%CI 0.25-1.71); among those receiving tamoxifen + surgery it was 0.65 (95% CI 0.20-2.1) and for the combination of RT, tamoxifen, and surgery it was 0.23 (95% CI 0.08-0.65, p=0.006). In a model that included these treatment parameters, patient age, DCIS size and grade were non-significant whereas the presence of free margins was highly significant (OR 0.27, 95%CI 0.10-0.71, p=0.008). Overall, 29% of new breast events were invasive. Contralateral events occurred in 14/467 women (3%) of BCT and mastectomy patients with hormone receptor positive DCIS, but eight of these were non-compliant.

Conclusions: The use of optimal DCIS therapy (complete excision, RT, tamoxifen) decreased the odds of ipsilateral breast events by 73%, whereas RT alone or tamoxifen alone in addition to surgery were less effective. Despite relatively high acceptance of tamoxifen, lack of compliance remains an issue and will be further examined.
Title: Accelerated partial breast irradiation through brachytherapy for DCIS: Utilization patterns, influencing factors, and outcomes

Ying Liu¹, Derek Schloemann¹, Min Lian¹ and Graham A Colditz¹. ¹Washington University School of Medicine, St Louis, MO.

Body: Background. Accelerated partial breast irradiation through brachytherapy (APBIb) is a new technique that delivers radiation to the tissue immediately surrounding the lumpectomy cavity, which is at the highest risk of recurrence. APBIb has been increasingly used since 2002 for invasive breast cancer as an alternative to whole breast irradiation (WBI). Little is known about APBIb use for ductal carcinoma in situ (DCIS) and its impact on local control of recurrence.

Methods. We used the Surveillance, Epidemiology, and End Results (SEER) database from 18 NCI-SEER Registries and identified 46,993 women diagnosed with primary DCIS between 2000 and 2011 who received WBI or APBIb after breast-conserving surgery. A multilevel logistic regression model was used to estimate the odds ratios (ORs) with 95% confidence intervals (95% CI) of APBIb use associated with patient- and neighborhood-related factors. A Cox proportional hazards regression analysis was performed to compare the associations of APBIb and WBI with local recurrence risk in patients with APBIb matched with patients with WBI on propensity scores.

Results. Overall, 4.7% of 46,993 patients received APBIb and 24.5% of them were in the American Society for Radiation Oncology guideline "unsuitable" category. During the average 4.5-year followup, 562 (1.5%) patients had ipsilateral recurrence. APBIb use increased from 0.1% in 2000 to 9.4% in 2008 and then decreased slowly to 7.7% in 2011. The factors independently associated with APBIb use included older age (OR=1.69, 95% CI=1.48-1.94 for ages 50-59; OR=1.90, 95% CI=1.66-2.18 for ages 60-69; OR=2.09, 95% CI=1.78-2.44 for ages 70-79; OR=2.09, 95% CI=1.61-2.72 for age 80 or older), racial and ethnic minority (OR=0.77, 95% CI=0.66-0.90 for non-Hispanic black; OR=0.59, 95% CI=0.47-0.73 for non-Hispanic Asian; OR=0.70, 95% CI=0.58-0.84 for Hispanic), larger tumor size (OR=0.53, 95% CI=0.43-0.65), estrogen receptor negativity (OR=0.84, 95% CI=0.73-0.98), and living in rural regions (OR=0.54, 95% CI=0.36-0.80). Marital status, nuclear grade, county-level socioeconomic deprivation, and density of radiation oncologists were not related to APBIb use. Compared with WBI, APBIb was associated with increased risk of local recurrence (hazard ratio=1.62, 95% CI=1.06-2.48) between propensity score-matched patients.

Conclusion. Although efficacy has not been determined, APBIb has been increasingly used for DCIS. APBIb use was more likely in older patients, but less likely in minority patients, those with larger tumors, those with ER negative tumors, and those living in rural regions. Treatment with APBIb compared with WBI resulted in higher local recurrence rates. Randomized clinical trials and observational studies with a long followup of a large number of patients are needed to determine the safety of APBIb for DCIS.
Title: The DCIS Score - Potential for healthcare savings?

Rebekah Young¹, Kimberly Gergelis¹, Shalom Kalnicki¹ and Jana L Fox¹. ¹Einstein/Montefiore Center for Cancer Care, Bronx, NY.

Body: Introduction
The Oncotype Dx Recurrence Score for DCIS (DCIS Score) is a 12-gene assay derived from the original Oncotype DX test that evaluates recurrence risk among women with invasive carcinoma of the breast. The DCIS Score provides a local recurrence risk estimate at 10 years following lumpectomy for ductal carcinoma in situ (DCIS). Results can help guide decisions regarding adjuvant radiation (RT). Foregoing RT can be a source of significant healthcare savings. We investigated the actual healthcare dollar savings to-date in our patient population.

Methods
We evaluated patients in whom the DCIS Score was ordered (x) and calculated total cost of testing. Potential cost of RT was that of IMRT as reimbursed by Medicare for a hypofractionated (16 fraction) course, multiplied by x. Many of our large-breasted patients require IMRT to increase dose homogeneity and to limit dose to normal tissues. We also calculated potential cost with 3D conformal (3D-CRT). Total potential cost was the sum of testing and treatment costs, determined for each modality. The number of patients ultimately treated (y) was also multiplied by these costs. Total actual cost was the sum of test expenses and actual treatment costs. Savings was the difference between the total actual and total potential cost.

Results
From February, 2012 to May, 2014 the DCIS Score was performed in 38 patients (x= 38). Median age was 66 (40-85). Grade was low in 39%, intermediate in 45%, and high in 16%. 50% had necrosis present, and the median size of DCIS was 0.5cm (0.1 - 3.1cm). The total cost of testing was $4125 * 38 = $156,750. IMRT reimburses at approximately $23,000 and 3D-CRT at approximately $11,000 per treatment. (Medicare reimbursement rates can vary among states.) The potential total cost of RT ranged from $418,000 - $874,000; testing brought the total potential costs to $574,750 - $1,030,750. Upon receipt of the test results, 12 (y) patients ultimately underwent therapy. IMRT was given in 11 patients and 3D-CRT in 1, for a total treatment cost of $264,000. Therefore, total actual expenditures were $420,750. Savings amounted to (574,750 – 420,750) = $154,000 to (1,030,750– 420,750) = $610,000.

Conclusions
In the era of rising healthcare costs, it is imperative to examine instances of possible over-treatment. The DCIS Score has the potential to save not only healthcare dollars, but to spare patients radiation side effects, time lost from work, and transportation expenses. While there are costs associated with the assay, if ordered judiciously, these can be offset by the subsequent savings from eliminating treatment.
Title: A class I histone deacetylase inhibitor, entinostat, enhances lapatinib efficacy in both HER2-overexpressing inflammatory and non-inflammatory breast cancer cells through FOXO3-mediated Bim1 expression

Jangsoon Lee¹, Chandra Bartholomeusz¹, Gabriel N Hortobagyi¹, Peter Ordentlich² and Naoto T Ueno¹. ¹UT MD Anderson Cancer Center, Houston, TX and ²Syndax Pharmaceuticals, Inc, Waltham, MA.

Body: Background: Human epidermal growth factor receptor 2 (HER2) overexpression has been reported in 15%-20% of breast cancers and is associated with shorter survival and worse clinical outcome. Inflammatory breast cancer (IBC), a very aggressive subtype of advanced breast cancer, accounts for approximately 2% of all breast cancers and 8%-10% of all breast cancer-related deaths in the United States. Recent study represented that about 60% of IBCs overexpress HER2. Although there are effective HER2-targeted agents, patients with HER2+ breast cancer often have intrinsic and acquired resistance to the anti-HER2 agents that are currently approved by the U.S. Food and Drug Administration. Therefore, novel combination strategies are needed to treat HER2+ breast cancers that develop drug resistance. To this end, we investigated the combinational effect of entinostat, an oral isoform-selective histone deacetylase type I inhibitor, and lapatinib, a HER2/epidermal growth factor receptor dual tyrosine kinase inhibitor, in HER2+ inflammatory breast cancer (IBC) and non-IBC breast cancer cells. Methods: We assessed the combinational synergistic effect and its mechanism via CellTiter Blue assay, flow cytometry, anchorage-independent growth, quantitative real-time polymerase chain reaction, small interfering RNA, Western blotting, and mammary fat pad xenograft mouse models. Results: We found that compared with entinostat or lapatinib alone, the two drugs in combination synergistically inhibited tumor cell proliferation (P < 0.001), reduced in vitro colony formation (P < 0.05), and resulted in significant in vivo tumor shrinkage or growth inhibition in both IBC and non-IBC xenograft mouse models (SUM190 and BT474, P < 0.001). The synergistic antitumor activity of the entinostat-lapatinib combination was due to downregulation of phosphorylated Akt, which induced the transcriptional activity of FOXO3, resulting in the induction of Bim1 (a BH3 domain-containing pro-apoptotic protein). Furthermore, entinostat sensitized trastuzumab/lapatinib-resistant HER2+ cells to the trastuzumab-lapatinib combination and enhanced the anti-proliferation effect compared with single-agent with lapatinib or combination treatment with lapatinib and trastuzumab. Conclusion: Taken together, our data provide evidence that entinostat has enhanced antitumor effect in combination with the HER2-targeted reagent lapatinib and results in the induction of apoptosis by FOXO3-mediated Bim1 expression. Our findings justify conducting a clinical trial (clinicaltrial.gov, NCT01434303) of combinational treatment with entinostat, lapatinib, and trastuzumab in patients with HER2+ IBC or non-IBC that is resistant to trastuzumab-based treatment.
Title: Genomic profiling by FoundationOne® analysis of inflammatory breast cancer cases reveals a high frequency of clinically relevant genomic alterations (GA)


Body: Background: Inflammatory breast cancer (IBC) is a distinct clinicopathologic entity that carries a worse prognosis relative to non-IBC breast cancer even when matched for biomarkers (ER/PR/HER2). Genomic profiling of IBC cases may identify alterations that suggest response to targeted therapies, but is best implemented by an integrated NGS assay capable of detecting all classes of genomic alterations (GA).

Methods: Hybridization capture of 3769 exons of 236 cancer-related genes and 47 introns from 19 genes that are frequently rearranged in cancer were fully sequenced to high, uniform coverage from a commercial CLIA-certified laboratory (Foundation Medicine).

Results: Of 2,208 clinical breast cancer cases assayed, 55 IBC cases were identified, and of the 50 cases for which hormone receptor and HER2/neu status were know, 34% were ER-/PR-/HER2- (TNBC). IBC cases harbored 274 GA with an average of 5.0 GA/tumor (range 1-15). At least one alteration associated with an FDA approved therapy or clinical trial was identified in 53/55 (96%) of cases, yielding an average of 2.6 clinically relevant GA/case. Genes most frequently altered were TP53 (60%), MYC (31%), PIK3CA (25%), ERBB2 (20%), FGFR1 (18%) and PTEN (16%). MYC amplifications were present in 24% of the 2,208 clinical breast carcinoma cases. In the TNBC subset of IBC, 8/19 (42%) pt samples showed MYC amplification (median copy number 8X, range 7-20) as compared to 9/36 (25%) in non-TNBC pt samples (median copy number 7X, range 6-21).

Within this prospective series, treatment decisions were made based on FoundationOne results. A 58-year old pt with likely secondary IBC harbored two ERBB2 activating base substitutions (V777L and S310F), but without amplification of ERBB2, and had durable response to lapatinib (Ali et al. JCO 2014). In another case, a 53 year old pt presented with ERBB2-amplified IBC now refractory to HER2-targeted therapy. FoundationOne testing revealed an activating EGFR mutation (L858R) as well as the previously described ERBB2 amplification, suggesting that the EGFR alteration may underlie the acquired resistance. The pt responded to erlotinib monotherapy for 8 months. (Ali et al. Clin Br Ca, 2013). Clinical follow-up for additional patients is ongoing.

