New Concepts in Breast Cancer Genomics

SABCS review
January 26, 2019

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Associate Professor
University of Wisconsin
M.E.B. declares the following:
Medical advisory board of Strata Oncology;
Research funding from Abbvie, Genetech, Puma, and Loxo Oncology.
OVERVIEW

• Single cell atlases of breast cancer (GS1-01, GS1-02)

• Metastatic breast cancer—further genetic differences from primary breast cancer (GS1-06, GS1-07, GS1-08)

• Late recurring ER+ subgroups, results from METABRIC (GS3-6)
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Landscape of the breast tumour microenvironment at single-cell resolution

A/Prof Alexander Swarbrick PhD
Garvan Institute of Medical Research
Sydney Australia
Solid cancers are complex cellular ‘ecosystems’
Multi-omic single cell analysis

DOI: 10.1101/424945
A breast cancer cell atlas

Clustering
~125,000 cells
25 tissues

Cell Type Proportions

Plasma
Epithelial 2
Myeloid
CAF 2
CAF 1
Endothelial
Epithelial 1
CD8 T
T Reg
CD4 T
B
Case study: Grade 3 TN IDC
Epithelial heterogeneity

Ki67

Cytokeratin 5

Cytokeratin 5 IHC
The breast cancer immune milieu
CITE-Seq analysis of 98 cell-surface markers

- **Lineage Markers**
  - CITE CD3
  - CITE CD4: T helper
  - CITE CD8: Cytotoxic T
  - CITE CD56: NK

- **Activation/Memory Markers**
  - CITE CD69: activated T
  - CITE CD45RA: Naive T
  - CITE CD45RO: Memory T
  - CITE CD103: Tissue resident T

- **Checkpoint**
  - CITE PD-1
  - CITE GITR
Towards A Human Breast Cell Atlas

Tapsi Kumar, M.S.
Navin Laboratory
Graduate Student, Department of Genetics
Program for Rapid Breast Tissue Collection & Processing

Tissue Sources
- Reduction Mammoplasty (UCI)
- Mastectomy (MDA)
- Post-mortem Tissues (MDA)

Surgery
Pathology
Tissue Dissociation

Data Analysis & Statistics
Next-Generation Sequencing
single cell RNA
QC Single Cell Suspensions

2 hours
Cell Types identified in Breast Tissues from 11 Women

Unbiased single cell RNA of 32148 cells from 11 normal breast tissues (mastectomies)
Fibroblasts and Epithelial cells are most abundant cell types, but their frequencies vary across women.

Unexpectedly, immune cell populations (T-cells, macrophages) were identified in many normal breast tissues and vary across women.

Adipocytes were removed during dissociation process and are not identified in the datasets.
Three Groups of Endothelial Cells

Lymphatic  Vascular-1  Vascular-2

Vascular-1
Jak Stat Signaling Pathway
Hematopoietic cell lineage
Antigen Processing and presentation

Vascular-2
PPAR Signaling Pathway
Focal Adhesion
ECM Receptor Interaction
• T cells mostly coming from 2 patients, different states of like activated, cytotoxic

• Macrophages & Monocyte group is most abundant immune population
My Conclusions/take home
My Conclusions/take home

Tumors consist of more than just cancer: CAFs, tumor endothelial cells, macrophages, T-cells, adipocytes.

Identifying differences between these non-cancer cells may provide important predictive biomarkers.

Multi-dimensional spatial-genomic analyses of tumors are on the way...

..but how will we use this information?
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THE GENOMIC LANDSCAPE OF METASTATIC BREAST CANCER

Study Design – CPCT-02

N = 442

- Lung: 12
- Liver: 199
- Soft tissue: 48
- Bone: 50
- Lymph node: 94
- Other / Unknown: 39

Fresh-frozen biopsy and blood control taken n = 552

- Tumor cell percentage <30%
  - n = 108
  - DNA yield too low for WGS
    - n = 2
- Tumor cell percentage >30%
  - n = 444
  - Successful WGS of biopsy and blood
    - n = 442

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Primary\(^\dagger\) versus metastatic BC

**Driver genes**

- TP53
- PIK3CA
- ERBB2
- ESR1
- GATA3
- PTEN
- KMT2C
- CDH1
- NF1
- MAP2K4
- ARID1A
- MAP3K1
- FOXA1
- NCOR1
- AKT1
- MAP3K1
- TBX3
- RB1
- CDKN1B
- GPS2
- CBFB
- RUNX1

