GENOMIC TESTING IN BREAST CANCER

Hatem A. Azim Jr, MD, PhD
Related Disclosures

• **Consultancy**: Nanostring

• **Research**: Genomic grade
A genomic test aims to detect heritable or somatic variation related to a disease
Genomic vs. Genetic

Somatic = not inheritable
E.g. somatic mutations (e.g. EGFR mutation)

Germline = inheritable
E.g. BRCA mutation
Points of discussion

• Introduction to gene expression profiling and BC subtypes

• Gene signatures and their clinical utility
  – Differences between different available tests
  – Prognostic and predictive value

• Genomic sequencing and its clinical utility
Gene expression profiling

Analysis of the expression of thousands of genes (mRNA) at once.

Graphically represented in “heat map”.

Each sample is represented by column, each gene represented by a row.

High expression = red
Low expression = green

Samples with similar pattern of expression are “clustered” together.
Breast cancer is not one disease

Diversity of gene expression correlates to clinical meaningful outcome

Basal-like
HER-2+
Luminal A
Luminal B

Sorlie T et al. PNAS 2001
Sotiriou C et al. PNAS 2003
Different pattern of BC subtypes according to age

- **≤ 40y**
  - Basal: 27%
  - Luminal-A: 34%
  - Luminal-B: 17%
  - HER2: 22%

- **41 – 52y**
  - Basal: 24%
  - Luminal-A: 28%
  - Luminal-B: 31%
  - HER2: 17%

- **53 – 64y**
  - Basal: 29%
  - Luminal-A: 21%
  - Luminal-B: 35%
  - HER2: 15%

Chi-square: p<0.0001

Azim HA Jr et al; Clin Cancer Res 2012
IHC surrogates for BC subtypes

**HER2-Negative**
- **ER-Negative**
- **ER-Positive**
  - Good Differentiation Low Ki-67
  - Poor Differentiation High Ki-67

**HER2-Positive**
- ER-Positive or ER-Negative

(PgR –ve)

Basal-like (%)

Luminal A (%)

Luminal B (%)

HER2-like (%)
Proliferation differentiate luminal-A from luminal-B

High Proliferation (luminal B)

Low Proliferation (luminal A)

ER-/HER2-

HER2+

ER+/HER2-

Low risk

High risk

Courtesy of Christos Sotiriou
Assessment of Ki67 in Breast Cancer: Recommendations from the International Ki67 in Breast Cancer Working Group


Manuscript received March 14, 2011; revised September 1, 2011; accepted September 2, 2011.

Correspondence to: Mitch Dowsett, BSc, PhD. Department of Biochemistry, Royal Marsden Hospital and Breakthrough Breast Cancer Centre, Fulham Rd, London SW3 6JJ, UK (e-mail: mitch.dowsett@icr.ac.uk).

Uncontrolled proliferation is a hallmark of cancer. In breast cancer, immunohistochemical assessment of the proportion of cells staining for the nuclear antigen Ki67 has become the most widely used method for comparing proliferation between tumor samples. Potential uses include prognosis, prediction of relative responsiveness or resistance to chemotherapy or endocrine therapy, estimation of residual risk in patients on standard therapy and as a dynamic biomarker of treatment efficacy in samples taken before, during, and after neoadjuvant therapy, particularly neoadjuvant endocrine therapy. Increasingly, Ki67 is measured in these scenarios for clinical research, including as a primary efficacy endpoint for clinical trials, and sometimes for clinical management. At present, the enormous variation in analytical practice markedly limits the value of Ki87 in each of these con-
Ki-67 Reproducibility among 8 reference labs worldwide

- On using 13.5% as a cut-off to define “Ki-67 low” versus “Ki-67 high tumors”, 30% of tumors were classified differently between one lab and the other !!!!
Triple negative BC is a basket of diverse subtypes !!

**GO Terms/Canonical Pathways**

**Basal-like 1**
- Cell Cycle
- DNA Replication Reactome
- G0 Pathway
- RNA Polymerase
- ATM/BRCA Pathway
- G0 to G1 Cell Cycle

**Basal-like 2**
- EGF Pathway
- NGF Pathway
- MET Pathway
- WNT β-catenin Pathway
- IGF1R Pathway
- Glioblastoma Glutamine Metabolism

**Immuno-modulatory**
- CTLA4 Pathway
- IL12 Pathway
- NK Cell Pathway
- Th1/Th2 Pathway
- IL7 Pathway
- Antigen Processing Presentation
- NFkB Pathway
- TNF Pathway
- T Cell Signaling Pathway
- DC Pathway
- BCR Signaling Pathway
- NK Cell Mediated Cytotoxicity
- JAK1/STAT Signaling Pathway
- ATM/BRCA Pathway

**Mesenchymal-like**
- IGF1/IGF2R Pathway
- ECM Pathway
- Regulation of Actin by RHOA
- WNT Pathway
- ALK Pathway
- TGFβ Pathway