Conclusions: 96% of IBC cases harbored at least one alteration that suggests responsiveness to agents that are FDA approved or being studied in clinical trials. IBC cases also frequently had MYC amplifications (31%), but this may reflect a high percentage of TNBC-IBC cases with MYC amplifications (42%) in this series. Given the limited treatment options and poor prognosis of patients with metastatic IBC, the FoundationOne assay with comprehensive NGS-based genomic profiling has the potential to identify new treatment paradigms and address an unmet clinical need for this disease.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-14-04
Average Grade: 5.00

Title: Targeting the platelet derived growth factor receptor alpha in inflammatory breast cancer

Kenneth L van Golen\textsuperscript{1,5,4}, Abhijit Ramachandran\textsuperscript{2}, Steven Van Laere\textsuperscript{3,5} and Madhura Joglekar\textsuperscript{1,5}. \textsuperscript{1}University of Delaware, Newark, DE; \textsuperscript{2}Arog Pharmaceuticals LLC, Dallas, TX; \textsuperscript{3}Translational Cancer Research Unit, Antwerp, Belgium; \textsuperscript{4}International Inflammatory Breast Cancer Consortium, Newark, DE and \textsuperscript{5}Helen F. Graham Cancer Center, Newark, DE.

Body: Inflammatory breast cancer (IBC) is the most lethal form of locally advanced breast cancer and carries a guarded prognosis. IBC presents with a "lumpless" primary tumor, which rapidly infiltrates and forms tumor emboli within the dermal lymphatic vessels of the skin overlying the breast. It is clear from the recent research advancements that IBC is a unique entity, not only in its clinical presentation, disease progression and response to therapy but also in its molecular expression profile. Therefore, it is essential to carry out molecular expression studies in IBC compared to non-IBC as well as to pursue these differences to study their effects on IBC phenotype. Our laboratory has demonstrated that the platelet derived growth factor receptor alpha (PDGFRa) is significantly over expressed in IBC patient samples (between 15-36\%) compared to non-IBC patient samples. Using multiple approaches we demonstrate the PDGFRa activation signature in IBC patient samples and cell lines. Our data demonstrates that PDGFRa is constitutively active and localized in the cytoplasm of IBC cells. Although the receptor is intracellular, PDGFRa signaling remains intact. Thus, PDGFRa is an attractive target for therapy. Here we show that a novel PDGFRa inhibitor, crenolanib, which specifically targets active PDGFR, is able to significantly inhibit IBC tumor growth and progression. In nude mice, 200 mm\textsuperscript{3} orthotopic IBC tumors did not increase in size when the mice were treated with crenolanib. In contrast, mice treated with vehicle control demonstrated a 4-fold increase in tumor growth over the 2-week course of the experiment. In addition, by using a novel in vitro IBC emboli growth model, we demonstrate that crenolanib prevents IBC tumor emboli formation and acts to sensitize IBC cells to chemotherapeutic agents. We were able to achieve a similar level of IBC cell killing with a 10-fold lower dose of chemotherapeutic agents compared to the agents by themselves. Together, these data demonstrate that PDGFRa is a viable target in IBC patients and that crenolanib is a potent and effective therapeutic agent against IBC cells.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-14-05
Average Grade: 4.20

Title: A novel link between anti-apoptotic signaling, NFκB, and SMAD7 in IBC pathobiology

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Body: Background: Inflammatory breast cancer (IBC) has the highest lethality amongst all subtypes of breast cancer and develops rapid therapeutic resistance. High NFκB activation has been identified as a distinct molecular determinant in IBC pathobiology; however, the precise sequence of its activation and functional consequence in IBC remains unknown. Our previous work identified increased expression of the X-linked inhibitor of apoptosis protein (XIAP) due to altered translation in IBC, while other studies have noted a crosstalk between XIAP and NFκB. We hypothesized that XIAP drives NFκB activation in IBC promoting therapeutic resistance and tumorigenesis.

Methods: NFκB phosphorylation, nuclear translocation, and target gene expression were evaluated in triple-negative SUM149 IBC cells with targeted overexpression or knockdown of XIAP. Using specific point mutants, we assessed the domain and mechanism of XIAP-mediated NFκB activation in IBC. We evaluated proliferation and viability in 2D and 3D culture of SUM149 cells treated with JSH-23, a small molecule inhibitor of NFκB nuclear translocation. We monitored the effects of XIAP overexpression or knockdown on in vivo tumorigenicity in IBC xenograft models by measuring tumor growth and NFκB signaling. IHC analysis of XIAP and NFκB was performed on tumor microarrays containing both non-IBC and IBC.

Results: Knockdown of XIAP significantly decreased NFκB activation in IBC cells. Domain analysis revealed the necessity of the BIR1 domain of XIAP and TAB1:IKKβ complex formation in activating NFκB. NFκB antagonism inhibited proliferation of cells and sensitized therapy-resistant, XIAP overexpressing cells to targeted therapy. Loss of XIAP inhibited tumor growth of SUM149 tumor cells, correlating with decreased ALDH activity and varied epithelial-mesenchymal characteristics in these cells, while overexpression of XIAP significantly enhanced tumor growth of SUM149 cells. Further analysis revealed altered SMAD7 expression in XIAP knockdown cells, revealing crosstalk between XIAP, NFκB, and TGFβ signaling in IBC. IHC analysis of XIAP expression in invasive non-IBC tumors correlated with triple-negative status as well as increased grade and stage of tumors. In IBC tumors, XIAP expression associated with increased NFκB.

Conclusions: In summary, our studies reveal that XIAP expression is necessary for NFκB activation in IBC and is critical for IBC development and progression. This study provides a novel insight into how an anti-apoptotic protein may regulate survival signaling and disease progression and may guide further research into innovative inhibitors of this interaction.
**2014 San Antonio Breast Cancer Symposium**

**Publication Number:** P6-14-06  
**Average Grade:** 5.40  

**Title:** CCR5 antagonists suppresses the migration and invasion of human inflammatory breast cancer cells

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**Body:**  
**Background:** Inflammatory breast cancer (IBC) is a rare and highly metastatic variant of breast cancer with a poor prognosis and lower survival rate. The disease has a peculiar pattern of early recurrence to soft tissue, bone and central nervous system irrespective of molecular subtype. Currently, there are no specific therapeutic options for IBC patients, particularly there are no therapeutic agents aimed at controlling metastatic spread. Chemokine CCL5 and its receptor CCR5 play a significant role in breast cancer progression and metastasis. We have recently shown that CCR5 promotes breast cancer invasiveness and metastatic potential, and CCR5 inhibition by the FDA approved CCR5 antagonists Maraviroc and Vicriviroc reduced \textit{in vivo} metastasis in a basal-like breast cancer model (Cancer Res 2012;72:3839-3850). Here, we examined the expression of CCR5, as well as the effects of CCR5 inhibition on the migration and invasion \textit{in vitro} in human IBC cells.

**Methods:** IBC cell lines SUM149, SUM190, and FC-IBC-02 derived from pleural effusion fluid of an IBC patient were examined for the CCR5 expression by immunofluorescence staining. The effects of CCR5 antagonists (Maraviroc and Vicriviroc) on cell migration and invasion \textit{in vitro} were examined using matrigel-coated Boyden chamber assay in FC-IBC-02 cells, which are triple-negative, basal-like, cancer stem cell phenotype, and rapidly developed primary tumors and metastasis \textit{in vivo} (Breast Cancer Res Treat 2013;140:23–33). Cells were treated by Maraviroc and Vicriviroc at 100 µM.

**Results:** CCR5 was expressed at low positive component comprising only about 5-7 % of the total cell population in SUM 149 cells. CCR5 expression was not detected in SUM190 cells. CCR5 was expressed at significantly higher levels with a higher percentage of positive population (50-60%) in FC-IBC-02 cells compared with SUM149 cells. FC-IBC-02 cells have a relatively high percentage of CCR5 positive cells in suspension culture. CCR5 inhibition by both CCR5 antagonists Maraviroc and Vicriviroc significantly inhibited the migration and invasion of IBC cells \textit{in vitro}. Maraviroc and Vicriviroc demonstrated effective agents in controlling migrations (55% and 60% relative inhibition).

**Conclusions:** CCR5 is highly expressed in human IBC cells. CCR5 antagonists demonstrated the inhibition of migration and invasion of IBC cells. Further studies are warranted to determine the effects of CCR5 antagonists in combination with standard treatment in the ability to control IBC progression and metastasis. These results may suggest a potential antimetastatic role for CCR5-inhibitors in IBC.
Title: High miR-19a serum levels correlate with favorable prognosis in patients with metastatic HER2+ inflammatory breast cancer and may result from an effective antibody-dependent cell-mediated cytotoxicity induced by trastuzumab

Simone Anfossi1,2, Antonio Giordano1,2, Lei Huo1,2, Ricardo H Alvarez1,2, Vicente Valero1,2, Gabriel N Hortobagyi1, Wendy A Woodward1,2, Naoto T Ueno1,2, George A Calin1 and James M Reuben1,2. 1University of Texas MD Anderson Cancer Center, Houston, TX and 2Morgan Welch Inflammatory Breast Cancer Research Program and Clinic, Houston, TX.

Body: Background: IBC is a rare but highly aggressive form of locally advanced breast cancer (5-year OS rate: 40.5% IBC vs 85% non-IBC patients) accounting for 10% of all breast cancer deaths. To date, no unique molecular diagnostic or prognostic biomarker has been identified for IBC. Increasing evidence supports the potential value of miRNA as prognostic and predictive serum biomarker in cancer. We found that IBC cells expressed high levels of miR-19a and patients with metastatic IBC HER2+ (MIBC HER2+) and high miR-19a serum levels had better prognosis than patients with MIBC HER2+ and low miR-19a serum levels. As one of the mechanisms of action of trastuzumab is the induction of antibody-dependent cell-mediated cytotoxicity (ADCC), we hypothesized that the increased miR-19a serum levels in MIBC HER2+ patients with favorable clinical outcome could result from an effective ADCC and be used as biomarker to monitor the response to trastuzumab.

Methods: Total RNA was isolated using the Total RNA Purification Kit (Invitrogen, Norgen Biotek). MiR-19a levels in tumor tissue, serum, cell lines and supernatants were evaluated by qRT-PCR (Applied Biosystems). ADCC was evaluated by Annexin V-FITC Apoptosis Detection Kit I (BD Pharmingen).

Results: Microarray was performed in IBC (n=23) and non-IBC (n=24) tumors and normal tissue (n=12). Microarray showed higher miR-19a expression in IBC compared with non-IBC (p=0.028) and normal tissue (p=0.0002). The two IBC cell lines SUM149 (triple receptor-negative) and KPL-4 (HER2-amplified) expressed higher levels of miR-19a compared with the non-IBC cell lines MDA-231 (triple receptor-negative), SKBR3 (HER2-amplified), and MCF-7 (HER2-non-amplified) (p<0.001, p<0.001, p<0.001 and p<0.05, p<0.05, p<0.01 respectively). To assess whether miR-19a could be released from IBC cells upon NK cell-mediated ADCC, we performed a NK cytotoxicity test using the NK-resistant KPL-4 cells and the NK-sensitive SKBR3 and the NK-sensitive MCF-7 cells as control. Co-incubation with trastuzumab induced increased MCF-7, SKBR3 and KPL-4 cell death (2.1-fold, 2.4-fold and 3.5-fold respectively) and accordingly increased miR-19a levels in their supernatants (MCF-7: 3-fold, p=0.017; SKBR3: 6-fold, p=0.005 and KPL-4: 8-fold, p=0.0001). The pattern of miR-19a levels in the supernatants correlated with that expressed at cellular levels (MCF-7<SKBR3<KPL-4). We measured miR-19a serum levels in patients with MIBC HER2+ (n=27) and metastatic nonIBC HER2+ (n=24). Patients with MIBC HER2+ and high miR-19a level had longer PFS (10.3 vs 3.2 months, p=0.022) and OS (median not reached vs 11.2 months, p=0.003) than patients with low miR-19a levels. Patients with metastatic nonIBC HER2+ and high miR-19a levels had longer OS (32.9 vs 13.3 months; p=0.015) than patients with low miR-19a levels (32.9 vs 13.3 months; p=0.015).