**Mutational signatures**

- Sig 8 (BRCA)
- Sig 1 (Age)
- Sig 2 (APOBEC)
- Sig 3 (BRCA)
- Sig 5 (Age)
- Sig 13 (APOBEC)
- Sig 16 (new in BC)
- Sig 9 (new in BC)
- Sig 17 (unknown)
- Sig 18 (unknown)
- Sig 30 (unknown)
- Sig 6 (MMR deficiency)


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Primary\textsuperscript{1} versus metastatic BC

Median TMB 1.29

Median TMB 2.97

\textsuperscript{1}Nik-Zainal et al. Nature. 2016;534(7605):47-54.
Genomic characterisation of metastatic breast cancers

Andre F, Filleron T, Ng C, Bertucci F, Le Tourneau C, Jacquet A,
Piscuoglio S, Jimenez M, Bachelot T

Institut Gustave Roussy, Villejuif; Centre Claudius Regault, Toulouse;
University Hospital Basel, Switzerland; Institut Curie, Paris;
Institut Paoli Calmette, Marseille; UNICANCER, Paris;
Centre Leon Berard, Lyon, France
mBC acquire ESR1 mutations under the pressure of endocrine therapy: Evidence of genome evolution

Beyond ESR1, does mBC acquire genomic alterations that contribute to disease progression?
Patients and Methods

**MOSCATO** (Massard et al, *Cancer Discov* 2017)
**SAFIR-02** (NCT: 02299999)
**PERMED-01** (NCT: 02342158)
**MATCH-R** (NCT: 02517892)

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DNA from **629** metastatic breast cancers and blood

- HiSeq (n=262), Novaseq (n=367)

Whole exome sequencing (100-120x for tumor DNA, 50 or 100x for normal DNA)

- Bioinfo Pipeline: Mutect (SNVs), strelka (indels), FACETS (CNAs)

- Genomic landscape of metastatic breast cancers
## Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Overall (n=629)</th>
<th>HR+/HER2- (n=387)</th>
<th>TNBC (n=186)</th>
<th>HER2+ (n=32)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at inclusion</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median (Range)</td>
<td>54 (26-83)</td>
<td>56 (26-82)</td>
<td>50 (27-83)</td>
<td>53 (37-73)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Site of biopsy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>272 (43.2)</td>
<td>205 (53%)</td>
<td>43 (23.1%)</td>
<td>9 (28.1%)</td>
<td></td>
</tr>
<tr>
<td>Lymph Node</td>
<td>111 (17.6%)</td>
<td>58 (15%)</td>
<td>43 (23.1%)</td>
<td>7 (21.9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Skin</td>
<td>76 (12.1%)</td>
<td>44 (11.4%)</td>
<td>24 (12.9%)</td>
<td>5 (15.6%)</td>
<td></td>
</tr>
<tr>
<td>Breast</td>
<td>74 (11.8%)</td>
<td>38 (9.8%)</td>
<td>33 (17.7%)</td>
<td>2 (6.3%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>96 (15.3%)</td>
<td>42 (10.8%)</td>
<td>43 (23.2%)</td>
<td>9 (28.1%)</td>
<td></td>
</tr>
<tr>
<td><strong>Previous Endocrine Therapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>403 (64.1%)</td>
<td>342 (88.4%)</td>
<td>28 (15.1%)</td>
<td>15 (46.9%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>Number of previous lines of Chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-1</td>
<td>427 (67.8%)</td>
<td>259 (66.9%)</td>
<td>140 (75.2%)</td>
<td>16 (50%)</td>
<td></td>
</tr>
<tr>
<td>&gt;1</td>
<td>182 (29%)</td>
<td>118 (30.5%)</td>
<td>38 (20.5%)</td>
<td>15 (47%)</td>
<td>0.0019</td>
</tr>
<tr>
<td>Missing</td>
<td>20 (3.2%)</td>
<td>10 (2.6%)</td>
<td>8 (4.3%)</td>
<td>1 (3%)</td>
<td></td>
</tr>
<tr>
<td><strong>Delay between biopsy and diagnosis of metastasis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-12 months</td>
<td>301 (47.9%)</td>
<td>162 (41.9%)</td>
<td>122 (65.6%)</td>
<td>15 (46.9%)</td>
<td></td>
</tr>
<tr>
<td>&gt; 12 months</td>
<td>183 (29%)</td>
<td>153 (39.5%)</td>
<td>23 (12.4%)</td>
<td>3 (9.3%)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Missing</td>
<td>145 (23%)</td>
<td>72 (18.6%)</td>
<td>41 (22%)</td>
<td>14 (43.8%)</td>
<td></td>
</tr>
</tbody>
</table>
Can we identify genomic alterations that drive progression of mBC?

