Conflict of Interest Statement

- C. Marchio: personal consultancy fees from Axiome Healthcare Strategies.
- F. Clatot: institutional research funds from Roche, institutional contracted research from Astra Zeneca & consulting fees from Roche, Astra Zeneca, BMS, Merck Sernono, Lilly.
- R. Salgado: consulting fees from BMS and Astra Zeneca and contracted research from Roche and Merck.
- All remaining authors declared no conflict of interest.

Unraveling lobular breast cancer progression and endocrine resistance mechanisms through the genomic and immune characterization of matched primary and metastatic samples

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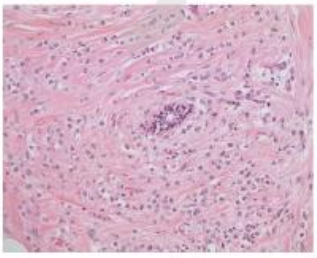
5th of December 2018

San Antonio Breast Cancer Symposium

Introduction

Invasive lobular breast cancer (ILC)



- 2nd most frequent histotype (5-15%)
- Small, discohesive epithelial cells
- Mostly ER+ and HER2-
- Characterized by the loss of CDH1
- Different relapse patterns over time & relapse sites as compared to invasive ductal carcinoma (IDC)

Desmedt *et al.*, Sem.Cancer Bio. 2017

Molecular characterization of primILC

Genomics

- Increased prevalence of *CDH1*, *PIK3CA*, *TBX3*, *FOXA1*, *HER2*, *HER3* & *AKT1* mutations.
- *HER2* and *AKT1* mutations: risk of early relapse.
- ILC histological subtypes differ at genomic level.

Gene expr.

- Multigene classifiers: ILC= mostly low-risk.
- Several gene expression clusters identified.

Immune

- Low levels of tumor infiltrating lymphocytes (TILs).
- Different immune composition.

Ciriello *et al.* Cell 2015, Desmedt *et al.* JCO 2016, Michaut *et al.* Sci Rep 2016, Pereira *et al.* Nat Com. 2016, Desmedt *et al.* JNCI 2018, Desmedt *et al.* Sem.Cancer Bio. 2017

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Molecular characterization of metILC

Retrospective studies

- Autopsy-based phylogenetic reconstruction.
- Retrospective collections.

Prospective initiatives

- Clinical trials (AURORA, PlasmaMATCH,…).
- Prospective institutional program (MSKCC-IMPACT).
- Patient-driven initiative (MBC project).

ILC-focused

- *ESR1* mutations: similar frequency & distribution
- Critical role of NF1 in endocrine resistance.

Cummings *et al.* J Path 2014; Juric *et al.* Nat 2015, Hoadley *et al.* Plos Med 2016; Savas *et al.* PloS Med 2016; Brown *et al.* Nat Comm 2017; Avigdor *et al.* JCI Inv 2017; Siegel *et al.* JCI 2018; Brastianos *et al.* Can Disc 2015; Yates *et al.* Cancer Cell 2017; Zehir *et al.* Nat Med 2017; Razavi *et al.* Cancer Cell 2018; www.mbcproject.org; Desmedt *et al.* NPJ BC In Press; Sokol *et al.* Ann Oncol 2018.

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Study aims

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1. Identify genomic alterations in cancer genes acquired during ILC progression.
2. Evaluate immune infiltration in metILC.
3. Identify clinical, pathological or genomic markers associated with relapse-free (RFS) and overall survival (OS) in ILC patients.

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Patients & Methods

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EuroILC

125 ER+ metILC patients from 6 European institutions

31 failed pathology review

94 ER+ metILC patients for TIL evaluation

21 with unsufficient DNA

73 ER+ metILC patients for genomic characterization:

1) Targeted sequencing (73 pts, 165 samples)
2) Low pass whole genome sequencing (63 pts, n=225 samples)

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MSKCC-IMPACT

Razavi *et al.* (Cancer Cell 2018)
1756 pts (1918 samples)

↓ *10 male pts*

1746 pts (1907 samples)

↓ *267 ER negative tumors*
28 ER Unk/ND tumors

1469 ER+ pts (1642 samples)

ER+ IDC	**ER+ ILC**
1120 pts (1230 samples)	**260 pts** (281 samples)
600P & 630M	132P & 149M
unmatched	unmatched

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ILC Patient & sample characteristics

EuroILC & MSKCC-IMPACT with met samples

Menopausal status			**PgR status_prim**		
Pre/peri	41 (44%)	73 (49%)	Negative	14 (15%)	23 (18%)
Post	53 (56%)	75 (51%)	Positive	77 (85%)	108 (32%)
Primary tumor size *			**HER2 status_prim**		
< 2 cm	21 (24%)	43 (35%)	Negative	78 (92%)	119 (94%)
≥ 2 cm	76 (76%)	79 (65%)	Positive	7 (8%)	7 (6%)
Nodal status			**Adjuvant chemotherapy****		
Negative	29 (31%)	40 (33%)	No	19 (20%)	77 (57%)
Positive	64 (69%)	82 (67%)	Yes	74 (80%)	58 (43%)
Histological subtype			**Adjuvant endocrine therapy****		
Classic	51 (56%)	NA	No	1 (1%)	31 (23%)
Non-classic	40 (44%)	NA	Yes	93 (99%)	104 (77%)
Histological grade_prim**			**Adjuvant radiotherapy**		
G1/G2	75 (81%)	34 (47%)	No	27 (30%)	NA
G3	18 (19%)	38 (53%)	Yes	64 (70%)	NA