Conclusion: High miR-19a serum levels are associated with favorable prognosis in patients with MIBC HER2+ and could result from an effective NK cell-mediated ADCC. MiR-19a may represent a novel serum biomarker to monitor the response to trastuzumab therapy and predict clinical outcome in patients with MIBC HER2+.
Title: Risk factors for developing inflammatory breast cancer: Unique trends among a single patient population

Randie E White¹, Laura E Warren², Jennifer R Bellon², Faina Nakhli³, Heather A Jacene¹, Eren D Yeh¹, Judith Hirshfield-Bartek¹, Beth Overmoyer¹ and Inflammatory Breast Cancer International Consortium³. ¹Dana-Farber Cancer Institute, Boston, MA; ²Brigham and Women's Hospital, Boston, MA and ³Inflammatory Breast Cancer International Consortium.

Body: Introduction: Inflammatory breast cancer (IBC) is a virulent form of breast cancer, characterized by skin erythema and edema associated with enlargement of the breast rapidly occurring within 3-6 months (mo). Because of its rarity (<5% incidence), there is a paucity of data identifying epidemiologic risk factors, which may shed light on the potential causes of IBC, and guide prevention strategies.

Methods: This study utilized retrospective data obtained from an IRB approved database of 275 patients (pts) with IBC evaluated at Dana Farber Cancer Institute (DFCI) from 1997-2012. Pts with confirmed invasive breast cancer had documented clinical characteristics of IBC clinically staged as T4d. The statistical software JMP 10 was used to perform statistical tests. Chi Square Tests, Fisher’s Exact Tests, and descriptive statistics were compiled.

Results: The mean age of diagnosis among our study population was 50.2 years (yrs). IBC pts were more frequently diagnosed when premenopausal (55%) versus (v) postmenopausal (45%); 25% of pts had metastases upon presentation. The majority of patients (77%) were overweight (BMI 25-29.9) or obese (BMI≥30). More premenopausal pts (80.3%) had a BMI >25 v postmenopausal pts (73.5%). We observed no association with BRCA status among those undergoing genetic testing (13% BRCA positive (pos); 54 tested); however 52% of pts had a family history of breast cancer (5% BRCA pos). The majority of pts (81%) did not undergo genetic testing. The most common IBC subtype was HER2 pos (40%); 19% were triple negative (neg), and 16% were hormone receptor (HR) pos/HER2 neg. We also observed a trend of a longer duration of symptoms prior to diagnosis among younger pts. The mean age of pts who experienced >6 months (mo) of symptoms prior to diagnosis was 42 yrs v the mean age of 51 yrs among those experiencing < 2 mo of symptoms prior to diagnosis. Pts also displayed a temporal trend in diagnosis dependent upon time of year. 57% of pts were diagnosed with IBC during the warmer temperatures (Mar 21 – Sept 20) compared with 43% diagnosed during the cooler temperatures (Sept 21– Mar 20).

Conclusion: This retrospective epidemiologic analysis demonstrated various trends in a single population of IBC pts. The association of high BMI and risk of developing IBC among premenopausal women contrasts with that seen in the non-IBC group, i.e. high BMI is a risk factor for non-IBC only among postmenopausal women. Targeting the obesity crisis may be a means of reducing the risk of developing IBC among younger women. A differentiating feature of IBC is the rapid onset of signs and symptoms of IBC, and yet, younger pts had a significant delay in diagnosis of >6 mo compared with older pts. This emphasizes the urgency to educate both pts and providers about IBC and facilitate rapid diagnostic procedures. The seasonal relationship with diagnosis observed in this cohort of IBC pts is intriguing. Investigators have hypothesized an infectious etiology contributing to the development of IBC, namely infection by viruses or bacterial pathogens may play a role in the pathophysiology of IBC. These unique trends seen in the DFCI IBC population deserve further investigation.
Title: Clinical outcomes of triple negative inflammatory breast cancer treated with contemporary anthracycline and taxane preoperative therapy support further investigation of therapeutic targets

Beth Overmoyer¹, Hao Guo¹, Laura E Warren², Jennifer R Bellon², Kornelia Polyak¹, Faina Nakhlis², Judith Hirshfield-Bartek¹, Heather Jacene¹, Eren D Yeh¹, Meredith Regan¹ and Inflammatory Breast Cancer International Consortium³. ¹Dana-Farber Cancer Institute, Boston, MA; ²Brigham and Women's Hospital, Boston, MA and ³Inflammatory Breast Cancer International Consortium.

Body: Background: Approximately 40-50% of inflammatory breast cancer (IBC) is triple negative (TN), defined as estrogen and progesterone receptor and HER2 negative. The poor prognosis associated with TN-IBC lends itself to active investigation of novel therapies to improve outcome. Dr. Kornelia Polyak’s laboratory has demonstrated a significant association of JAK2/STAT3 pathway activity in TN-IBC (Overmoyer, Cancer Res 2012). In preparation of designing a clinical trial investigating the effect of JAK2 inhibition by ruxolitinib on biologic parameters and subsequent use in neoadjuvant chemotherapy (NAC) for TN-IBC, we determined historical outcomes resulting from a single institution’s contemporary standard treatment of TN-IBC.

Methods: Among the 273 pts enrolled in the IRB approved IBC database at the Dana Farber Cancer Institute, 28 pts were identified with Stage III (T4d,NX,M0)TN-IBC diagnosed from 1/1/1999 to 12/31/2011 who were treated with standard NAC including anthracycline, cyclophosphamide and taxane. Time to treatment failure (TTF) was defined from diagnosis (dx) to first progression or recurrence; time to distant metastasis (TDM) was defined from dx to first metastasis at a distant site; overall survival was defined from dx to death from any cause. Subsequent to NAC, 25 pts underwent modified radical mastectomy (MRM) and radiation. For those 25 pts, disease-free survival (DFS) was defined from MRM to first recurrence or death; time to local/regional recurrence (LRR) was defined from MRM with death as competing risk. All time-to event endpoints were censored at the date last known alive if an event was not observed.

Results: Among 28 patients, the median TTF was 19 months (mo) with 67% free from progression/recurrence at 1 year (yr) after dx (95% CI, 51-87%). Median TDM was 20 mo with 78% free from distant metastases at 1 yr (CI 64-95%). Most patients (13/21) had multiple sites of first distant metastasis including: lung (7), contralateral axilla (7), bone (6), liver (3) and CNS (3). Median OS was 34 mo since dx (Table). Among 25 patients who underwent MRM, median DFS was 15 mo with 1-yr DFS of 58% (42-82%); the cumulative probability of LRR was 13% and 33% at 1 and 2 yr.

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<td>OS probability</td>
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<td>1-year</td>
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<td>2-year</td>
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<td>3-year</td>
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Conclusions: This retrospective analysis of clinical outcomes of Stage III TN-IBC treated with contemporary anthracycline/taxane regimes is consistent with previously reported outcomes using historical NAC regimens. (Li, et al, the Oncologist 2012). These dismal rates of 49% 3-year OS from diagnosis and 58% 1-yr DFS following MRM demand more active investigation into novel targeting agents which can be combined with standard NAC specifically for the treatment of TN-IBC; a disease that has no known therapeutic target. For this reason DFCI is actively investigating the role of inhibiting the JAK2/STAT3 pathway using ruxolitinib in conjunction with standard weekly paclitaxel followed by AC as NAC for TN-IBC. Clinical trial information: NCT01796197.
Expression of chemokine and macrophage infiltration in tumor microenvironment: Role in breast cancer metastasis

Brij Nath Tewari¹, Chakrapani Tripathi², Khemraj S Baghel², Mehraj U-Din Lone², Richa Shrivastava², Sanjeev Misra¹, Madanlal B Bhatt¹, Vijay Kumar¹, Nuzhat Husain¹, Madhumati Goel¹ and Smriti Bhadauria². ¹King George Medical University, Lucknow, Uttar Pradesh, India and ²Central Drug Research Institute, Lucknow, Uttar Pradesh, India.

Body: Breast cancer is one of the most common invasive cancers and the second leading cause of cancer related deaths among women in the across world. Breast cancer-related mortality is associated with the development of metastatic potential of primary tumor lesions. Breast Cancer is a complex and intrinsically heterogeneous disease with different morphologies, molecular profiles and clinical behaviour which require different treatments. The metastatic of breast cancer (MBC) has been the issue of intense scrutiny of cancer. The most common sites for metastasis are the lung, liver, bones and brain. The brain is one of the most common organs affected in the spread of breast cancer that eventually results in lethal development of the cancer disease. Brain metastasis is an progressively more common obstacle in breast cancer patients about 15%–30% of breast cancer patients develop brain metastasis.

Human breast tumor specimen were collected after informed consent from 83 patients who underwent surgery (MRM) for breast cancer at Department of surgical oncology, king George Medical University, Lucknow (India) approved by our, Institutional ethics committee the study protocol (ECM IIB/P17) which followed the Helsinki Declaration. 83 Sample of breast cancer patients, tumor tissue and adjacent normal tissue was obtained by MRM of the patient earlier these patients were diagnosed with carcinoma of breast cancer.

The clinicopathological data were retrieved from medical records at the same institution. The most important prognostic factors in breast cancer are age, tumor size, status TNM, and pathological grade, histological type of the tumor, and hormone-receptor status ER, PR, her2neu and macrophage surface marker via CD68. A large number of other factors have been investigated for their potentiating to predict the outcome of breast cancer metastasis. We have analysed the expression level chemokine via RT-PCR. Our finding clinical as well as biological indicated that chemokine is a potent chemo-attractant for breast cancer patient. The results of our study indicate that significant associations exist between CCL2, NF-kB, TNF-α & IL-1β in breast cancer, along different stages of breast cancer patients.
A comparative analysis of primary tumor resection in men and women with stage IV breast cancer

Nasreen A Vohra, Swapnil D Kachare, Timothy L Fitzgerald, Jan H Wong and Mahvish Muzaffar. East Carolina University, Brody School of Medicine, Greenville, NC.

Background: Primary tumor resection (PTR) for metastatic female breast cancer continues to be debated. Given the rarity of male breast cancer, treatment paradigms for female breast cancer are extended to the management of male breast cancer. Whether the role of PTR in men with metastatic breast cancer is similar to that in women remains unclear. We sought to compare these two populations using a large, national database.

Methods:
All patients with Stage IV breast cancer between the years 1988-2011 in the SEER database were identified. Uni and multivariate descriptive and survival analyses were performed.