**Mesenchymal Stem-like**
- ECM Receptor Interaction
- TGFβ Pathway
- WNT β-catenin
- Focal Adhesion
- Inositol Phosphate Metabolism
- NFkB Pathway
- EGF Pathway
- ALK Pathway
- GPCR Pathway
- EKR1/2 Pathway
- Integrin Mediated Adhesion
- ABC Transporters General
- RHO Pathway
- Smooth Muscle Contraction
- Calcium Signaling Pathway
- Adipocytokine Signaling Pathway
- PDGF Pathway
- TGFβ Pathway

**Luminal AR**
- Pentose/Phosphonate Interconversion
- Glutathione Metabolism
- Folate Metabolism
- Steroid Biosynthesis
- Purine Metabolism
- Androgen and Estrogen Metabolism
- Glycosphingolipid Metabolism
- Flagellar Assembly
- Citrate Cycle TCA
- Phenylalanine Metabolism
- ATP Synthesis
- Starch and Sucrose Metabolism
- Arginine and Proline Metabolism
- Mitochondrial Respiratory Electron Transport Pathway
- Arginine and Proline Metabolism
- Fatty Acid Metabolism
- Alanine, Aspartate and Glutamate metabolism
- Glutathione metabolism
- Cysteine and Cystine metabolism
- Pantothenate and Coenzyme A biosynthesis
- Thiamine metabolism

**DNA repair**

**Immune**

**Stem Cell**

**Androgen**

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Lehmann B et al; J Clin Inv 2011
Points of discussion

• Introduction to BC subtypes and gene expression profiling

• Gene signatures and their clinical utility
  – Differences between different available tests
  – Prognostic and predictive value

• Genomic sequencing and its clinical utility
Gene expression signatures

Courtesy of C. Sotiriou
Genomic signatures are only informative in ER+ BC

Wirapati P et al; Breast Cancer Res 2008
They are highly comparable in terms of prognostic performance.

<table>
<thead>
<tr>
<th>Has it been retrospectively validated on prospective phase III trials?</th>
<th>Oncotype Dx</th>
<th>MammaPrint</th>
<th>PAM50 ROR</th>
<th>EndoPredict</th>
<th>Breast Cancer Index (BCI)</th>
<th>Genomic grade</th>
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<tbody>
<tr>
<td>B-20</td>
<td>x</td>
<td>ATAC</td>
<td>ABCSG6</td>
<td>ATAC</td>
<td>BIG 1–98</td>
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<tr>
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</table>

<table>
<thead>
<tr>
<th>Can it predict early recurrence (0–5 years)?</th>
<th>Can it predict late recurrence (after 5 years)?</th>
<th>Can it be tested on FFPE tissue?</th>
<th>Can the test be decentralized with established reproducibility data?</th>
<th>Is it subjected to a randomized prospective trial to demonstrate clinical utility?</th>
</tr>
</thead>
<tbody>
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Table 1. Different genomic tests that are currently available to refine prognosis of patients with ER-positive HER2-negative primary breast cancer.

+ Test subjected to prospective validation is on frozen tissue.
+ FFPE, formalin-fixed paraffin-embedded.
Assay Description:
The Prosigna™ breast cancer gene signature assay measures the expression of 50 different genes in tumor subtype and reports a Risk of Recurrence Score (ROR), which is used to assign patients to predefined risk groups. These reports are derived from a proprietary algorithm based on the PAM50 gene signature, intrinsic subtype, and clinical variables in a reference panel with nodal status.

Risk of Recurrence:
- Low risk
- Intermediate risk
- High risk

Subtype: luminal A

Probability of Distant Recurrence:
In the clinical validation studies, patients who were node-negative, luminal A subtype, with an ROR score of 25 were in the low-risk group. This group averaged a 4% probability of distant recurrence at 10 years.

The Prosigna algorithm has been validated by 2 randomized clinical trials including more than 2400 patients with varying rates of distant recurrence. An analysis of these 2 clinical validation studies shows that the probability of distant recurrence for the low-risk population is 4%, while the high-risk population has a significantly greater probability of distant recurrence.

Summary of Results: HIGH RISK LUMINAL-TYPE (B)

MammaPrint 70-Gene Risk of Recurrence
BluePrint 60-Gene Molecular Subtype

Patient's MammaPrint Result: HIGH RISK
Average 10-year Risk of Recurrence Unrelied: 23%
Patient's MammaPrint Index: MPI = 0.355

NanoString Technologies, Inc. 500 Fairview Avenue N | Suite 2000 | Seattle, Washington 98109 | 1 206 376 6296 | nanostring.com

© 2013 NanoString Technologies, Inc. For more information, visit PROSIGNA.com or e-mail biosupport@nanostring.com.
TAILORx: Study Design

Pre-REGISTER

Oncotype DX Assay

REGISTER
Specimen Banking

Secondary Study Group 1
RS < 11
~29% of population

ARM A
Hormonal therapy alone

~ 15%

Primary Study Group
RS 11-25
~44% of population

RANDOMIZE
Stratification factors:
tumor size, menopausal status, planned chemo, planned radiation