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ILC Patient & sample characteristics

EuroILC & MSKCC-IMPACT with met samples

Time to relapse			**Timing metastatic biopsy**		
Median	4.69 yrs	4.65 yrs	<1yr diagnosis	75 (84%)	NA
De novo metastatic	14 (15%)	24 (18%)	Later	14 (16%)	NA
Relapsed <5 yrs	34 (36%)	46 (34%)	**Endocrine treat. (ET) before met. sampl.***		
Relapsed >5 but <10 yrs	31 (33%)	36 (27%)	SERM only	39 (41%)	34 (25%)
Relapsed >10 yrs	15 (16%)	29 (21%)	AI only	20 (21%)	39 (29%)
ER status_met			SERM and AI	28 (30%)	35 (26%)
Negative	9 (11%)	18 (14%)	No endocrine treat.	7 (7%)	27 (20%)
Positive	73 (89%)	115 (86%)	**Duration ET before met. sampling**		
PgR status_met			<2 years	17 (20%)	16 (15%)
Negative	38 (48%)	63 (49%)	2-4 years	20 (23%)	22 (20%)
Positive	41 (52%)	65 (51%)	> 4 years	50 (57%)	70 (65%)
HER2 status_met			**Deceased****		
Negative	75 (95%)	118 (92%)	No	43 (46%)	108 (72%)
Positive	4 (5%)	10 (8%)	Yes	51 (54%)	41 (28%)

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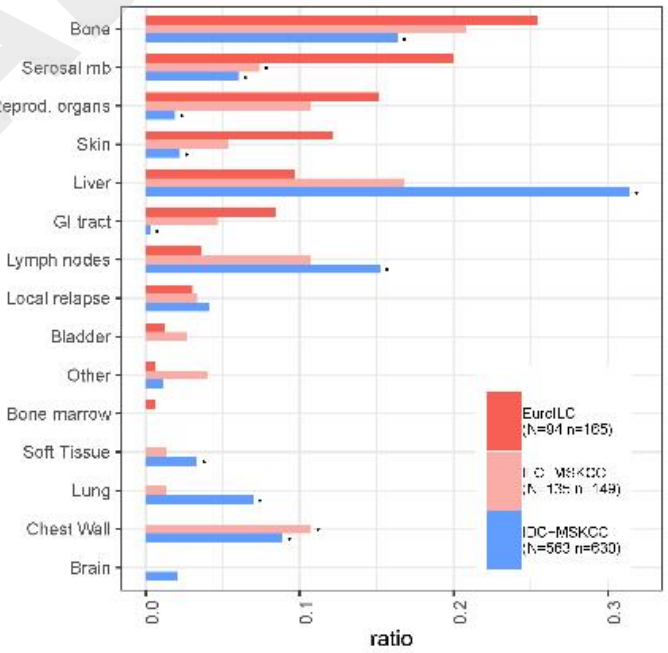
Distribution metastatic samples



Methods EuroILC (1/3)

- Centralized pathology review for histology. ER, PgR, HER2, grade and Ki67 status are the local ones.
- Stromal TILs assessed by 2 experienced pathologists on H&E slides using standardized protocols[1].
- Statistical analyses: associations assessed by Fisher exact test, or linear regression models. Survival analysis assessed using Cox models. All tests are two sided and corrected for multiple testing by FDR.

Salgado *et al.* Ann Oncol 2014

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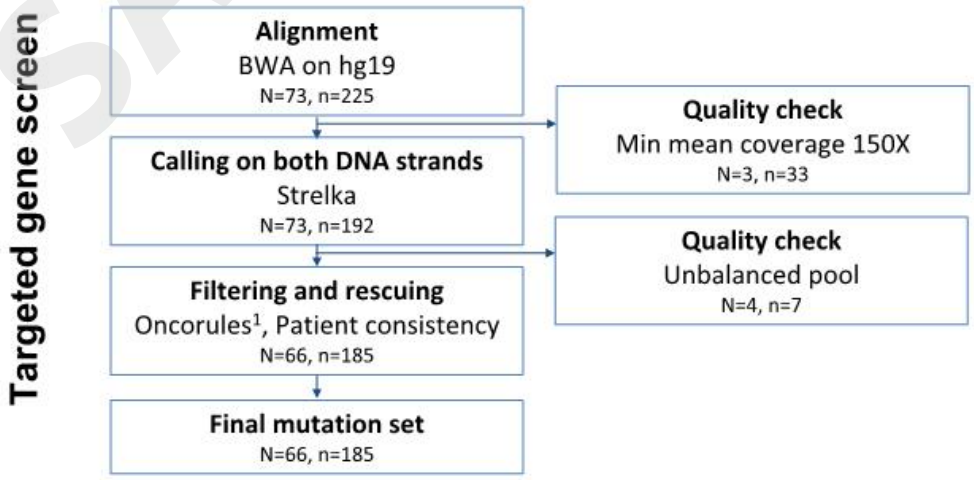
Methods EuroILC (2/3):