Results: A total of 41,601 patients with stage IV breast cancer were identified; 98.9% (n=41,162) females, 1.1% (n=439) males. On average, female patients were younger (63 vs. 66y) and more often White (78 vs. 74%), p≤0.02. Tumors in male patients were more likely to be hormonally positive, with varying breakdown of T and N-stages and histologic subtypes as compared to tumors in females, p≤0.05. Males were more likely to undergo PTR (51 vs.40%, p<0.05), however both males and females had similar rates of radiotherapy (35 vs. 32%, p=0.35). Among male patients, those who received PTR were of similar age to those who did not receive PTR (p=0.64), but had a greater representation of White patients (p=0.04). There were differences in T-stage, N-stage and hormonal status between men who did and did not receive PTR, p≤0.05. Men receiving surgery were also more likely to receive radiation therapy (38 vs. 32%, p=0.003). In women, all demographic and tumor-related factors were significantly different between those who did and did not undergo PTR. On univariate analysis, surgery was associated with improved disease-specific median survival in both men (36 vs. 21 mths) and women (34 vs. 18 mths), p<0.05. Younger age, White race, lower T and N-stage, lower grade, hormonal positivity, mucinous histology, and radiation therapy were associated with improved DSS in men. On multivariate analysis, a lack of resection of the primary tumor remained independently associated with increased mortality in men (HR 1.91, p<0.05) and women (HR 1.6, p<0.05). Over the study period there was a decrease in the rate of surgery in both men and women, p≤0.0006, but only women were found to have a statistically significant improvement in DSS with surgery over time.

Conclusion: Regardless of gender, patients with metastatic breast cancer who underwent primary tumor resection had a significant improvement in DSS. Factors associated with DSS varied between male and female patients, but the reasons for this difference are unclear. A well designed randomized trial including both genders will help determine the utility of PTR in stage IV breast cancer patients.

Characteristics of Stage IV Breast Cancer in Males: Surgery vs. No surgery (S = ≤ p.05)

<table>
<thead>
<tr>
<th></th>
<th>Surgery (%)</th>
<th>No surgery (%)</th>
<th>P-value</th>
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<tbody>
<tr>
<td>White race</td>
<td>78.4</td>
<td>68.7</td>
<td>S</td>
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<tr>
<td>T1 stage</td>
<td>15.8</td>
<td>7.4</td>
<td>S</td>
</tr>
<tr>
<td>N1 stage</td>
<td>27.5</td>
<td>38.6</td>
<td>S</td>
</tr>
<tr>
<td>ER +</td>
<td>72.5</td>
<td>57.6</td>
<td>S</td>
</tr>
<tr>
<td>PR +</td>
<td>57.2</td>
<td>45.2</td>
<td>S</td>
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<tr>
<td>Her2 +</td>
<td>1.4</td>
<td>3.7</td>
<td>0.25</td>
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<tr>
<td>Grade II</td>
<td>34.7</td>
<td>26.3</td>
<td>S</td>
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<tr>
<td>Infiltrating ductal carcinoma</td>
<td>76.6</td>
<td>56.2</td>
<td>S</td>
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<tr>
<td>Radiation therapy</td>
<td>37.8</td>
<td>32.3</td>
<td>S</td>
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<tr>
<td>Year of diagnosis</td>
<td>8.1</td>
<td>7.4</td>
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<tr>
<td>1988-1992</td>
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<tr>
<td>2008-2011</td>
<td>25.2</td>
<td>36.9</td>
<td>S</td>
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Bisrat G Debeb, Lara Lacerda, Simone Anfossi, Parmeswaran Diagaradjane, Khoi Chu, Lei Huo, Caimiao Wei, Richard A Larson, Adam R Wolfe, Wei Xu, Daniel L Smith, Li Li, Cristina Ivan, Pamela K Allen, Xiang H Zhang, George A Calin, Savitri Krishnamurthy, Naoto T Ueno, Thomas A Buchholz, James M Reuben and Wendy A Woodward. 1University of Texas MD Anderson Cancer Center, Houston, TX and 2Baylor College of Medicine, Houston, TX.

**Body:**

**Purpose:** Brain metastasis poses a major treatment challenge and remains an unmet clinical need. Finding novel therapies to prevent and treat brain metastases requires an understanding of the biology and molecular basis of the process, which currently is constrained by a dearth of experimental models and specific therapeutic targets. The purpose of this study was to develop preclinical models and identify molecular mediators of brain metastasis from breast cancer.

**Methods:** We used MDA-MB-IBC3 (ER-/HER2+), SUM149 (ER-/HER2-), MCF7 (ER+/HER2-), SUM159 (ER-/HER2-) and MDA-MB-231 (ER-/HER2-) cell lines for this study. GFP-labeled cells were injected via tail vein into SCID/beige mice and metastatic colonization to the brain and lung evaluated by fluorescent stereomicroscope and histology 8-weeks after injection. miRNA microarray was performed with miRNA 3.0 Array. Stable knockdown of miR-141 was achieved with the lentiviral miRZip system. MiR-141 serum levels in 105 breast cancer patients were measured using quantitative PCR.

**Results:** We developed novel brain metastasis models in which tail-vein injection of both triple-negative and a HER2-overexpressing inflammatory breast cancer lines led to a high rate of brain metastases (67%) in SCID/Beige mice (SUM149, 6 of 9 mice; MDA-MB-IBC3, 10 of 15 mice). Sub-lines derived from lung or brain metastases in these models were morphologically and molecularly distinct. The brain metastasis-derived sublines showed epithelial morphology and overexpressed epithelial markers and miR-141 while sublines from lung metastases showed mesenchymal morphology and overexpressed mesenchymal markers. Knockdown of miR-141 significantly inhibited metastatic colonization to the brain compared to controls (miR-141 knockdown vs. control: SUM149, 0 of 8 mice vs. 6 of 9 mice, p=0.009; MDA-MB-IBC3, 2 of 14 mice vs. 10 of 15 mice, p=0.007) but it did not affect colonization to the lung. Importantly, ectopic expression of miR-141 in non-expressing MDA-MB-231 significantly enhanced brain metastatic colonization (5 of 9 mice vs. 0 of 10 mice, P=0.02). On multivariate analyses high serum level of miR-141 was an independent predictor of progression free survival [HR 4.8 (95%CI, 2.6-8.7), P<0.001] and overall survival [HR 7.0 (95%CI 3.5-15.1), P<0.001] in patients with metastatic breast cancer.

**Conclusion:** We demonstrated high rates of brain metastases from a heterogeneous group of cell lines that have not previously been associated with brain metastases, demonstrated miR-141 as a key regulator of brain metastasis and provided clinical evidence supporting the prognostic relevance of miR-141. We propose that miR-141 should be examined as a biomarker and potential target in the prevention and treatment of brain metastases from breast cancer.
Oncolytic viral therapy enhances the survival of mice in a novel model of breast cancer brain metastases

W Hans Meisen1, Samuel Dubin1, Steven Sizemore1, Haritha Mathsyaraja1, Katie Thies1, Norm Lehman1, Peter Boyer1, Alena C Jaime-Ramirez1, J Bradley Elder1, Kimberly Powell1, Michael Ostrowski1 and Balveen Kaur1. 1James Comprehensive Cancer Center The Ohio State University Medical Center, Columbus, OH.

Body: Breast cancer (BC) is one of the leading causes of brain metastases. The 2 year survival rate of patients with breast cancer brain metastases (BM) is less than 2%. Oncolytic viruses exploit the aberrant molecular and genetic pathways found in cancer cells to selectively replicate in and destroy tumors while sparing normal tissues. Here, we demonstrate the oncolytic Herpes Simplex Virus [HSV-1], 34.5ENVE, can specifically target and destroy BC brain metastases. The 34.5ENVE virus expresses anti-angiogenic Vstat120 and its replication is transcriptionally driven by the cancer specific promoter Nestin. Vstat120 expression is reduced in brain, renal, and gastric cancers, however its expression status in BC is not known. Analysis of The Cancer Genome Atlas revealed a 52% reduction in Vstat120 expression in invasive ductal breast carcinomas (n=69) compared to normal breast tissue (n=389; P<0.0001). Reduced Vstat120 expression was also associated with decreased disease free survival in BC patients (n=324; P<0.03). An examination of Vstat120 expression in 50 breast cancer cell lines from the Neve et al dataset showed Vstat120 mRNA levels were reduced in 38% of BC cell lines compared to the MCF-10A epithelial cell line (19 of 50 cell lines). These analyses suggested BC patients may benefit from the re-expression of anti-angiogenic Vstat120. Nestin is up-regulated in several metastatic cancers, and its expression correlates with decreased survival in BC patients. In a cohort of 166 patients stratified by median Nestin expression, we observed Nestin to be significantly associated with an increased incidence of brain and lung metastases (n=164; P<0.02). Additional analysis of the Neve et al microarray dataset showed Nestin was upregulated in 100% of the BC cell lines examined (50 of 50). These results suggest that Nestin expression may be a strong therapeutic target for BC. 34.5ENVE was cytotoxic to human BC cells of varying subtypes in vitro including the HER2+ and triple negative BCs known to frequently metastasize to the brain. Since 34.5ENVE replication is driven by a Nestin promoter, we compared the cytotoxicity of 34.5ENVE with a similar virus lacking Nestin driven ICP34.5 expression. We observed a 54.14% and 24.44% increase in killing in the MDA-MB-468 and MDA-MB-231 cell lines in the Nestin-driven 34.5ENVE virus as compared to a virus lacking ICP34.5, respectively (P<0.001). This is the first study to specifically use Nestin expression to target BC. To test the therapeutic efficacy of 34.5ENVE against BM in vivo, we created a novel, immune competent BC BM model using Met-1 and DB-7 murine BC cell lines. Intracranial implantation of these cells resulted in tumors which recapitulated the human BM tumor biology. Treatment of mice with established Met-1 BM tumors with a single, intratumoral dose of 34.5ENVE resulted in significant tumor regression [via MRI] and increased survival. Similarly, mice bearing intracranial DB-7 tumors treated with a single dose of 34.5ENVE showed a doubling of median survival compared to control treated mice [median survival 17 days vs 34 days, respectively; P<0.0004]. The results of these studies warrant further investigation of oncolytic 34.5ENVE viral therapy to treat established BC brain metastases.
2014 San Antonio Breast Cancer Symposium

Publication Number: P6-16-03
Average Grade: 7.25

Title: Intrinsic subtypes and MRI patterns in brain metastasis associated with breast cancer

Nicole Williams¹, Vinay Varadan², Aditi Vadodkar³, Kristy Miskimen¹, Stephanie Kim¹, Shaveta Vinayak¹, Paula Silverman¹, Jill Barnholtz-Sloan³, Andrew Sloan¹, Cheryl Thompson², Lisa Rogers¹, Hannah Gilmore¹ and Lyndsay Harris¹. ¹University Hospitals/Case Western Reserve, Cleveland, OH; ²Case Comprehensive Cancer Center, Cleveland, OH and ³Case Western Reserve University, Cleveland, OH.

Body: Background: Breast cancer is the 2nd most common cancer to metastasize to the brain. The development of brain metastasis (BM) is associated with a lower median survival compared with other locations of metastasis and carries with it high morbidity and reduced quality of life. There are limited treatment options and virtually no approved targeted therapies for this disease. We have previously reported specific pathways enriched in BM compared to primary tumors and now further this study to examine pathways by intrinsic subtype and location of and number of BM.

Methods: Archival FFPE material was obtained from BM using pathology records; clinical data, including MRI images, was retrieved from medical records and institutional tumor registry under an IRB protocol. Tumor DNA/RNA was extracted from 2 mm cores macrodissected from the FFPE tissue blocks using the Qiagen AllPrep DNA/RNA FFPE kit. Gene expression profiling was performed using Affymetrix Human Transcriptome Array 2.0 microarray on BM samples for which sufficient RNA was available. Gene-level expression quantification was derived after RMA normalization using the Affymetrix Transcriptome Analysis Console. PAM50 subtypes were assigned by clustering samples using median-subtracted PAM50 gene expression levels. Differential gene expression was estimated using the non-parametric Mann-Whitney test, followed by assessment of false discovery rate using the Benjamini-Hochberg FDR methodology. Pathway enrichment analysis was performed using the NCI Pathway Interaction Database.

