Discussion of Abstracts #230, #410, #420

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DISCLOSURE

Nothing to disclose that is relevant to these abstracts
Abstracts

• Primary Breast Cancers
  • Gene expression profiles for patterns of metastatic spread (Lawler et al)

• Relapsed Breast Cancers
  • Targeted NGS to detect mutation of a single gene in blood versus tissue (De Laere et al)

• Primary Triple-Negative Breast Cancers
  • Cytogenetic scars from aberrant DNA repair (Grigoriadis et al)
Clinical patterns of metastatic spread from formalin-fixed, paraffin-embedded (FFPE) expression profiles:
a case-control study of 1,357 breast cancer patients

Katherine Lawler, Efterpi Papouli, Andrew Tutt, Tony Ng, Sarah Pinder, Peter Parker, Lars Holmberg, Cheryl E. Gillett, Anita Grigoriadis, Arnie Purushotham

King’s College London
Gene expression of FFPE primary tumours: case-control patient selection and RNA extraction

**Incidence-based controls**
- no recurrence at calendar date

**Case-control pairs**
- random selection

**RNA extraction**
- n=1357 patients

**Discovery cohort enriched for each type of metastasis**
- Validation cohort represents usual clinical population

**Case selection:** primary tumour samples with recorded metastatic recurrence

<table>
<thead>
<tr>
<th>Metastatic group (first site)</th>
<th>No. of patients</th>
<th>% of metastatic population</th>
<th>No. of cases randomly selected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone only</td>
<td>413</td>
<td>26%</td>
<td>400</td>
</tr>
<tr>
<td>Bone + visceral within 6 month period</td>
<td>438</td>
<td>27%</td>
<td>400</td>
</tr>
<tr>
<td>Visceral only</td>
<td>747</td>
<td>47%</td>
<td>400</td>
</tr>
<tr>
<td><strong>Total, all metastases:</strong> 1598</td>
<td><strong>100%</strong></td>
<td></td>
<td><strong>n=1200</strong></td>
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</tbody>
</table>

**Discovery set**
- n=537 patients

**Validation set**
- n=146 patients

**1357 cases extracted ➔ 683 expression arrays**

**Why?**
- 23% ➔ mets
Genes associated with specific patterns of metastatic spread

Case-control pairs

Gene expression specific to metastatic group

Metastatic group vs. No metastasis

ER-positive

<table>
<thead>
<tr>
<th>Metastatic group</th>
<th>Density</th>
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<tbody>
<tr>
<td>Bone+ Visceral</td>
<td>54 genes</td>
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<tr>
<td>Visceral only</td>
<td>386 genes</td>
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<tr>
<td>Bone only</td>
<td>2 genes</td>
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ER-negative

<table>
<thead>
<tr>
<th>Metastatic group</th>
<th>Density</th>
</tr>
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<tbody>
<tr>
<td>Bone+ Visceral</td>
<td>30 genes</td>
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<tr>
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<td>8 genes</td>
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<tr>
<td>Bone only</td>
<td>14 genes</td>
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</table>

IMPAKT 2015, Lawler
Prognosis for specific patterns of metastatic spread in an ER-positive breast cancer population

Metastatic group-specific expression scores

Discovery set

<table>
<thead>
<tr>
<th>Metastatic group</th>
<th>Prob. survival</th>
<th>p-value</th>
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<tbody>
<tr>
<td>Bone+ Visceral</td>
<td>low score</td>
<td>5e-10</td>
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<tr>
<td>Visceral only</td>
<td>med score</td>
<td></td>
</tr>
<tr>
<td></td>
<td>high score</td>
<td></td>
</tr>
<tr>
<td>Bone only</td>
<td>low score</td>
<td>8e-6</td>
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<tr>
<td></td>
<td>med score</td>
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</tr>
<tr>
<td></td>
<td>high score</td>
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Validation set

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<th>Metastatic group</th>
<th>Prob. survival</th>
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<td>Bone only</td>
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Multivariable survival analysis with competing risks

10yr follow-up

IMPAKT 2015, Lawler
Comments

- Difficult model: time-dependent risk for metastases
  - Slower for ER+
  - Patterns differ by phenotype
  - When can you be sure a control is really a control?

- Difficult methodology: use of FFPE samples
  - A major challenge for discovery of gene expression signatures (arrays or RNAseq)

- Difficult challenge: site-specific signatures for metastases
  - This shows some feasibility in ER+
  - Is a larger study required to test this for ER-negative?
Exploring the intra-patient *PIK3CA* mutational heterogeneity of circulating tumour cells by massive parallel sequencing in patients with metastatic hormone receptor-positive breast cancer.

Authors: De Laere B 1, 2, Peeters D J E 1, 2, Salgado R 2, 3, Vermeulen P B 1, 2, van Dam P A 1, Van Laere S J 1, 2 4 and Dirix L Y 1, 2

1 Center for Oncological Research, Faculty of Medicine & Health Sciences, University of Antwerp, Antwerp, Belgium
2 GZA Hospitals Sint-Augustinus, Antwerp, Belgium
3 Breast International Group (BIG), Brussels, Belgium
4 Department Oncology, KU Leuven, Leuven, Belgium
Hotspot *PIK3CA* mutational heterogeneity in CTC

A.

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- **P539R**
- **E542K**
- **E545K**
- **H1047R**

B.