II. Comparison with genomic landscape of early Breast Cancers

Comparison with TCGA (Lefebvre, PLoS Med), Fisher’s exact test adjusted for multiple comparisons (<0.05)
Which genomic alterations are enriched in mBC as compared to eBC?

Nine gene alterations enriched in the overall population:
- Transcription and splicing
- Signal transduction
- Migration, invasion

Ten gene alterations enriched in HR+/Her2- mBC:
- MethylTransferase that regulates ER activity
- Transcription factor Co-repressor of ER
- MEK pathway
- G1-S phase Downstream CDK4

What is the clinical relevance of detecting these alterations (HR+/Her2-)?
Clinical relevance of RB1 & NF1 mutations

**RB1**

HR: 2.29 95%CI[1.16; 4.53], p=0.017

**NF1**

HR: 1.92 95%CI[1.15; 3.21], p=0.013

HR: Hazard Ratio (multivariate)
Tumor Mutational Burden
Are mBC presenting more mutations than eBC?

HR death (HR+/Her2-): p=0.039
Q2: HR: 1.37, 95%CI[0.95-1.99]
Q3: HR: 1.57, 95%CI[1.10-2.24]

****: p<0.0001
My Conclusions/take home

Metastatic tumors have profile of mutations that are different from primary cancer.

Are these due to:
  - selective pressure?
  - higher propensity to mutation in high TMB tumors with additional mutations
Unraveling lobular breast cancer progression and endocrine resistance mechanisms through the genomic and immune characterization of matched primary and metastatic samples


5th of December 2018
San Antonio Breast Cancer Symposium
EuroILC

125 ER+ metILC patients from 6 European institutions

31 failed pathology review

94 ER+ metILC patients for TIL evaluation

21 with insufficient 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)
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
1120 pts (1230 samples)
600P & 630M
unmatched

ER+ ILC
260 pts (281 samples)
132P & 149M
unmatched
## ILC Patient & Sample Characteristics

**EuroILC & MSKCC-IMPACT with met samples**

<table>
<thead>
<tr>
<th>Menopausal Status</th>
<th>PgR Status <em>prim</em></th>
<th>HER2 Status <em>prim</em></th>
<th>Adjuvant Chemotherapy**</th>
<th>Adjuvant Endocrine Therapy**</th>
<th>Adjuvant Radiotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre/peri</td>
<td>41 (44%)</td>
<td>73 (49%)</td>
<td>Negative</td>
<td>14 (15%)</td>
<td>23 (18%)</td>
</tr>
<tr>
<td>Post</td>
<td>53 (56%)</td>
<td>75 (51%)</td>
<td>Positive</td>
<td>77 (85%)</td>
<td>108 (32%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Primary Tumor Size *</th>
<th>Negative</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 cm</td>
<td>21 (24%)</td>
<td>43 (35%)</td>
</tr>
<tr>
<td>≥ 2 cm</td>
<td>76 (76%)</td>
<td>79 (65%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nodal Status</th>
<th>Adjuvant Chemotherapy**</th>
<th>Adjuvant Endocrine Therapy**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>29 (31%)</td>
<td>19 (20%)</td>
</tr>
<tr>
<td>Positive</td>
<td>64 (69%)</td>
<td>74 (80%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histological Subtype</th>
<th>Adjuvant Endocrine Therapy**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Classic</td>
<td>No</td>
</tr>
<tr>
<td>Non-classic</td>
<td>Yes</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Histological Grade _prim**</th>
<th>Adjuvant Radiotherapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1/G2</td>
<td>34 (47%)</td>
</tr>
<tr>
<td>G3</td>
<td>38 (53%)</td>
</tr>
</tbody>
</table>

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Distribution metastatic samples

- Bone
- Serosal mb
- Reprod. organs
- Skin
- Liver
- GI tract
- Lymph nodes
- Local relapse
- Bladder
- Other
- Bone marrow
- Soft Tissue
- Lung
- Chest Wall
- Brain

**Source:**
- Eurolc (N=84 n=165)
- ILC–MSKCC (N=135 n=149)
- IDC–MSKCC (N=563 n=630)
Comparison prim EuroILC with public prim ILC (ER+ only)

Increased prevalence in ILC from recurring patients: *ESR1, IGF1R, MAP3K1, NF1, RB1, TP53*
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%)
My Conclusions/take home

Metastatic tumors have profile of mutations that are different from primary cancer.