Results: Gene expression profiling showed the following intrinsic subtype distribution among all BM: luminal A 32% (19/59), luminal B 31% (18/59), HER2 enriched 7% (4/59), and basal subtype 31% (18/59). At time of development of BM 64% (38/59) of patients presented with a single lesion compared to 36% (21/59) of patients who presented with multiple lesions (p=0.12). Thirty-nine percent (7/18) of patients with basal subtypes were observed to present with multiple BM compared to 61% (11/18) non-basal subtypes (p=0.25). In addition, 12% (13/59) of BM were found to be exclusively dural-based lesions. They appeared more frequently in the luminal subtypes [11/13 vs 2/13; p=0.06]. A total of 314 genes were differentially expressed (Wilcox pval < 0.05; FDR < 0.01) between the basal and luminal subtypes. As expected, we found that the FOXA transcription factor network was up-regulated in luminal when compared to the basal subtype, whereas the FOXM1 transcription factor network was up-regulated in the basals. If we also found a total of 28 genes to be significantly differentially expressed (Wilcox pval < 0.05; FDR < 0.01) between the dural and non-dural BM. The beta1 integrin and syndecan-1 pathways were significantly enriched, along with angiogenesis and lymphatic endothelium pathways. Key genes in these pathways (COL1A1, COL1A2, COL3A1, CDH11, were found to be at least 2-fold up-regulated in the dural BM compared to non-dural BM.

Conclusion: Identifying pathways that are differentially expressed between intrinsic subtypes may help us develop new targeted therapies to provide more treatment options for breast cancer patients with brain metastasis.
Title: Phase 1/2a study of glutathione PEGylated liposomal doxorubicin (2B3-101) in breast cancer patients with brain metastases

Philippe G Aftimos1, Bojana Milojkovic-Kerklaan2, Véronique Diéras3, Sevilay Altintas4, Carey Anders5, Monica Arnedos6, Hans Gelderblom7, Patricia Soetekouw8, Werner Gladdines9, Pieter Gaillard9, Carlos de Sousa9, Agnes Jager10, Myra van Linde11, Ahmad Awada1, Jan Schellens2 and Dieta Brandsma12. 1Institut Jules Bordet, Université Libre de Bruxelles, Medical Oncology Clinic, Brussels, Belgium; 2Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands; 3Institute Curie,, Paris, France; 4Antwerp University Hospital, Antwerp, Belgium; 5Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC; 6Gustave Roussy Cancer Institute, Villejuif, France; 7Leiden University Medical Center, Leiden, Netherlands; 8Maastricht University Medical Center,, Maastricht, Netherlands; 9to-BBB technologies B.V., Leiden, Netherlands; 10Erasmus MC Daniel den Hoed Cancer Center, Rotterdam, Netherlands; 11VU Medical Center, Amsterdam, Noord-Holland, Netherlands and 12Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Amsterdam, Noord-Holland, Netherlands.

Body: Background:
The incidence of brain metastases (BM) in breast cancer (BC) patients (pts) has increased over the past decade. The brain is regarded as a sanctuary site for metastatic cells which are partially protected from drugs by the blood-brain barrier (BBB). 2B3-101 is a doxorubicin (DOX) liposomal formulation that uses glutathione transporters on the BBB to penetrate the brain. Non-clinical studies have shown a 5-fold enhanced delivery of DOX to the brain after IV administration of 2B3-101 compared to liposomal DOX, without signs of cardio- or neurotoxicity.

Methods:
This phase 1/2a open label study assessed the safety, tolerability, MTD, PK, and anti-tumor activity of single agent 2B3-101 in pts with BM of solid tumors, or high-grade gliomas. For this analyses BCBM pts (n=25) were included. These pts received 2B3-101 at a starting dose of either 40 (n=3) or 50 (n=22) mg/m² IV every 3 weeks, until disease progression or unacceptable toxicity. Anti-tumor activity was assessed by RECIST 1.1. Patients with HER2-positive BCBM were treated with concurrent trastuzumab.

Results:
As of May 30, 2014, 88 cycles (median 2, range 1-10) of 2B3-101 alone or with trastuzumab were administered to 25 heavily pretreated BC pts, 3 pts are still on treatment. Median age was 47 (33–61) years and 18 (72%) pts had HER2+ disease. Pts had received a median of 7 (4–15) prior regimens; 18 (72%) had received prior radiation therapy to the brain. In phase 1, 2B3-101 alone or with trastuzumab was well tolerated up to a dose intensity of 15 mg/m²/wk. Cycle 1 MTD was not reached. Phase 2a dose of 50 mg/m² was selected based upon tolerability after repeated dosing. The most frequent reported treatment emergent AEs are qualitatively consistent with conventional PEGylated liposomal DOX and were (≥ grade 2): neutropenia (59%), palmar plantar erythrodysesthesia (PPE) (50%), fatigue (45%), stomatitis (22%), and infusion reaction (18%). Notable Grade 3–4 AEs were neutropenia (35%) and PPE (13%). All AEs were transient and manageable with dose delays, reductions and standard medication. 57% of pts had 2B3-101 dose delays and 39% of pts required dose reductions. 2B3-101 showed no neuro- or cardiotoxicity. PK data showed non-linear exposure of 2B3-101 without signs of accumulation upon repeat dosing, a mean half-life of 69h (range 43-120h) and independent of trastuzumab co-treatment.

Best overall and intracranial tumor responses of 2B3-101 in 23 evaluable BCBM pts (92% of total) are summarized in Table 1.

Table 1: Anti-tumor activity results of 2B3-101 in BCBM patients

<table>
<thead>
<tr>
<th>Population</th>
<th>Number of evaluable pts</th>
<th>Overall partial response</th>
<th>Overall stable disease</th>
<th>Intracranial tumor response of ≥ 20%.</th>
<th>12-weeks overall PFS rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>23</td>
<td>2 (9%)</td>
<td>11 (48%)</td>
<td>4 (17%)</td>
<td>48%</td>
</tr>
<tr>
<td>&quot;Luminal&quot;</td>
<td>4</td>
<td>0 (0%)</td>
<td>2 (50%)</td>
<td>1 (25%)</td>
<td>50%</td>
</tr>
<tr>
<td>HER2 positive</td>
<td>16</td>
<td>2 (13%)</td>
<td>9 (56%)</td>
<td>3 (19%)</td>
<td>56%</td>
</tr>
<tr>
<td>---------------</td>
<td>----</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>-----</td>
</tr>
<tr>
<td>TNBC</td>
<td>3</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0%</td>
</tr>
</tbody>
</table>

**Conclusions:**
2B3-101 alone or with trastuzumab is safe and well tolerated and shows intra- and extracranial anti-tumor activity in heavily pretreated BC pts. A 12-week PFS rate of 56% in HER2+ BCBM was observed, which warrants further clinical studies. An international, multicenter, randomized, controlled phase IIb study in HER2+ BCBM is being planned. NCT01386580, sponsored by to-BBB technologies BV.
Title: New graded prognostic assessment (GPA) in breast cancer brain metastases in a contemporary cohort

Manmeet S Ahluwalia, Ming Chi, Vyshak Alva Venur, Thomas Budd, Lilyana Angelov, Samuel Chao, Paul Elson, Gene H Barnett and Jame Abraham. 'Cleveland Clinic, Cleveland, OH.

Body:

Background:
Breast cancer is the second most common cause of brain metastases (BCBM). We evaluated prognostic factors for overall survival (OS) in contemporary cohort of BCBM patients treated at a tertiary care institution.

Methods:
With IRB approval, Cleveland Clinic's database was used to identify BCBM patients treated between 2000 and 2013. OS from the diagnosis of BCBM was the primary end point. Cox proportional hazards models with stepwise variable selection were used for data analysis.

Results:
562 female patients were included for this analysis. Karnofsky performance scale (KPS) was 90-100 in 204 patients (41%), 70-80 in 223 (44%) and <70 in 76 (15%) patients. Two hundred, eighty nine (52%) patients were treated with whole brain radiation therapy (WBRT), 89 (16%) with stereotactic radiosurgery (SRS), 66 (12%) with WBRT and SRS, 44 (8%) underwent surgery and WBRT, 16 (3%) underwent surgery and SRS. Median OS for the entire cohort was 11.2 months (95% C.I., 10.0-13.1). Median OS in Luminal B (triple positive), Her 2 positive, Luminal A (ER/PR positive, Her 2 negative) and basal (triple negative) patients was 23.3, 14.5, 10.0 and 8.3 months respectively. Disease specific graded prognostic assessment (GPA) for BCBM is based on KPS, age at diagnosis, and subtype. Overall it was prognostic for OS (p <.0001), however separation between groups was variable. Starting de-novo, KPS, age at diagnosis of BCBM, and subtype were again identified as prognostic, although the scoring was different. Additional independent predictors were the number of sites of extra- cranial metastases, leptomeningeal disease, control of primary cancer and location of BCBM were found to be independently prognostic for survival. A new GPA was formulated by assigning "points" (weights) to the levels within each of these seven factors that are proportional to the regression coefficient estimates in the final model and then adding the total number of points present.

Prognostic factors in BCBM on multivariate analysis

<table>
<thead>
<tr>
<th>Factor</th>
<th>Points</th>
<th>Hazard Ratio</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPS</td>
<td></td>
<td>0.41 (0.32-0.52)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>80-100</td>
<td>13</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;70</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breast Cancer Subtype ( basal as reference)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luminal B</td>
<td>10</td>
<td>0.49 (0.32-0.74)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HER2</td>
<td>8</td>
<td>0.58 (0.43-0.77)</td>
<td>0.007</td>
</tr>
<tr>
<td>Luminal A</td>
<td>6</td>
<td>0.63 (0.48-0.77)</td>
<td>0.002</td>
</tr>
<tr>
<td>Basal</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of sites of Extra-Cranial Metastases</td>
<td></td>
<td>0.65 (0.56-0.77)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>0 or 1</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;3</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptomeningeal Disease</td>
<td></td>
<td>0.51 (0.38-0.67)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>No</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Number of points</td>
<td>Number</td>
<td>Median OS (months)</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------</td>
<td>--------</td>
<td>-------------------</td>
</tr>
<tr>
<td><strong>Primary Controlled</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td></td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Age at Diagnosis of BCBM</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;50</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>&gt;=50</td>
<td></td>
<td>0</td>
<td></td>
</tr>
<tr>
<td><strong>Location of Brain Metastasis</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infratentorial or Supratentorial</td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Both</td>
<td></td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

The new GPA consists of four groups: unfavorable, intermediate 1, intermediate 2 and favorable with OS of 2.7, 9.1, 18.5 and 29.5 months respectively.

**New GPA and Conventional GPA**

<table>
<thead>
<tr>
<th>Group</th>
<th>Number of points</th>
<th>Number</th>
<th>Median OS (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New GPA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfavorable</td>
<td>&lt;=21</td>
<td>84 (19%)</td>
<td>2.7</td>
</tr>
<tr>
<td>Intermediate-1</td>
<td>22-36</td>
<td>153 (34%)</td>
<td>9.1</td>
</tr>
<tr>
<td>Intermediate-2</td>
<td>37-45</td>
<td>133 (30%)</td>
<td>18.5</td>
</tr>
<tr>
<td>Favorable</td>
<td>&gt;45</td>
<td>78 (17%)</td>
<td>29.5</td>
</tr>
<tr>
<td><strong>Conventional GPA</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unfavorable</td>
<td>0-1</td>
<td>55 (12%)</td>
<td>4.8</td>
</tr>
<tr>
<td>Intermediate-1</td>
<td>1.5-2</td>
<td>130 (29%)</td>
<td>9.1</td>
</tr>
<tr>
<td>Intermediate-2</td>
<td>2.5-3</td>
<td>148 (33%)</td>
<td>12.9</td>
</tr>
<tr>
<td>Favorable</td>
<td>3.5-4</td>
<td>116 (26%)</td>
<td>20.4</td>
</tr>
</tbody>
</table>

**Conclusions:**

A new GPA for BCBM is proposed.
Title: Impact of early detection of brain metastasis in metastatic breast cancer patients: A single institutional experience

Satomi Matsuo¹, Junichiro Watanabe¹, Koichi Mitsuya¹ and Yoko Nakasu¹. ¹Shizuoka Cancer Center, Shizuoka, Japan.