- **Percentage of CTC**
  - **WT**
  - **MT clonal (>75%)**
  - **MT subclonal (<25%)**
  - **MIX**

**Legend:**
- WT
- HS1
- HS2
- DM
Comparative PIK3CA mutational analysis: Early versus advanced disease

- Overall higher level of agreement between CTC and PT/META

- PT versus cfDNA versus CTC (n=18)
  - Plasma ‘failed’ to detect 2 mutant patients (2/18, 11%)
    - Patients 889 and 3546
    - Mutation was present in PT/META and CTC

- Disparity between early and advanced disease in 4 patients (4/25, 16%)
  - Patient 1529, 2139, 2648, and 3516
Another view of these pilot data

If Tumor Tissue Has Mutated Status

<table>
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<th>WT</th>
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<td>1</td>
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<tr>
<td>cfDNA</td>
<td>2</td>
<td>0</td>
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If Tumor Tissue Has Wild-type Status

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<thead>
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<th>MT</th>
<th>WT</th>
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<td>4</td>
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<tr>
<td>cfDNA</td>
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</table>
Comments

- Feasibility demonstrated for single CTC measurements
- PIK3CA genomic status in tumor tissue appears to be more concordant with status in CTCs than with status in circulating free DNA

- Sometimes the PIK3CA genomic status was different in the circulation from the status in the tumor tissue
  - Observed more often if tumor tissue had WT status
  - May be more common with cfDNA than with CTCs

- Look forward to a larger study with matched primary and metastatic tissues, and blood samples
Profiles of genome complexity identify HORMAD1 as a driver of homologous recombination deficiency and platinum therapy response in triple-negative breast cancer

Anita Grigoriadis, Daniel Weekes, Johnathan Watkins, Patrycja Gazinska, Jessica Frankum, Chris Lord, Alan Ashworth, James Ford, Melinda Telli, Andrew Tutt

Breakthrough Breast Cancer Research Unit

King’s College London, UK
Scores of Chromosomal Instability Scarring (SCINS) - measures of structural aberrations

Allelic Imbalanced Copy Number Aberration (SAlCNA)

Non-homologous end joining (NHEJ)
Non-allelic homologous recombination (HR)

Copy neutral Loss of Heterozygosity (ScnLOH)

Non-conservative allelic homologous recombination (HR)

Allelic Balanced Copy Number Aberration (SAbCNA)

Whole genome duplication
Extent and nature of genomic aberrations differ across TNBCs

- METABRIC TNBCs
- TCGA TNBCs
- KCL TNBCs
- PrECOG TNBCs

Scores

- AbCNA...Allelic Balance
- AiCNA.....Allelic Imbalance
- CnLOH....copy-neutral LOH
$S_{AiCNA}$ is associated with platinum-based chemotherapy sensitivity in wildtype and mutant $BRCA1$ TNBCS

$S_{AiCNA} \ldots$ Allelic Imbalance

$S_{CnLOH} \ldots$ CnLOH

Phase II Study of Gemcitabine, Carboplatin, and Iniparib As Neoadjuvant Therapy for Triple-Negative and $BRCA1/2$ Mutation–Associated Breast Cancer With Assessment of a Tumor-Based Measure of Genomic Instability: PrECOG 0105

SCINS-defined tumour classification identifies *HORMAD1* in high AiCNA TNBCs

SCINS-based class discovery

![Graphs and tables showing the expression levels of HORMAD1 in KCL and METABRIC TNBCs]
**HORMAD1**, a Cancer/Testis Antigen, displays bimodal expression specifically in TNBCs

Bimodal expression in KCL TNBCs

![Graph showing bimodal expression in TCGA TNBC RNA-Seq](image)

Bimodal expression in PrECOG trial TNBCs

![Graph showing bimodal expression in PrECOG trial TNBCs](image)

Prediction of clinical outcome (PrECOG)

![Graph showing prediction of clinical outcome](image)

TNBC, Testis, ER-Pos BC

**IMPACT**

**BRCA1/2**

**BRCA1/2/HORMAD1**

**S_{AICNA} (21)**

**BRCA1/2/S_{AICNA} (22)**

HORMAD1

BRCA1/2

BRCA1/2-HORMAD1

S_{AICNA} (21)

BRCA1/2-S_{AICNA} (22)

**Balanced Accuracy**

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<th>0.2</th>
<th>0.4</th>
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<tbody>
<tr>
<td>HORMAD1</td>
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<tr>
<td>BRCA1/2</td>
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<tr>
<td>BRCA1/2-HORMAD1</td>
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<tr>
<td>S_{AICNA} (21)</td>
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<tr>
<td>BRCA1/2-S_{AICNA} (22)</td>
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</table>

- **All TNBCs**: Balanced Accuracy = 0.62
- **BRCA1/2 wildtype TNBCs**: Balanced Accuracy = 0.73

**P = 0.0502**

**OR = 2.91 (1.02-8.75 CI)**
Comments

• Meiosis ≈ genomic instability (diversity)
• Mitosis ≈ genomic fidelity

• BRCA1/2 aberrations lead to genomic instability
• SCINS ≈ indicate genomic instability
• Exciting potential for wild-type BRCA-1/2 TNBC
  • Genomic instability + High proliferation
  • Allelic imbalanced CNAs
    • Possible response to DNA damaging Rx
    • Induced by HORMAD1 (meiosis-associated gene)

• The translational implications of this early and innovative work are very interesting