Gene panel: *AKT1, ARID1A, CDH1, ERBB2, ERBB3, ESR1, FOXA1, GATA3, IGF1R, JAK2, MAP2K4, MAP3K1, NF1, PIK3CA, PTEN, RB1, RUNX1, STAT3, TBX3, TP53.*

Desmedt *et al.* JCO 2016

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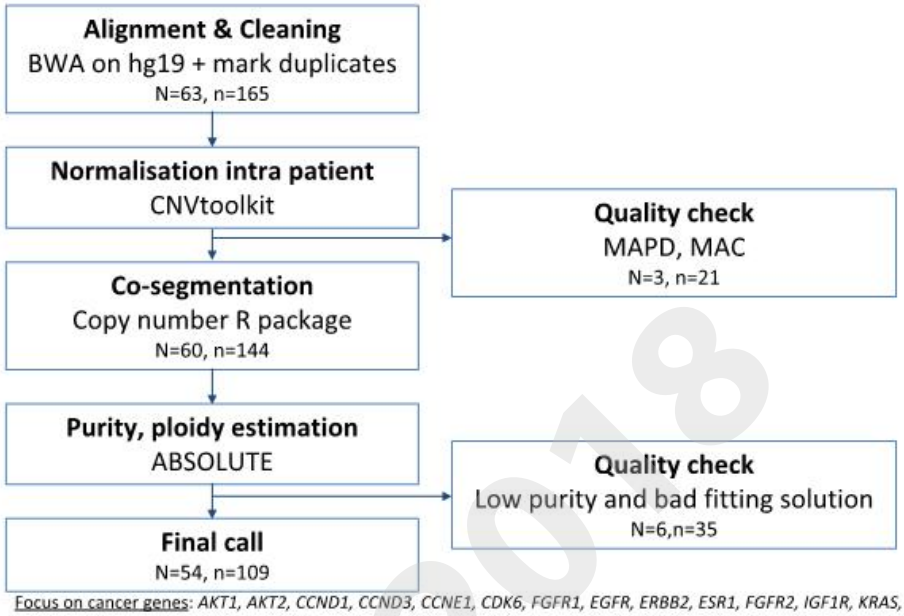
Methods EuroILC (3/3):

Low pass whole genome sequencing



Focus on cancer genes: *AKT1, AKT2, CCND1, CCND3, CCNE1, CDK6, FGFR1, EGFR, ERBB2, ESR1, FGFR2, IGF1R, KRAS, MDM2, MYC, PDGFRA, PIK3CA, ZNF217, BRCA2, CDH1, CDKN2A, CDKN2B, DNMT3A, MAP2K4, MAP3K1, NCOR1, NF1, PTEN, RB1, SMAD4, TP53* (Nik-Zainal *et al.* Nature 2016)

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Results

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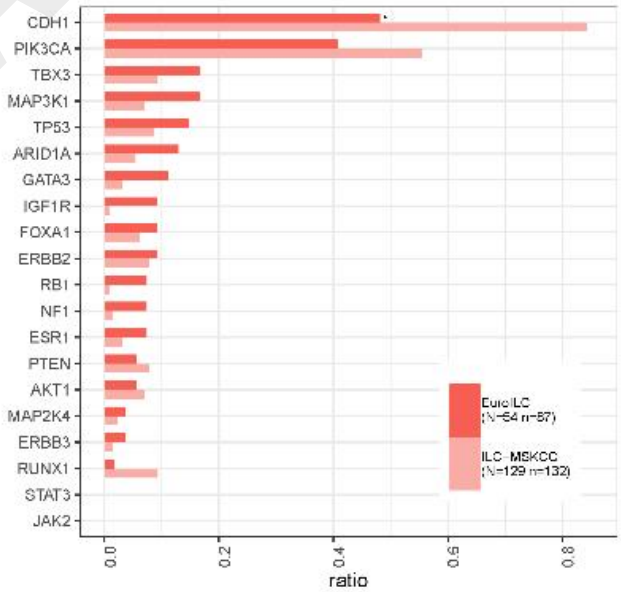
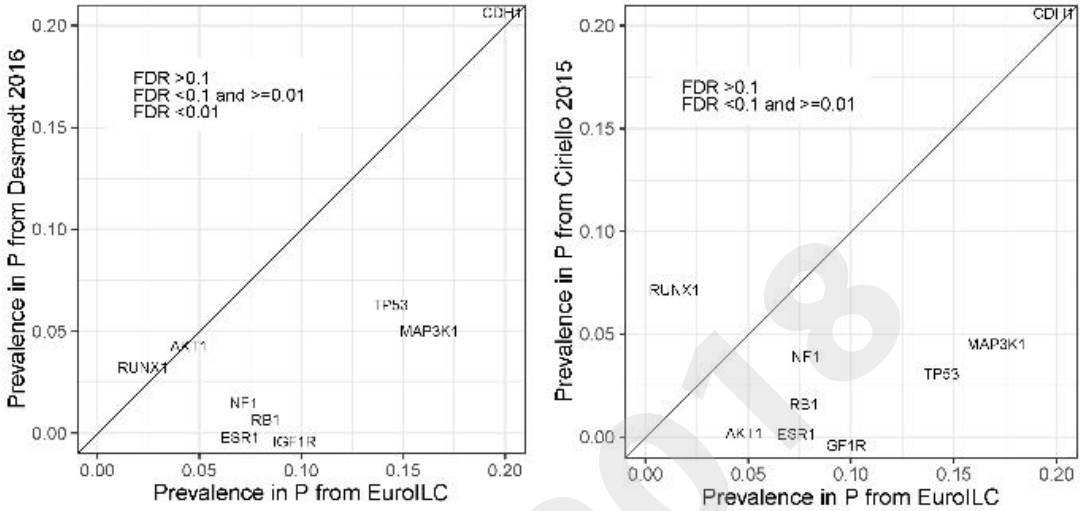
Results