Body: Background/Introduction
Since brain metastasis (BM) is commonly seen in metastatic breast cancer (MBC) patients (pts), it may be an important prognostic factor. It has been believed that screening MRI would not improve a pt's outcome, however, an appropriate diagnosis and early initiation of therapy, especially screening MRI followed by stereotactic irradiation (STI), might contribute to a pt's survival.

Patients and methods
We reviewed our medical records for 589 MBC pts who were treated between September 2002 and March 2014. COX regression analyses were applied to identify the survival risk factors, and the Kaplan-Meier method with a log-rank test was utilized to evaluate the survival rates.

Results
Upon checking our medical records, 187 pts, or 37.1% of MBC pts, developed BM with the median time to BM of 585.0 days (95% confidence interval [CI] 501.0-647.0). The tumor subtypes of primary lesion were luminal, 44.9%; luminal-HER2, 14.9%; HER2+, 21.3%; triple-negative (TN), 16.0% and unavailable, 2.6%. The median overall survival (OS) from the diagnosis of BM was as follows: all pts, 292.0 days (95%CI 220.0-345.0); luminal, 212.0 days (150.0-293.0); luminal-HER2, 400.0 days (258.0-613.0); HER2+, 531.0 days (423.0-670.0) and TN, 127.0 days (43.0-185.0). Accompanying visceral and/or bone lesion(s) at the diagnosis of BM were as follows: lung, 49.7%; liver, 45.9% and bone, 64.7%. The development of BM in the HER2+ pt was significantly less frequently associated with progression of known visceral/bone lesions than other subtypes (HER2+ 41.0% versus luminal 64.2% or TN 70.0%; P<0.01, Chi-square test), and the pts who developed BM without progression of other lesion(s) showed a significantly superior OS (hazard ratio [HR] 0.48, 95%CI 0.32-0.70, P<0.001) regardless of the subtype. The 91 pts with BM (48.6%) whose lesions were detected by screening MRI showed a significantly superior OS from BM than the pts with BM with any symptoms (median 359.0 days, 95%CI 249.0-439.0 versus 222.0 days, 95%CI 159.0-292.0; P<0.05). STI was performed as initial therapy in 20.3% of pts, and improved their OS from BM in all subtypes (median 439.0 days with STI, 293.0 days without STI; P<0.05), STI or the use of a tyrosine kinase inhibitor (TKI) significantly improved the OS from BM in HER2+ pts (median 661.0 days with STI versus 423.0 days without STI; P<0.05, median 622.0 days with TKI versus 287.0 days without TKI; P<0.01) The univariate COX analysis indicated that HER2+, 2>ECOG performance status at the diagnosis of BM, no other progressive lesions at the diagnosis of BM, <5 brain lesions and STI were favorable factors for the OS. When limited to HER2+ pts, the multivariate COX analysis showed that the lack of other progressive lesions at the diagnosis of BM (HR 0.20, 95%CI 0.09-0.47, P<0.001) and STI (HR 0.18, 95%CI 0.06-0.56, P<0.01) markedly decreased the risk of death for HER2+ pts.

Conclusions
From our institutional review, there seemed to be no special strategy for improving the OS of luminal or TN pts, however, HER2+ pts showed an improved OS after BM following the early detection and appropriate therapies for the BM. We conclude that HER2+MBC pts are good candidates for a BM screening program.
Title: Predict subsequent brain metastasis in patients with metastatic breast cancer: External validation of a published nomogram and competing risk regression analysis

Ludivine Genre, Henri Roché, Monia Ouali, Thomas Filleron and Florence Dalenc. 1Claudius Regaud Institute, Toulouse, France; 2Claudius Regaud Institute, Toulouse, France and 3Claudius Regaud Institut, Toulouse, France.

Body: Background: Brain metastasis (BM) is a fatal event that nonetheless alter seriously survival of patients suffering from breast cancer but also reduce their quality of life. Selection of an enriched patients population at high risk for BM is warranted to develope preventive strategies and/or to evaluate the impact early treatment of BM in prospective trials.

Methods: Electronic medical records of patients, treated in the Institut Claudius Regaud, with metastatic breast cancer and without BM at stage IV diagnosis or in the first month, were retrospectively reviewed for the period between January 2005 and December 2012. We first study the Graesslin’s nomogram (J Clin Oncol., 2010; 28(12): 2032-37) characteristics in our patients. The discrimination prediction of subsequent BM was evaluated by the area under the receiver operating characteristic curve (AUC) and we performed the calibration. Moreover, we have evaluated average and maximal errors between predictions and observations obtained from the calibration curve. Then, competing risk analysis was used to identify prognostic factors with time to BM appearance and death before BM.

Results: We identified 446 patients without BM at stage IV diagnosis or in the first month, 70 of them developed subsequent BM. Young age (≤ 50 years) at the diagnosis of breast cancer (p=0.01), ductal carcinoma (p=0.02), negative status of hormone receptor (p<0.0001), HER2 overexpression/amplification (p<0.0001), grade III of primary tumour (p=0.04) and number of metastatic sites (p=0.05) were significatively associated with subsequent BM. The external validation of Graesslin’s nomogram shows a good discrimination with an AUC of 0.695 [95%CI, 0.61-0.77]. The calibration is correct with an E max = 0.076 and an E avg = 0.37. Interestingly, in our study the cumulative incidence of BM is 5.48%, 12.95% and 18.15% at 1, 3 and 5 years after the diagnosis of stage IV. In contrast, the cumulative incidence of death before the diagnosis of BM is 16.08%, 43.97% and 56.40% respectively. In multivariate analysis, HER2 overexpression/amplification (p<0.0001), triple negative status (p=0.027) and number of metastasis sites (p=0.037) are associated with BM, while age > 50 years (p=0.015) and triple negative status (p<0.001) are associated with death before the BM diagnosis.

Conclusion: We have validate the robustness of Graesslin’s nomogram to predict subsequent BM in patients with metastatic breast cancer and suggest the best utility in patients with HER2+ breast cancer, looking forward new systemic therapies to control non BM in triple negative breast carcinoma.
Title: Prognostic factor of HER2-positive breast cancer patients developed brain metastasis: A multicenter retrospective analysis

Naoki Hayashi1, Naoki Niikura2, Norikazu Masuda3, Seiki Takashima4, Rikiya Nakamura5, Ken-Ichi Watanabe6, Chizuko Kanbayashi7, Mayumi Ishida8, Yasuo Hozumi9, Michiko Tsuneizumi10, Naoto Kondo11, Yoichi Naito12,13, Yayoi Honda14, Akira Matsui15, Tomomi Fujisawa16, Risa Oshitani15, Hiroyuki Yasojima1, Hideko Yamauchi1, Shigehira Sai17 and Hiroji Iwata1.

1St Luke's International Hospital, Tokyo, Japan; 2Tokai University School of Medicine, Kanagawa, Japan; 3Breast Oncology, NHO Osaka National Hospital, Osaka, Japan; 4National Hospital Organization Shikoku Cancer Center, Japan; 5Chiba Cancer Center Hospital, Chiba, Japan; 6Hokkaido Cancer Center, Japan; 7Niigata Cancer Center Hospital, Niigata, Japan; 8National Kyushu Cancer Center, Japan; 9Jichi Medical University, Japan; 10Shizuoka General Hospital, Shizuoka, Japan; 11Aichi Cancer Center, Nagoya, Japan; 12National Cancer Center Exploratory Oncology Research and Clinical Trial Center, Japan; 13National Cancer Center Hospital East, Japan; 14Cancer and Infectious Diseases Center, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; 15National Hospital Organization, Tokyo Medical Center, Japan; 16Gunma Prefectural Cancer Center, Ota, Gunma, Japan and 17Kyoto University Graduate School of Medicine, Japan.

Body: Background:
HER2-positive breast cancer has a high risk of developing brain metastasis compared to other subtypes of breast cancer. However, the clinical course and prognostic factors of HER2-positive breast cancer patients with brain metastases are not well known because of the relatively small population. The aim of this study was to determine clinicopathological factors associated with prognosis of HER2-positive patients developed brain metastasis.

Methods:
A retrospective large dataset of 432 HER2-positive patients who were diagnosed with brain metastases between 2001 and 2012 were collected from 24 institutions of the Japan Clinical Oncology Group: Breast Cancer Study Group. We assessed the clinicopathological factors associated with prognosis of these populations with brain metastases.

Results:
The median age of the 432 patients was 54 years (range, 20–86 years). Of the patients, 162 patients (37.5%) had ER-positive/HER2-positive (ER+HER2+) breast cancer and 270 patients (62.5%) had ER-negative/HER2-positive (ER-HER2+) breast cancer. Nineteen of the 162 patients with ER+HER2+ (12%) and 53 of the 270 patients with ER-HER2+ (20%) underwent surgery for brain metastases. After the diagnosis of brain metastasis, 108 patients with ER+HER2+ (63%) and 175 patients with ER-HER2+ (64%) received HER2-targeting agents, including trastuzumab and/or lapatinib.

The median brain metastasis-free survival period from the diagnosis of primary breast cancer was 33.5 month in both subtypes. In 63.4% of patients with ER+HER2+ subtype and 75.6% of patients with ER-HER2+, brain metastases were detected within 2 years after development of first distant metastasis. Eighty-four patients with ER+HER2+ subtype (52%) and 133 patients with ER-HER2+ (49%) had more than 3 brain metastases at the diagnosis.

The median survival period after developing brain metastasis was 16.5 months (95% confidence interval [CI], 11.9–21.1 months) in patients with ER+HER2+ and 11.5 months (95% CI, 9.1–13.8 months) in patients with ER-HER2+ (p = 0.117). Patients with more than 3 brain metastases had significantly shorter OS period than patients with equal or less than 3 brain metastases in both of ER+HER2+ (p < 0.001) and ER-HER2+ (p = 0.018). According to receiving HER2-targeting agents, patients receiving both of trastuzumab and lapatinib had significantly longer survival period than patients who had received trastuzumab alone, lapatinib alone, or no HER2-targeting agent (p < 0.001).

Conclusions:
Our results showed that HER2-positive patients with more than 3 brain metastases at the diagnosis had poor prognosis regardless of ER-positivity, and receiving both of trastuzumab and lapatinib might improve their survival. Further studies are needed to determine the best treatment strategy including these HER2-targeting agents for these populations.
Title: SPARC expression in brain metastases of breast cancer patients

Isabell Witzel¹, Jakob Matschke², Markus Glatzel¹, Karin Milde-Langosch¹, Elena Laakmann¹, Sibylle Loibl¹, Berit M Pfitzner³, Carsten Denkert³ and Volkmar Müller¹. ¹University Medical Center, Hamburg, Germany; ²Institute of Neuropathology, University Medical Center, Hamburg, Germany; ³Institute of Pathology, University Medicine of Berlin, Berlin, Germany and ⁴German Breast Group, Neu-Iseburg, Hessen, Germany.

Body: Background: The incidence of brain metastases in breast cancer patients is rising and has become a major clinical challenge in the last years with so far limited therapeutic options. Therefore, further insights into the biology of brain tropism are important. Secreted protein acidic and rich in cysteine (SPARC) is an albumin-binding protein. A differential expression of SPARC in breast tumor tissue and its surrounding stroma compared to normal tissues has been described and might be associated with poor prognosis. In this study, tissues of brain metastases were evaluated for SPARC expression by immunohistochemistry using a standardized immunoreactive score (IRS).