In addition to ESR1, these include AKT1 and HER2 mutations.

• ongoing trials of AKT1 inhibitors (e.g. NCI Match), HER2 inhibitors (e.g. SUMMIT)

These differences are likely due to acquired mutations.
OVERVIEW

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• Late recurring ER+ subgroups, results from METABRIC (GS3-6)
Dynamics of breast cancer relapse reveal molecularly defined late recurring ER-positive subgroups: Results from the METABRIC study

Oscar M. Rueda, Stephen-John Sammut, Suet-Feung Chin, Jennifer L Caswell-Jin, Jose A Seoane, Maurizio Callari, Rajbir Batra, Bernard Pereira, Alejandra Bruna, H Raza Ali, Elena Provenzano, Bin Liu, Michelle Parisien, Cheryl Gillett, Steven McKinney, Andrew R. Green, Leigh Murphy, Arnie Purushotham, Ian O. Ellis, Paul D. Pharoah, Cristina Rueda, Samuel AJR Aparicio, Carlos Caldas, Christina Curtis
Background: breast cancer recurrence

- While prognosis for early stage breast cancer (BC) has improved dramatically, 20-30% of patients recur with incurable disease.
- Spatial and temporal patterns of relapse are unknown and difficult to predict.
- A subset of women with early stage ER+ BC have a persistent risk of recurrence and death up to 20 years post-diagnosis (Pan et al. NEJM 2017).
- Critical need to identify tumor characteristics that are more predictive of risk of recurrence than standard clinical covariates (nodal status, tumor size, grade).
METABRIC Cohort Overview

- 3240 breast cancer patients derived from 5 tumor banks in the UK and Canada diagnosed between 1977-2005

- Genomic data on primary tumors from 1980 patients:
  - Copy Number, mRNA Expression (Curtis et al. *Nature* 2012)
  - miRNA Expression (Dvinge et al. *Nature* 2013)

- Long term clinical follow-up; median 14 years

- Relapse: date of first relapse for all patients (n=1079/1980), dates and sites of every relapse for 57% of patients (n=618)
The integrative subgroups have distinct ‘drivers’

Curtis et al. *Nature* 2012
Mapping Integrative Subtypes

Russnes et al. *Am J Path* 2017
The integrative subtypes have varied risk of relapse.
High risk of late distant relapse in four ER+/HER2-integrative subtypes

Probability of distant relapse/cancer death
Years after surgery

Model
clinical + IHC
clinical + IntClust

San Antonio Breast Cancer Symposium, December 4-8, 2018

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Distinct genomic drivers in high-risk ER+ subtypes

ER+ cases:
- 8% IC1 (17q23) with S6K1
- 5.5% IC6 (8p12) with FGFR1, ZNF703
- 8% IC9 (8q24) with MYC
- 4.5% IC2 (11q13/14) with FGFR3, CCND1, RSF1, EMSY
Differential risk of late distant relapse: IntClust relative to PAM50 subtypes

San Antonio Breast Cancer Symposium, December 4-8, 2018

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Integrative subtypes improve prediction of late distant relapse beyond clinical covariates

<table>
<thead>
<tr>
<th></th>
<th>METABRIC</th>
<th>VALIDATION COHORT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>10 years</td>
<td>15 years</td>
</tr>
<tr>
<td>C-index (clinical +IHC)</td>
<td>0.618</td>
<td>0.612</td>
</tr>
<tr>
<td>C-index (clinical + IntClust)</td>
<td>0.672</td>
<td>0.647</td>
</tr>
</tbody>
</table>

*Analysis performed on ER+/HER2- patients who were relapse free at 5 years
My Conclusions/take home

Genomic analyses can identify patients at high risk of late relapse, accounting for 26% of ER+ tumors

These provide an opportunity for identifying patients most at risk of late relapse

These are driven by S6K1 (IC1), FGFR1 (IC6), MYC (IC9) and FGF3, CCND1, RSF1, EMSY (IC12)
Breast cancer genomics summary

• Multidimensional spatial cell atlases of breast cancer are on the way... but the challenge is how to use these clinically.

• Metastatic breast cancer has more common mutations in potential drug targets such as HER2 and AKT1 compared with primary cancer...
  NGS in metastatic cancer or cfDNA (for bone only disease) is becoming clinically relevant

• Genomics may identify patients at high risk of rate relapse
  • prolonged endocrine therapy
  • New drug targets (e.g. FGFR1 in IC6)