1. Genomic alterations in metastatic ILC patients.
2. Genomic alterations acquired during progression.
3. Immune infiltration in metastatic ILC.
4. Clinical, pathological or genomic markers associated with the time to relapse.

Mutational landscape primary tumors from recurring ILC patients



Comparison prim EuroILC with public prim ILC (ER+ only)



Increased prevalence in ILC from recurring patients: *ESR1*, *IGF1R*, *MAP3K1, NF1, RB1, TP53*

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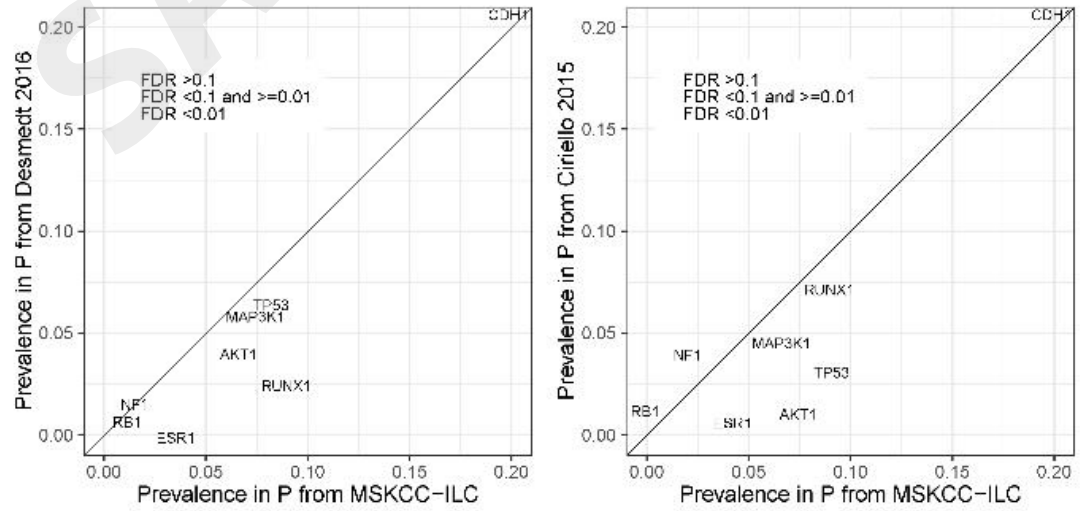
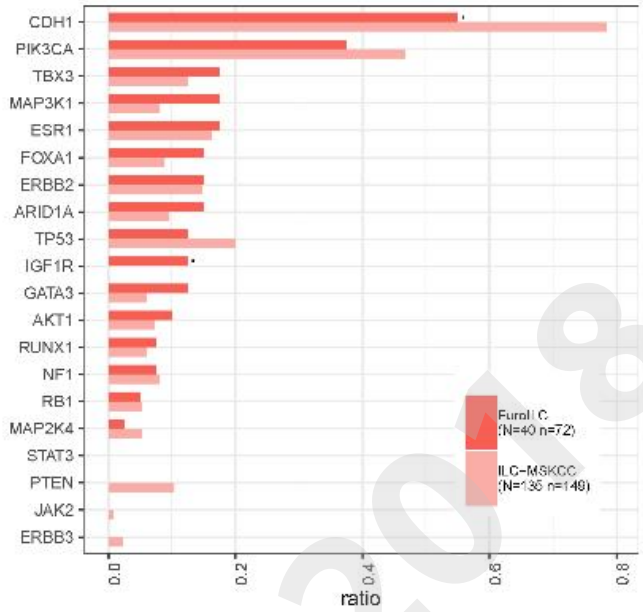
Comparison prim ILC MSKCC with public prim ILC (ER+ only)



Increased prevalence in ILC from recurring patients: *AKT1, CDH1*, *ESR1*, *RUNX1*