Methods: 138 tissue samples of brain metastases were available for construction of a tissue-microarray (TMA) and evaluation of SPARC expression. Immunohistochemical staining for SPARC was carried out as previously described (Sinn et al. Ann Onc, 2014) using the antibody Novocastra NCL-O-NECTIN; Clone: 15G12; 1:100). For stromal SPARC expression, the intensity of staining was evaluated by a four-tier scoring system (negative, weak, moderate and strong). Cytoplasmic SPARC expression was evaluated by the percentage of tumor cells with cytoplasmatic staining (0 % = "0", 1-10 % = "1", 11-50 % = "2", 51-80 % = "3", 81-100 % = "4"). The staining intensity was evaluated as negative (0), weak (1), moderate (2) or strong (3). The numeric values were multiplied, resulting in an immunoreactivity score (IRS) ranging from 0 to 12. The definition for high cytoplasmic SPARC was IRS ≥ 3 (at least weak intensity in > 50 % of tumor cells or at least strong intensity in 1-10 % of tumor cells). Staining of normal brain tissue and comparison to primary breast tumors is still ongoing.

Results: Cytoplasmic SPARC expression was detectable in 104 cases (78%), 30 cases were negative (22%). Stromal SPARC intensity was strong in 65 (63%) cases, moderate in 35 (34%) cases and low in 4 cases (4%). There was a weak association between stromal and cytoplasmic SPARC expression (r=0.22, p=0.010). No significant association between SPARC expression and clinicopathological parameters could be observed. 43.5% (n=54) of brain metastases were HER2-positive, 39.5% (n=49) were triple-negative and 16.9 % (n=21) were HR+/HER2 negative. Cytoplasmic SPARC expression was slightly higher in HER2 positive compared to triple-negative brain metastases. Regarding survival analysis, strong stromal SPARC expression was associated with shorter overall survival from first diagnosis of brain metastases but did not reach statistical significance (HR 4.2, 95%-CI 0.5 – 34, p=0.18).

Conclusion: SPARC is frequently expressed in brain metastases of breast cancer patients. It might provide additional prognostic information in patients with brain metastases.
Title: Activity of T-DM1 in HER2-positive breast cancer brain metastases

Rupert Bartsch¹, Anna S Berghoff¹, Ursula Vogl², Margaretha Rudas¹, Elisabeth Bergen¹, Michael Gnant¹, Karin Dieckmann¹, Katja Pinker¹, Zsuzsanna Bago-Horvath¹, Arik Galid³, Leopold Oehler², Christoph C Zielinski¹, Guenther G Steger¹ and Matthias Preusser¹. ¹Medical University of Vienna; ²St Joseph Hospital Vienna and ³Hanusch Hospital Vienna.

Body: Background
Different local therapy options such as radiotherapy, radiosurgery and neurosurgery remain the mainstay of brain metastases (BM) management, especially in case of oligometastatic or symptomatic disease. Recently, the LANDSCAPE study established lapatinib plus capecitabine (LapCap) as potential standard option for primary systemic treatment in oligosymptomatic patients (pts) with multiple Her2-positive BM. Limited evidence exists with regards to the potential activity of antibodies in BM. Due to a disruption of the blood-brain-barrier at the metastatic site, it is anticipated that even large molecules such as antibodies may penetrate into the central-nervous system (CNS). On the other hand, the CNS is an immune-privileged organ which may hamper activity of trastuzumab. This problem may be overcome by the use of T-DM1.

T-DM1 is an antibody-drug conjugate linking trastuzumab (T) to an anti-microtubule agent providing higher activity and lower toxicity as compared to LapCap. Here, we investigated the activity of T-DM1 in newly diagnosed or progressive BM.

Patients and Methods
Nine pts (median age 55 years) with Her2-positive BM treated at two Austrian centres were included. All pts had received prior treatment with T, five pts (55.6%) had already received lapatinib, and two pts (22.2%) pertuzumab as well.
In two asymptomatic pts, T-DM1 was administered as primary therapy, while seven pts had documented CNS progression upon prior local treatment. T-DM1 was administered every three weeks at a dose of 3.6 mg/kg. Restaging was conducted every twelve weeks with MRI or whenever symptoms of disease progression occurred.

Results
Median follow-up was 6 months and median brain metastases-free survival 11 months. Seven pts (two with primary treatment and five receiving T-DM1 upon CNS progression) are currently assessable for CNS response. 3/7 pts (42.9%) had partial remission, one patient progressing upon prior local therapy had stable disease lasting for 10 months, and one patient had stable disease for 5 month. One patient progressing of prior WBRT had minor response of BM on MRI but no reduction of brain oedema and increasing cortisol doses and was therefore deemed PD. The other patient with primary PD was also progressing after WBRT.

Conclusion
This prospectively sampled case series again indicates that systemic therapy offers activity in Her2-positive BM. LapCap remains the standard of care but T-DM1 offers relevant clinical activity and should be investigated within the context of larger clinical studies.
Title: Heregulin/HER3 signaling increases invasive behavior of HER2+ breast cancer cells

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Body: Breast cancer is a heterogeneous disease with distinct histological and molecular subtypes differing in prognosis and metastatic behavior. Colonization of the brain is arguably the most aggressive manifestation of metastatic breast cancer. Brain metastasis is a growing public health problem associated with a high degree of morbidity. The management of brain metastases is an unmet medical need for which establishment of more effective preventive and therapeutic treatment strategies are of paramount importance. In order to achieve that, we need to deepen our knowledge and insights about the molecular mechanisms and signal transduction pathways underlying initiation and progression of brain metastases.

Invasion of breast tumor cells across the basement membrane and endothelium is a critical early requirement for metastasis. Disseminated cells must then undergo extravasation at distant tissue sites. In the case of brain metastases, cells must breach the highly specialized and impermeable blood-brain-barrier (BBB). Improving our understanding of the molecular mechanisms underlying these processes may reveal new drug targets to prevent development or suppress growth of these lethal tumors. Of all the breast cancer subtypes, patients with HER2-positive disease exhibit the highest frequency of brain metastases. Both HER2 and its obligate dimerization partner, HER3 (ErbB3), are associated with development of brain metastases, though a mechanistic link has not yet been established. The main goal of this project was to elucidate the molecular mechanisms by which the HER2/HER3 heterodimer could promote metastatic progression and more specifically, development of brain metastases. In order to achieve this, activation of the HER2/HER3 signaling axis was assessed in a panel of HER2+ breast cancer cell lines (MDA-MB-361, MCF7 and SKBr3) after treatment with the HER3 ligand heregulin (HRG). The effects of HRG on proliferation, migration, invasion and transendothelial migration (TEM) across a tight layer of primary human brain microvascular endothelial cells were also assessed as functional readouts of this axis in vitro. Finally, the molecular mechanisms underlying some of the HRG-induced changes (proliferation and TEM) were investigated.

The results of this study showed that HRG increased proliferation of luminal HER2+ breast cancer cells via induction of cyclin D1 and down-regulation of p27 proteins. In addition, HRG-induced mesenchymal differentiation, migration and invasive capabilities in these cells in vitro, and this was associated with induction of matrix metalloproteinase-9 (MMP-9) and cathepsin B. Moreover, HRG induced TEM activity of these cells in a HER3- and MMP-dependent manner, raising the possibility that HRG could play a role in proteolytic permeabilization of the BBB in brain metastases from HER2+ breast cancer. Further in vivo studies are required to determine if this mechanism is involved in the initial seeding of micrometastases in the brain and/or in maintaining a favorable microenvironment to sustain tumor growth. The results of this study have potential implications in oncology, where targeting HRG/HER3 signaling could be exploited in preventive and therapeutic strategies for management of brain metastases.
Title: Graded prognostic assessment for triple negative breast cancer brain metastases

Ming Chi¹, Vyshak Alva Venur¹, Jame Abraham¹, Thomas G Budd¹, Paul Elson¹ and Manmeet S Ahluwalia¹. ¹Taussig Cancer Institute, Cleveland Clinic, Cleveland, OH.

Body: Background:
Brain metastases is a serious complication of triple negative breast cancer (TBCBM). We evaluated prognostic factors for overall survival (OS) in contemporary cohort of TBCBM patients treated at a tertiary care institution.

Methods:
With IRB approval, Cleveland Clinic’s database was used to identify BCBM patients treated between 2000 and 2013. OS from the diagnosis of TBCBM was the primary end point. Cox proportional hazards models with stepwise variable selection were used for data analysis.

Results:
One hundred forty three female patients were included for this analysis. Karnofsky performance scale (KPS) was 90-100 in 52 patients (39%), 70-80 in 50 (38%) and <70 in 30 (23%) patients. Sixty eight (49%) patients were treated with whole brain radiation therapy (WBRT), 26 (19%) with stereotactic radiosurgery (SRS), 18 (13%) with WBRT and SRS, 13 (9%) underwent surgery and WBRT, 5 (4%) underwent surgery and SRS. Seventy nine percent (113/143) of patients are reported to have died. Overall survival was 8.3 months (95% C.I. 5.5-10.6). Disease specific graded prognostic assessment (GPA) for breast cancer brain metastases is based on KPS, age at diagnosis, and subtype. By definition triple negative patients fall into the lowest two GPA score groups. GPA was prognostic for survival (p=.05), however separation between groups was variable. Starting de-novo, KPS (but different coding than in the GPA), leptomeningeal disease, and the number of extracranial sites of disease at the time of diagnosis of TBCBM were found to be independently prognostic for survival (proportional hazards model, stratified by when brain metastases were diagnosed, and a stepwise selection algorithm). A new GPA was formulated by assigning "points" (weights) to the levels within each of these three factors that are proportional to the regression coefficient estimates in the final model and then adding the total number of points present.

Prognostic Factors Impacting Survival of TBCBM

<table>
<thead>
<tr>
<th>Factor</th>
<th>Number of points</th>
<th>Hazard Ratio</th>
<th>p</th>
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<tr>
<td>KPS</td>
<td></td>
<td>0.31 (0.21-0.48)</td>
<td>&lt;.0001</td>
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<tr>
<td>80-100</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;= 70</td>
<td>0</td>
<td></td>
<td></td>
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<tr>
<td>No. Extra-Cranial Mets</td>
<td></td>
<td>0.42 (0.28-0.64)</td>
<td>&lt;.0001</td>
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<tr>
<td>0 or 1</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;= 2</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptomeningeal Disease</td>
<td></td>
<td>0.43 (0.26-0.71)</td>
<td>0.001</td>
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<tr>
<td>No</td>
<td>2</td>
<td></td>
<td></td>
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<tr>
<td>Yes</td>
<td>0</td>
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The new GPA consists of three groups: unfavorable, intermediate and favorable with OS of 2.7, 9.1 and 13.6 months respectively.

