S6-02 OCCURRENCE OF NATURAL ESR1 MUTATIONS DURING ACQUISITION OF ENDOCRINE RESISTANCE IN BREAST CANCERS AND WIDELY USED ER+ CELL LINES.  Speaker: Pascal Gellert

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San Antonio Breast Cancer Symposium, December 8-12, 2015

Occurrence of natural ESR1 mutations during acquisition of endocrine resistance in breast cancers and widely used ER+ cell lines

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Aims

- To identify changes in the mutational profile of ER+ breast cancers at progression on Aromatase Inhibitor treatment

- To identify concomitant mutations occurring in cell line models of resistance to E-deprivation

ER+ Breast Cancer

- Mutational landscape of ER+ primary BC well described

- Few studies on mutational landscape of metastatic BC

- Landscape changes between primary and metastatic BC likely to determine the integration of diagnostic and treatment

Frequently mutated genes in ER+ primary BS

Gellert et al, SABCS 2014
TCGA, Nature 2012
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**The Hallmarks of AI resistance**

- **ER pathway**
  - Loss of ER expression
  - ESR1 mutation or amplification

- **Tumor microenvironment**
  - ECM & Cellular components

- **Growth factor receptor pathway**
  - e.g. HER2 mutation or amplification

- **Apoptosis and senescence**
  - e.g. mutation of TP53

- **Cell cycle machinery**
  - e.g. amplification of CCND1

- **Secondary messengers**
  - Mutations in
    - PI3K pathway
    - MAPK pathway

- **Epithelial-mesenchymal transition**
  - Notch, Hedgehog, WNT, TWIST1

*modified from Ma et al, Nature 2015*

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**ESR1 mutations in metastases**

**Primary disease**

<table>
<thead>
<tr>
<th>Samples</th>
<th>% ESR1 mutated</th>
<th>Pairs</th>
</tr>
</thead>
<tbody>
<tr>
<td>261 primary</td>
<td>3.3% primary</td>
<td>47 pairs</td>
</tr>
<tr>
<td>195 mets</td>
<td>13.3% mets</td>
<td></td>
</tr>
</tbody>
</table>

- Li et al, Cell Rep 2013
- Meenach-Larrin et al, Cancer Res 2013
- Toy et al, Nature Genet 2013
- Robinson et al, Nature Genet 2013
- Jeselsohn et al, Clin Cancer Res 2014

**Potential factors**

- Time, treatment, metastasis, mutational driver

- Small number of pairs
- Great treatment heterogeneity

*Modified from Segal and Dowsett, CCR 2014*
Patients and Methods

Arnedos M et al, Annals Oncology 2014:
- 55 patients selected retrospectively (2004-2009, Royal Marsden Hospital)
- Paired primary and recurrences available
- ER+
- Locally advanced or metastatic setting
- Relapsed or progression during AI treatment

Immunohistochemical analysis:
- 7% ER- in recurrences
- Decrease of PgR
- Higher Ki67
- 5% gained HER2 amplification

48 pairs for targeted sequencing from FFPE blocks

Genes of Interest

ER pathway
- Loss of ER expression
- ESR1 mutation or amplification

Tumor microenvironment
- ECM & Cellular components

ESR1

Growth factor receptor pathway
- e.g. HER2 mutation or amplification
- HER2, KIT, KRAS, BRAF

Secondary messengers
- PI3K pathway
- MAPK pathway
- PIK3CA, PTEN, AKT, PIK3R1
- MAP3K1, MAP2K4, GATA3

Apoptosis and senescence
- e.g. mutation of TP53

Cell cycle machinery
- e.g. amplification of CCND1

AI resistance

Whole gene Hotspot
- RUNX1, SF3B1

Epithelial-mesenchymal transition
- Notch, Hedgehog, WNT, TWIST1

CDH1

modified from Ma et al, Nature 2015
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Results

- Good quality data was available on 41 pairs
- Median depth was 782-fold
- A total of 89 tier 1 mutations were identified
- No significant difference in the number of mutations between the pre and post AI lesions

![Graph showing mutation percentages for different genes](image)

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Mutational overview

Mutations were found in 29 patients

- 12 patients with private mutations
- Mutations found in post only for HER2, MAP2K4 and ESR1
- No mutations found in 12 patients, loss/reduction of ER and gain of HER2 amplification in these

![Mutational overview matrix](image)
In vitro model for AI resistance

- Long term estrogen deprivation (LTED) to model relapse on an AI
- Study mutational changes of ESR1 between wild type and resistant breast cancer cell lines
Acquisition of ESR1 mutations in vitro

SUM44

Expression of endogenous E-regulated genes

Phenotypic analysis

RIME shows altered ER-proteome

ChIP-seq WT versus LTED

Anti-proliferative effect of fulvestrant

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Acquisition of ESR1 mutations in vitro

MCF7

1.3x10^6 WT-MCF7 were WT for ESR1, suggesting the mutation is absent or at extremely low YAF

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Conclusions

- Recurrences after AI but not primary ER+ tumors may contain ESR1 mutations that could influence clinical decision making.
- Other than ESR1 there are no consistent acquisition of mutations.
- High variability of genotype and phenotype requires individual interpretation for personalised treatment.
- In vitro ESR1 mutations can be acquired under estrogen deprivation resulting in a ligand independent phenotype.
- Anti-proliferative effect of SERDs remains effective.

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Patients involved in the study

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