Mutational landscape ILC metastases



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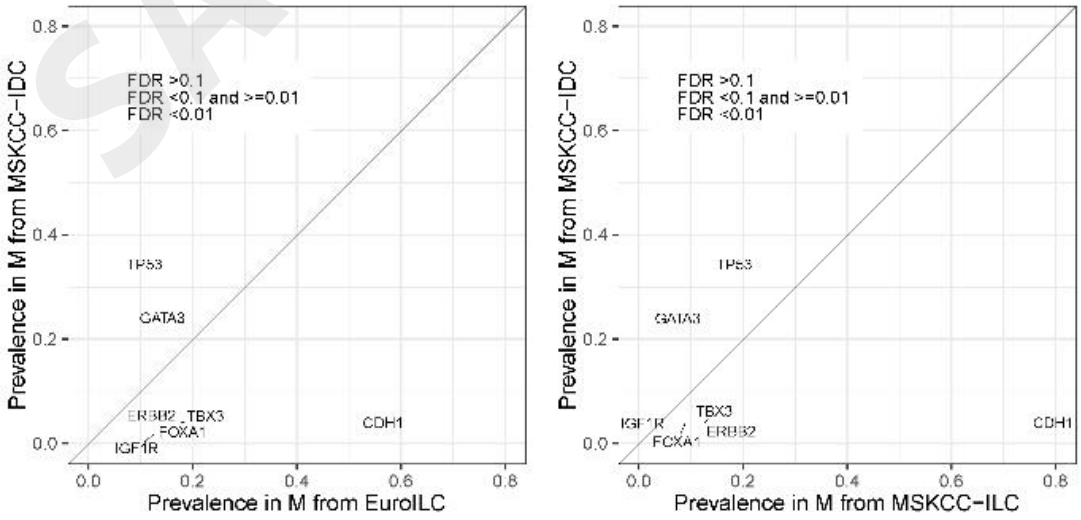
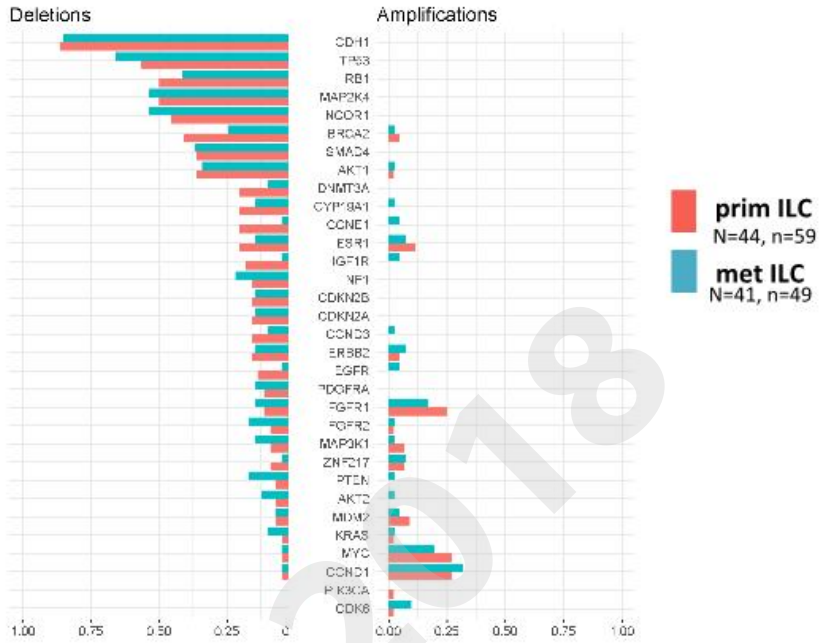
Comparison M IDC vs M ILC (ER+ only)



Increased prevalence in ILC: *ERBB2*, *CDH1*, *FOXA1*, *TBX3* (+*IGF1R*)
Decreased prevalence in ILC: *TP53*, *GATA3*

Frequency copy number aberrations



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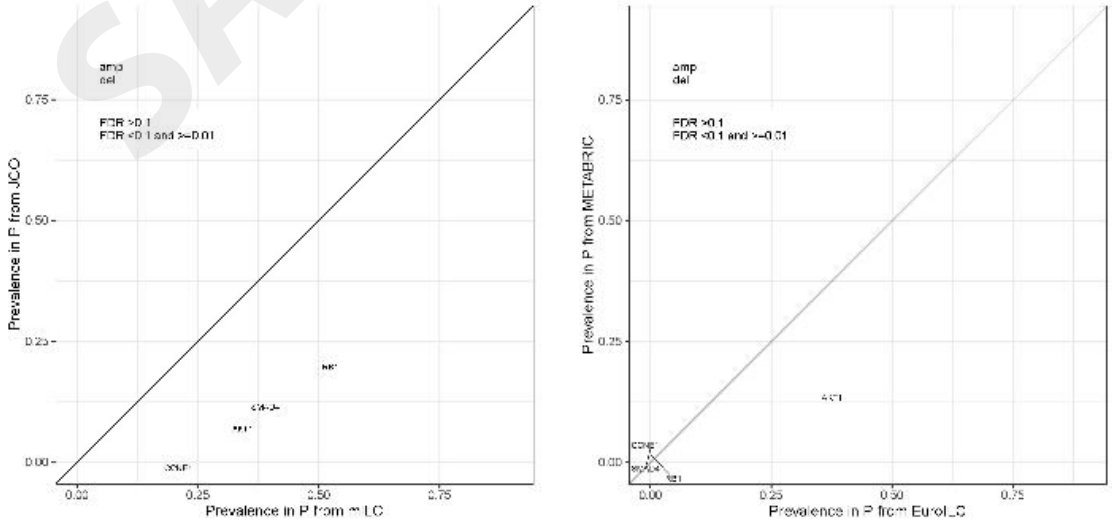
Comparison prim EuroILC with public prim ILC (ER+ only)