New GPA

<table>
<thead>
<tr>
<th>GPA</th>
<th>Number of points</th>
<th>Number</th>
<th>Median OS ( months)</th>
<th>P</th>
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<tr>
<td>Favorable</td>
<td>7</td>
<td>36 (27%)</td>
<td>13.6</td>
<td></td>
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<tr>
<td>Intermediate</td>
<td>4-5</td>
<td>59 (45%)</td>
<td>9.1</td>
<td></td>
</tr>
<tr>
<td>Unfavorable</td>
<td>&lt;=3</td>
<td>36 (27%)</td>
<td>2.7</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>&lt;0.001</td>
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Conclusions:
A novel GPA for TBCBM is proposed.
Title: MicroRNA-34a suppresses breast cancer bone metastasis by inhibiting osteoclastogenesis and targeting tgif2

Jing Y Krzeszinski¹, Wei Wei¹, HoangDinh Huynh¹, Zixue Jin¹, Xunde Wang¹, Tsung-Cheng Chang², Xian-jin Xie³,⁴, Lin He⁵, Lingegowda S Mangala⁶,⁷, Gabriel Lopez-Berestein⁷,⁸, Anil K Sood⁶,⁷,⁹, Joshua T Mendell²,³ and Yihong Wan¹,³. ¹University of Texas Southwestern Medical Center, Dallas, TX; ²University of Texas Southwestern Medical Center, Dallas, TX; ³Simmons Cancer Center, University of Texas Southwestern Medical Center, Dallas, TX; ⁴University of Texas Southwestern Medical Center, Dallas, TX; ⁵University of California at Berkeley, Berkeley, CA; ⁶University of Texas MD Anderson Cancer Center, Houston, TX; ⁷Center for RNA Interference and Non-Coding RNA, University of Texas MD Anderson Cancer Center, Houston, TX; ⁸University of Texas MD Anderson Cancer Center, Houston, TX and ⁹University of Texas MD Anderson Cancer Center, Houston, TX.

Body: Introduction:
Approximately 70% of people living with metastatic breast cancer have metastases to their bones. Osteolytic bone metastasis is a common, debilitating and essentially incurable skeletal complication of breast cancer, in which tumor cells migrate to and destroy bones, causing extreme pain, fractures, life-threatening hypercalcemia, limited mobility and eventually mortality. The bone resorbing osteoclasts significantly contribute to this process. MicroRNAs (miRNAs) play important roles in physiology and disease, and present tremendous therapeutic potential. Nonetheless, how miRNAs regulate skeletal biology is underexplored. We hypothesize that miRNAs that can suppress osteoclast function may ameliorate breast cancer bone metastasis.

Methods:
A strategy of ex vivo osteoclast differentiation from mouse bone marrow cells was used to examine the levels of several cancer-related miRNAs during a time course of osteoclastogenesis, and test the effects of miR-34a mimic or inhibitor. Micro-Computed Tomography was conducted to quantify bone mass. ELISA analyses were performed to measure serum bone resorption and bone formation markers. Ovariectomy was employed as a model for postmenopausal osteoporosis. As a model for bone metastases, luciferase labelled bone-metastasis prone MDA-MB-231 human breast cancer cell subline were injected into the left cardiac ventrical of nu/nu mice. Bone metastases were detected and quantified weekly post injection by bioluminescence imaging.

Results:
MiR-34a is down-regulated during osteoclast differentiation. Osteoclastic miR-34a over-expressing transgenic mice exhibit lower bone resorption and higher bone mass. Conversely, miR-34a knockout and heterozygous mice exhibit elevated bone resorption and reduced bone mass. Consequently, ovariectomy-induced osteoporosis, as well as bone metastasis of breast cancer are diminished in osteoclastic miR-34a transgenic mice. Pharmacologically, systemic delivery of miR-34a mimics via a chitosan nanoparticle vehicle (miR-34a-CH) can target multiple tissues. Our bio-distribution analysis shows that miR-34a-CH delivery to the bone marrow is among the highest compared to other tissues. Administration of miR-34a nanoparticles not only diminished ovariectomy induced bone loss but also attenuated breast cancer bone metastasis. Systemic miR-34a-CH delivery affected neither tumor growth when cancer cells were injected subcutaneously nor tumor metastasis to other organs such as lung when cancer cells were injected intravenously. These results further support the notion that the suppression of bone metastases by miR-34a-CH was mediated by its inhibition of the bone metastatic niche rather than the cancer cells. Mechanistically, we identify the homeodomain transcription factor Tgif2 as an essential direct miR-34a target that is pro-osteoclastogenic. Tgif2 deletion reduces bone resorption and abolishes miR-34a regulation.

Conclusion:
Using mouse genetic, pharmacological and disease models, we have identified mir-34a as a novel and critical suppressor of osteoclastogenesis, bone resorption and the bone metastatic niche, revealing miR-34a as a potential new therapeutic strategy to confer skeletal protection and ameliorate bone metastasis of breast cancer.
Title: Inhibition of osteolytic tumor growth by 5-FdU-alendronate, a bisphosphonate conjugate that maintains bone formation: Implications for treatment of osteolytic bone lesions

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Body: Patients with breast cancer bone metastases suffer significant morbidity from skeletal-related events but an effective agent that inhibits bone resorption while maintaining bone formation has not been identified. In this study we tested the therapeutic efficacy of a novel drug, 5-FdU-ale, a conjugate between the anti-metabolite 5-FdU and the bisphosphonate alendronate, in a mouse model of breast cancer bone metastases. Administration of 5-FdU-ale in vitro induces cell cycle arrest similar to treatment with unconjugated 5-FdU. In vivo, mice harboring bone lesions treated with 5-FdU-ale showed a reduction in tumor size not observed with administration of either Alendronate or 5-FdU. Since osteolysis mediated release of growth factors from bone contribute to tumor growth, we show 5-FdU-ale treatment significantly reduces bone resorption, although uniquely, the inhibition of osteoclast activity is not mediated through inhibition of prenylation of small GTPases. Furthermore, and in contrast to Alendronate, there is no concomitant decrease in bone formation activity, as determined by serum osteocalcin levels. This finding is supported by micro-CT analyses which reveal significantly higher bone volume and histologically, pockets of tumor cells are observed largely confined to regions of marrow space between endochondral and trabecular bone. Taken together, we conclude that 5-FdU-ale has potent anti-tumor efficacy in osteolytic bone lesions mediated uniquely through bone formation activity of osteoblasts and inhibition of bone resorption. The study identifies the important role of osteoblast in not only preventing the osteolytic metastatic phenotype but as a means of harnessing the therapeutic potential of bone formation to treat osteolytic lesions.
Title: Re-introduction of tumor suppressor miR-34a harbors therapeutic efficacy in triple negative breast cancer

Brian D Adams¹, Lajos Pusztai¹, David L Rimm¹ and Frank J Slack¹. ¹Yale University, New Haven, CT.

Body: Place Holder Abstract

Triple negative breast cancer accounts for a disproportionate share of the total breast cancer morbidity because of its aggressive behavior, increased incidence in younger women, and lack of effective targeted therapies. MicroRNAs are global regulators of survival and proliferation pathways important in cancer development and tumor maintenance. MicroRNAs function as tumor suppressors and oncogenes, and are highly dysregulated in cancer. Here, we identified miR-34a to be aberrantly lost in triple negative cancer cell lines when compared to both a luminal cancer subtype as well as normal breast cells. Re-introduction of miR-34a in triple negative cancer lines results in inhibition of cell proliferation, reactivation of senescence, and enhances sensitivity to apoptotic-inducing agents. Furthermore, intratumoral delivery of miR-34a into subcutaneously implanted tumors in nude mice delays tumor growth as compared to a scrambled control. In conclusion re-introduction of miR-34a in triple negative breast cancer promotes anti-tumorigenic phenotypes both in vitro and in vivo, and could therefore be used as a therapeutic agent to treat the disease.
Title: Atomic force microscopy of triple negative breast cancer cells: A predictive value of mechanical phenotype

Pawel A Osmulski and Maria Gaczynska. 'University of Texas Health Science Center, San Antonio, TX.

Body: We applied Quantitative Nanomechanical Measurements with Atomic Force Microscopy (QNM-AFM) to test a response of triple negative breast cancer (TNBC) cells to treatment with inhibitors of proteasome. Genetic screens identified TNBCs as addicted to the activity of ubiquitin-proteasome pathway, the major intracellular venue of regulated protein degradation, served by the essential protease, the proteasome. Here we tested the effects of treatment of two proteasome inhibitors on the canonical triple-negative cell line, MDA-MB-231. The inhibitors represent two very distinct types of mechanism. Bortezomib (Velcade®) is a competitive inhibitor that targets active sites of the enzyme. On the other hand, B1 is a novel noncompetitive allosteric drug lead that interferes with interactions between the subcomplexes of proteasome, the 20S catalytic core and the 19S regulatory particle ("cap"). In a search for test to predict the putative value of inhibitors we turned to a mechanical phenotype. Mechanical properties of cells constitute a sensitive indicator of physiological status of cells. The best-known and most explored mechanical parameters are elasticity ("softness") represented by the Young modulus, and surface adhesiveness ("stickiness"). It has been well established that cancer cell are much softer and less sticky than the healthy counterparts. These changes in physical phenotype of cancer cells are usually linked to remodeling of their cytoskeleton and altered expression of membrane proteins. Such remodeling may be a very early indicator of the cells' response to a drug, a less explored but potentially very useful feature. Indeed, it has been shown that often treatment of cancer cells with anticancer drugs at least partially reverses their mechanical phenotype resembling healthy cells. Here we found that 24-hrs exposure of the cells to 10 nM bortezomib increased their stiffness and adhesiveness about two times. Even more striking, a treatment of the cells with 10 nM B1 induced almost threefold increase of stiffness with twofold increase in adhesion. Strikingly, treatments with such concentrations of the inhibitors alone did not significantly influence the viability of the cells, whereas their combination reduced the population of live cells to 50% or less of the control values. The strong effects of the inhibitor treatment on the mechanical phenotype are in stark contrast with little or no effects on cell viability. The results point at extraordinary sensitivity of the mechanical phenotype in detection of cell response to anticancer drugs. We are exploring the potential predictive value of AFM-based cell surface studies in a search for effective drugs or drug combinations.
Title: Histone deacetylase inhibitors as an alternative therapeutic approach for human breast cancer treatment, in vitro and in vivo

Soha H Nosir¹ and Ahmed S Sultan¹. ¹Alexandria University, Alexandria, Egypt.

Body: Despite major recent advances in therapy, more effective approaches to the treatment and prevention are necessary. Histone deacetylase inhibitors (HDACIs) provide an alternative therapeutic approach for the treatment of breast cancer. Recently, our promising data showed that HDACIs might be a single anti-cancer drug that given the range of molecular and biological responses and have a minimal toxicity to normal cells.

In the present study, we investigated and compared the anticancer effects of two structurally distinct HDACIs, Vorinostat (SAHA) and Valproic Acid (VPA). We tested the effect of VPA/SAHA as a combination treatment, using two different human breast cancer cell lines, MDA-MB-231 and T-47D respectively. Anticancer effects of tested drugs were assessed by MTT assay, western blotting analysis of expression levels of Cav-1, p53, E-cadherin, β-catenin and cyclin D1. Furthermore, SAHA treated cells for 48 h alone and/or VPA showed a strong inhibitory effect on cyclin D1 expression and enhanced effect on E-cadherin/beta-catenin complex expression compared to control and the affects were strongly detected in SAHA compared to VPA treated cells.

Furthermore, VPA/SAHA combined treatment induced morphological changes, apoptosis induction and DNA fragmentations after 48h in both MDA-MB-231 and T47D cell lines respectively. Furthermore, Cav-1 and p53 expression levels were up-regulated and beta-catenin expression level was down-regulated after treatment with SAHA alone and /or VPA in T-47D cells but not in MDA-MB-231 cells.

In vivo model, the antitumor effect of SAHA alone and/or VPA was also confirmed by immunohistochemical analysis to detect Cav-1, p53, beta-catenin and cyclin D1 expression levels in tumor tissues of MNU-induced breast carcinoma. The tumor size of MNU-induced breast carcinoma in rats was significantly decreased after combined treatment with SAHA and VPA. Furthermore, nuclear localization of cyclin D1 and beta-catenin were significantly decreased upon SAHA treatment compared to VPA treatment. Our data suggest that the combined treatment of SAHA and VPA exerts significant antitumor activity and could be promising therapeutic candidates for human breast cancer treatment.