Genomic and Immune characterization of metastatic breast cancer (mBC): An ancillary study of the SAFIR01 & MOSCATO trials

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Extensive efforts have been done in order to profile primary breast cancers.

Mutational landscape may evolve and some arguments suggest that metastatic “lethal” breast cancer dramatically differ from primary.

The molecular landscape of metastatic breast cancer is unknown.

The aim of this study is to analyze the genomic and immunologic profiles of «lethal» metastatic breast cancers in order to identify new targets and unmet medical needs.
Outline

• Whole exome sequencing
  – Genomic landscape
  – Mutational signatures

• Immunological markers
  – MHC I
  – TIL
  – PDL1/PD1
Whole Exome Sequencing

HiSeq technology (Integragen)

- Normal DNA
- SNP/Indels

Normal: 50x
Whole exome sequencing
Tumor: 100x

Patient

- Tumor DNA
- SNP/Indels

Quality control with Sanger

<table>
<thead>
<tr>
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<th>Mean coverage +/- SD</th>
<th>% of the exome covered &gt;25X</th>
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<tbody>
<tr>
<td>Tumor samples</td>
<td>102x</td>
<td>89%</td>
</tr>
<tr>
<td>Normal samples</td>
<td>53x</td>
<td>79%</td>
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- Compare genotype
- Determine variant status

PIK3CA: 14/18
AKT: 3/4

CASAVA1.8.2 (Illumina Software)
Identification of significantly mutated genes

Somatic mutations

Mutation Filters

Significantly mutated according to MuSic and drGAP (FDR < 15% in both analyses)

significantly mutated genes

BWA + mutect + IndelGenotyper + GATK haplotyperCaller

>10% allele frequency in tumor (with at least 5 supporting reads)
< 2% allele frequency in blood (with at most 5 supporting reads)
403 metastatic biopsies from SAFIR01 trial

143 blood samples

102 pairs biopsies/blood SAFIR01

86 pairs

16 tumors <30% cancer cells

7 pairs metastases-blood from MOSCATO trial (>30% cancer cells)

93 pairs metastases-blood analyzed

52 additional metastases not matched with normal DNA

ESR1 & prognosis
Mutational landscape and significantly mutated genes

- ESR1, TSC1/2 and DOT1L are found mutated in at least 5% of mBC but <1% early breast cancers (TCGA)

- Using a 15% FDR as cut-off, we could not identify other recurrent «metastasis-specific» drivers

FDR<15%
Recurrent alterations
ESR1 mutations & patient outcome

ESR1 mutations are associated with poor outcome

Multivariate analysis:
HR=4.60 (2.04;10.35), p<0.0001

Median OS:
11 months

ESR1 WT (n=75, 83%)
ESR1 mutations (n=16, 17%)
Analysis of mutation signatures (EMu algorithm) revealed two mutational processes.

**Signature 1:** C>T

**Signature 2:** TpC>G/T
Cluster of patients present with ER+, PIK3CA mutations, high mutation rate, TpC>G/T mutations,
Mutation number & patient outcome

- Highly mutated (n=17)
- Low mutation rate (n=18)
- Intermediate (n=17)

p=0.09

p=0.02
Outline

• Whole exome sequencing
  – Genomic landscape
  – Mutational signatures

• Immunological markers
  – MHC I
  – TIL
  – PDL1/PD1
Methods: Immune characterization

- 333 samples were stained for immune analyses
- Intratumoral and stromal TILs was assessed according to criteria previously described and published by Denkert et al.\(^1\)
- PD-L1 and PD-1 were performed by Medimmune using internal protocols
  - PD-L1: anti-human PD-L1 monoclonal antibody (CAMP-1). Samples were considered PD-L1 (+) if 5% or more of the tumor cells showed staining at the surface membrane
  - PD-1: mouse anti-human PD-1 (clone Nat105). Average number of cells were then categorized in a 0-3 scoring system.
- MHC class I: mouse monoclonal antibody (clone EMR8-5).
  - Semi-quantitative evaluation of percentage of stained cells (moderate to strong intensity). Internal positive control (lymphocytes and endothelial cells)