Increased prevalence of *AKT1* (+*CCNE1*, *RB1*, *SMAD4*) deletions in ILC from recurring patients

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Results

1. Genomic alterations in metastatic ILC.
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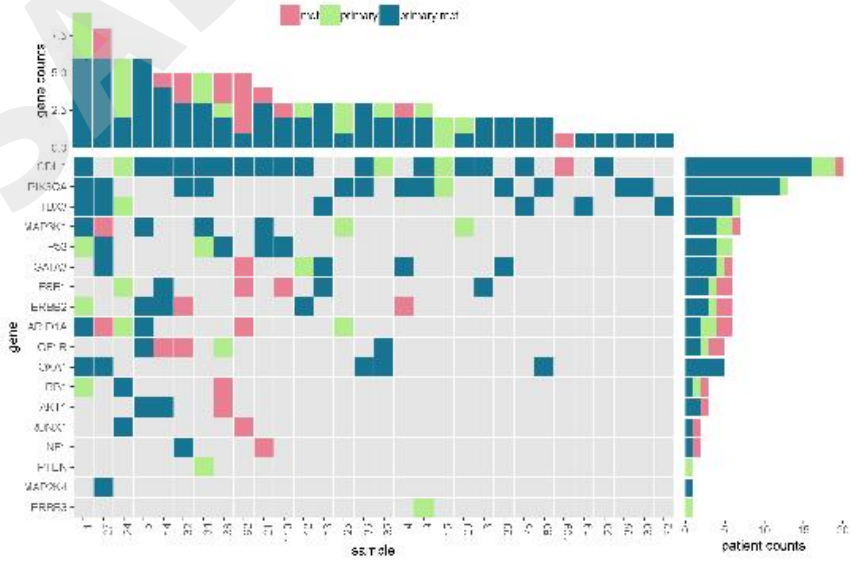
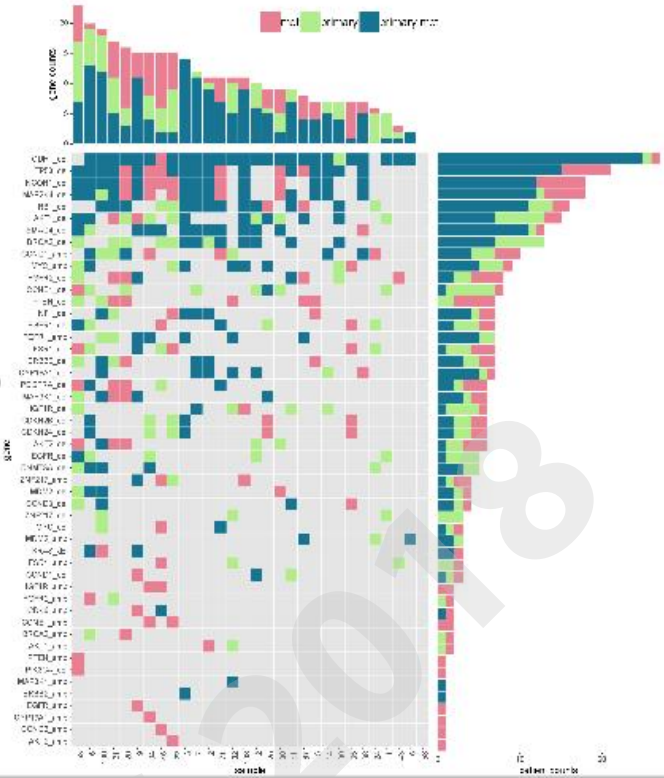
4. Clinical, pathological, immune or genomic markers associated with survival.

Mutations in paired samples



Mutations private to the metastasis: *ERBB2*, *ESR1* & *AKT1 (7%)*, *CDH1*, *NF1*, *MAP3K1* & *RUNX1 (3%)*

CNAs in paired samples



Alterations private to the metastasis: *TP53* del (20%), *PTEN* del (17%), *CCND1* ampl (10%) & *ESR1* del, *CCNE1* ampl, *IGF1R* ampl, *NF1* del (7%)

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	Pt12 *de novo met*	Pt34 *RFS= 4.4 yrs*	Pt21 *RFS= 10.8 yrs*	Pt67 *RFS= 15.1 yrs*
Primary disease	Postmenop. pT2, pN0 Mixed NC ER+/PR-/HER2- Ki67 15%	Postmenop. pT2, pN1 Classic ER+/PR+/HER2 NA Ki67 NA	Postmenop. pT2, pNx Mixed NC ER+/PR+/HER2- Ki67 40%	Posmenop. pT1, pN3 Classic ER+/PR+/HER2- Ki67 15%
Metastasis	Bone ER+/PR+/HER2- Ki67 NA	Local & pleura ER+/PR-/HER2- Ki67 5%	Bone ER+/PR+/HER2- Ki67 25%	Peritoneum ER+/PR+/HER2- Ki67 16%
Common alterations	*CDH1* mut+del *HER2* mut *RB1* del	*CDH1* mut+del *PIK3CA* mut *MAP3K1* mut *FGFR1* ampl	*CDH1* mut+del *TP53* mut *MAP3K1* mut *RB1* del	*CDH1* mut+del *FGFR1* ampl
Alterations private to M	/	*CCND1* ampl *CCNE1* ampl *IGF1R* ampl *TP53* del	*NF1* mut *PTEN* del	3 *ESR1* muts *PTEN* del *RB1* del *TP53* del

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Results

1. Genomic alterations in metastatic ILC.
2. Genomic alterations acquired during progression.
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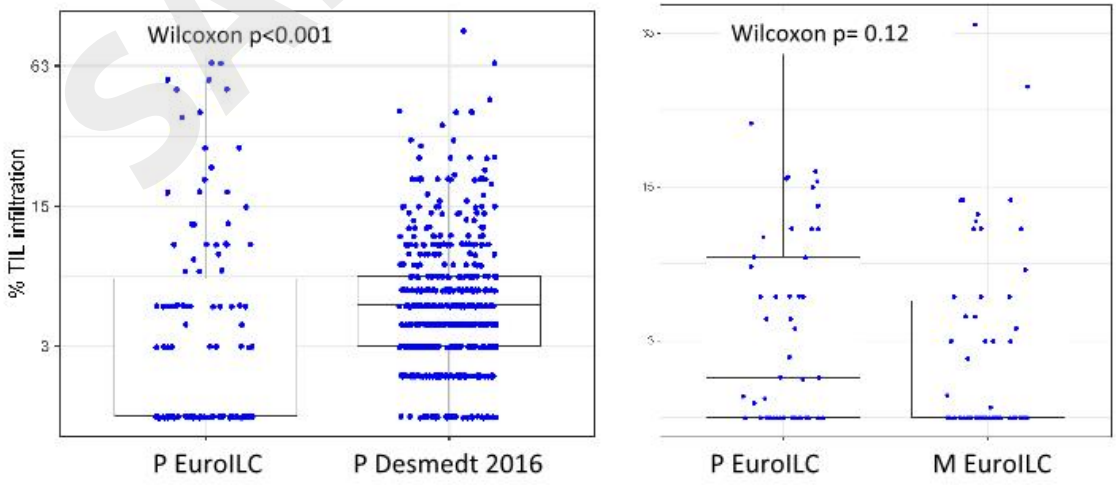
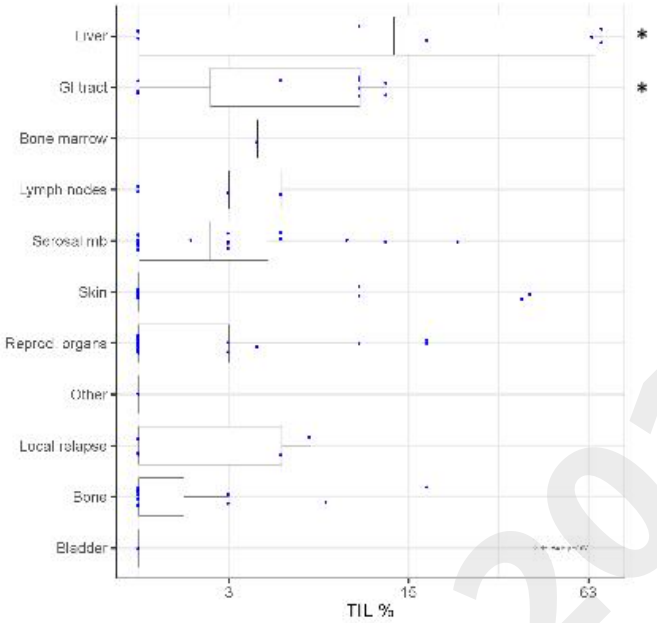
4. Clinical, pathological or genomic markers associated with survival.

TIL levels



- **Lower TIL levels** in primILC from patients who relapsed compared to published consecutive primILC cohort.
- **Lower TIL levels** in primary versus metastases in matched analysis (not stat. significant).