Question: which patients could be eligible to modulators of immune checkpoints and expansion of adaptive immune response
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MHC I: can the cancer cell be targeted by the CTL?
A majority of metastatic breast cancers have lost MHC I expression
MHC class I expression by metastatic breast cancers

Correlation with ER status

<table>
<thead>
<tr>
<th></th>
<th>ER-(n=110)</th>
<th>ER+ (n=178)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC I</td>
<td>p=0.0324</td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>30%</td>
<td>10%</td>
</tr>
<tr>
<td>Range</td>
<td>0-100</td>
<td>0-100</td>
</tr>
<tr>
<td>Missing</td>
<td>31</td>
<td>47</td>
</tr>
</tbody>
</table>

MHC I expression is higher in ER-negative breast cancer
MHC class I expression by metastatic breast cancers

% cancer cells stained

n=247

MHC I expression is lower in heavily pretreated patients

Correlation with number of previous chemotherapy

<table>
<thead>
<tr>
<th></th>
<th>No chemo</th>
<th>1 Line</th>
<th>2 Lines</th>
<th>&gt;2 Lines</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>109</td>
<td>83</td>
<td>52</td>
<td>46</td>
</tr>
<tr>
<td>MHC I p</td>
<td>0.0696</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>20.0</td>
<td>20.0</td>
<td>15.0</td>
<td>5.0</td>
</tr>
<tr>
<td>(Range)</td>
<td>(0.0:100.0)</td>
<td>(0.0:100.0)</td>
<td>(0.0:100.0)</td>
<td>(0.0:100.0)</td>
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Question: which patients could be eligible to modulators of immune checkpoints and expansion of adaptive immune response

Are the lymphocytes in the tumor site (TILs)?
Stromal TIL and metastatic breast cancers

Correlation between stTIL and nb mutations (spearm: 0.338, p=0.008)

<table>
<thead>
<tr>
<th>ER+/HER2-</th>
<th>ER-/HER2-</th>
<th>HER2+</th>
</tr>
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<tbody>
<tr>
<td>Median</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>Q3</td>
<td>15%</td>
<td>20%</td>
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p<0.001

p<0.1
Questions: which patients could be eligible to modulators of immune checkpoints and adaptive immune response

Is PDL1 the immunosuppressive network in mBC?
### PD1/PDL1 expression in metastatic breast cancers

<table>
<thead>
<tr>
<th></th>
<th>ER+/Her2- (n=145)</th>
<th>TNBC (n=66)</th>
<th>Her2-overexpressed (n=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PDL1 cancer cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;5%)</td>
<td>2 (1%)</td>
<td>2 (3%)</td>
<td>3 (8%)</td>
</tr>
<tr>
<td><strong>PDL1 immune cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;0 cell)</td>
<td>104 (71%)</td>
<td>46 (69%)</td>
<td>25 (69%)</td>
</tr>
<tr>
<td><strong>PD1 immune cells</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(&gt;0 cell)</td>
<td>30 (20%)</td>
<td>20 (31%)</td>
<td>13 (36%)</td>
</tr>
</tbody>
</table>
Conclusion

- ESR1, TSC1/2 and DOT1L mutations are enriched in metastatic samples.

- In this preliminary analysis (93 samples), we could not identify additional « metastasis-specific » drivers.

- ESR1 mutation is associated with poor outcome.

- A subset of PIK3CA mutated mBC clusters in a group defined by high mutation rate and TpC>G/T mutational signature.

- Ideal population to develop immunotherapeutics could TNBC / Her2+++ treated with <2 lines chemotherapy (TIL+ / MHC I+)
Questions generated by the study

• Is it possible to identify new recurrent « metastases-specific » drivers in metastatic samples? Need for more samples before excluding they exist (aim >200 Q1 2015)

• Does ESR1 mutated BC define a genomic segment with very poor outcome that would deserve drug approval based on phase II?

• Should PIK3CA mutated mBC be stratified according to the mutational process?

• Should trials on immunotherapeutic stratify the patients based on MHC I?

• Should trials on immunotherapeutics include interferon in the strategy?

• Which immune targets in mBC? (CD73, NK0)
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