TILs in metILC (N=67, n=107)



Metastases in liver and GI tract have significantly higher TIL levels (when looking at 1 vs All Other)

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Results

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3. Immune infiltration in metastatic ILC.
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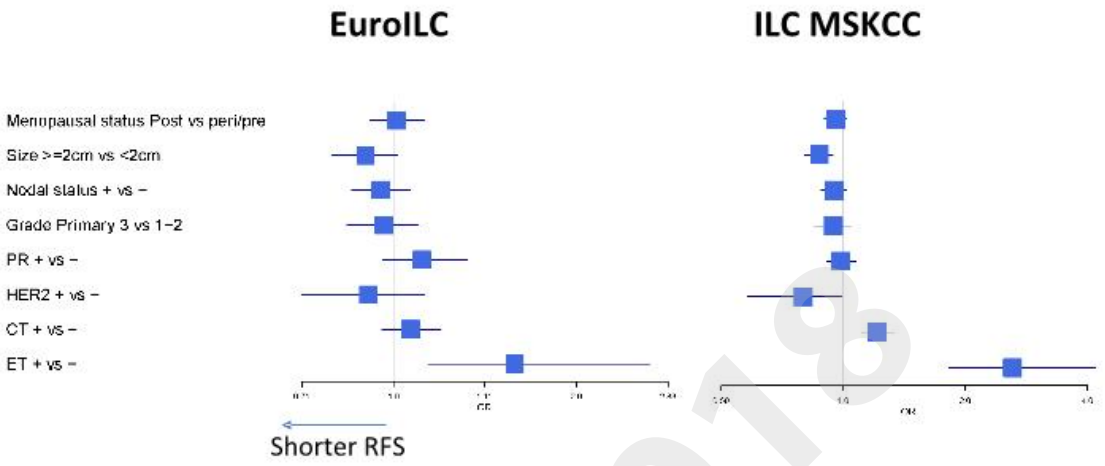
Clinico-pathological variables & RFS



Longer RFS: use of endocrine therapy and chemotherapy.
Shorter RFS: larger tumor size.

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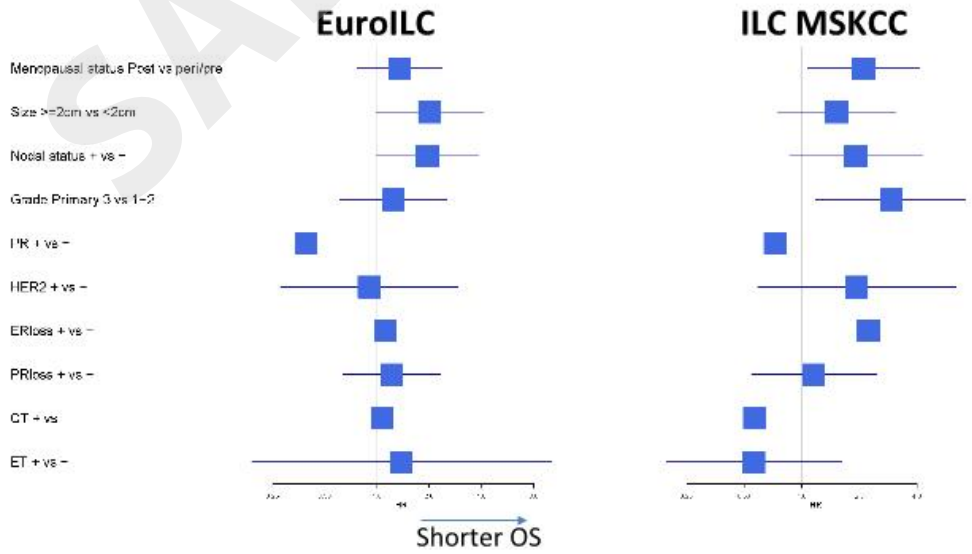
Clinico-pathological variables & OS



Longer OS: PR positive status primary tumor.
Shorter OS: larger tumor size, positive nodal status.

Genomic alterations & survival (exploratory)

RFS		
EuroILC	*ERBB2* (7.5%)	OR= 0.48 (95% CI: 0.18- 0.99)
OS		
EuroILC	*RUNX1* (7.5%) *ESR1* (17.5%) *RB1* (7.4%)	HR= 9.42 (95% CI: 1.1- 80.7) HR= 3.29 (95% CI: 1.0-11.2) HR= 2.28 (95% CI: 2.0- 50.1)
MSKCC-IMPACT	*TP53* (20%)	HR= 2.28 (95% CI: 1.14-4.54)

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Conclusions

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Conclusions (1/2)

1. Primary tumors from ILC patients who relapsed present an increased mutational frequency for several genes (*AKT1*, *IGF1R*, *MAP3K1*, *NF1*, *RB1*, *RUNX1*, *TP53*).

2. Metastases from ER+ ILC and IDC are different:
   - More ***CDH1***, ***ERBB2***, *FOXA1*, *IGF1R*, ***TBX3***
   - Less ***GATA3***, *TP53*

3. Unique matched prim/met cohort allowed the identification of alterations private to the metastasis:
   - *ERBB2*, *ESR1* & *AKT1 (7%)*, *CDH1*, *NF1*, *MAP3K1* & *RUNX1 (3%)*
   - *TP53* del (20%), *PTEN* del (17%), *CCND1* ampl (10%) & *ESR1* del, *CCNE1* ampl, *IGF1R* ampl, *NF1* del (7%).

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Conclusions (2/2)

4. TIL levels are lower in primary tumors from patients who relapsed as compared to consecutive primary ILCs & TIL levels are lower in metastases compared to the primary in a matched analysis.

5. Exploratory analyses revealed that some alterations could be associated with RFS or OS.

6. Given the relatively low prevalence of the majority of the detected genomic alterations, additional cohorts of matched primary/metastastic ILC samples are needed to confirm the present findings.

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All patients & their families