ARM B
Hormonal therapy alone

~ 70%

ARM C
Chemotherapy plus hormonal therapy

Secondary Study Group 2
RS > 25
~27% of population

ARM D
Chemotherapy plus hormonal therapy

~ 15%
Oncotype DX low risk (~15%)  
5-years of endocrine therapy (ET)

5 years of endocrine therapy is a very effective treatment for patients with ER+, node negative BC and low recurrence score (<11)

This constitutes around 15% of patients in the US
Oncotype DX intermediate risk (~70%)
Chemo + ET vs. ET

Chemotherapy does not add benefit in patients with ER+, node negative BC and intermediate recurrence score (11 - 26)

This constitutes around 70% of patients in the US

Freedom from BC recurrence @ 9 years
Chemo + ET = 95.0%
ET only = 94.5%
Potential use of Genomic tests to predict long-term recurrence

Can we get better outcomes!!?

30% are node-positive

Estimated distant recurrence-free survival (95% CI)

- **Low**:
  - 10-yr DRFS: 98.7 (96.9 - 99.5)
  - 15-yr DRFS: 97.6 (94.7 - 98.9)
- **Intermediate**:
  - 10-yr DRFS: 95.2 (92.3 - 97.0)
  - 15-yr DRFS: 90.9 (85.9 - 94.2)
- **High**:
  - 10-yr DRFS: 91.5 (87.8 - 94.1)
  - 15-yr DRFS: 82.5 (74.8 - 88.1)

Patients at risk:
- Low: 460, 447, 439, 412, 331, 250, 188, 125, 81, 50, 25
- High: 370, 347, 330, 301, 238, 198, 153, 119, 82, 43, 24

Gnant M; IMPAKT 203 & Filipits M et al; Clin Cancer Res 2014
Challenges in Treating Premenopausal Women with Endocrine-Sensitive Breast Cancer

Hatem A. Azim Jr., MD, PhD, Nancy E. Davidson, MD, and Kathryn J. Ruddy, MD, MPH

Studies needed to confirm this concept
Points of discussion

• Introduction to gene expression profiling and BC subtypes

• Gene signatures and their clinical utility
  – Differences between different available tests
  – Prognostic and predictive value

• Genomic sequencing and its clinical utility
The Central Dogma

DNA → Transcription: the synthesis of an RNA copy of a segment of DNA → RNA → Translation → Protein

Sequencing
Mutations

Gene expression profiling
“transcriptomics”
Gene signatures (e.g. mammaprint, PAM50)

Immunohistochemistry
ER, PR, HER2, Ki67
DNA Sequencing

• Process of determining the precise order of nucleotides

• Change in the normal sequence is called a mutation

• There are different types of mutations
Types of sequencing

- Whole genome sequencing

- Exome sequencing (2% of whole genome)

- Targeted gene sequencing
The landscape of cancer genes and mutational processes in breast cancer

Genomic aberrations in young and elderly breast cancer patients

Hatem A. Azim Jr, Bastien Nguyen, Sylvain Brohée, Gabriele Zoppoli and Christos Sotiriou

≤ 45 y

- PIK3CA: 28.8%
- GATA3: 15.2%
- TP53: 27.9%
- TNN: 13.5%
- Others, each <10%

46 – 69 y

- PIK3CA: 32.7%
- TP53: 33.4%
- CDH1: 13.1%
- TTN: 15.1%
- Others, each <10%

≥ 70 y

- PIK3CA: 41.9%
- TP53: 23.2%
- CDH1: 14.8%
- TTN: 29%
- Others, each <10%

Azim HA Jr et al; BMC Med 2015

Do not duplicate or distribute without permission of ESO and the author.
Approximately 90% of PIK3CA mutations occur in ER+ tumors.
A key intracellular signal transduction pathway that promotes proliferation, cell survival, growth and angiogenesis in response to extracellular signals.
PIK3CA mutation could inform on benefit of PIK3CA inhibitors in mBC

SOLAR-1 phase 3 trial

PIK3CA mutant

Median PFS, months:
- Alpelisib + fulvestrant (n=85) 11.1 (95% CI: 7.3–16.8)
- Placebo + fulvestrant (n=88) 3.7 (95% CI: 2.1–5.6)

PIK3CA wild

Median PFS, months:
- Alpelisib + fulvestrant (n=115) 7.4 (95% CI: 5.4–9.3)
- Placebo + fulvestrant (n=116) 5.6 (95% CI: 3.9–6.1)

André F et al; ESMO 2018
Role of PI3K targeting post CDK4/6 failure?
Tumor evolves over time
Primary ≠ metastases
8 Genes more frequently mutated in metastatic BC compared to early BC
ESR1 mutations rarely acquired during adjuvant AI, frequently during metastatic AI, associated with worse outcome

Schiavon G et al; Sci Transl Med 2015

Do not duplicate or distribute without permission of ESO and the author
8 Genes more frequently mutated in metastatic BC compared to early BC

N = 216 MBC, 771 early BC
Sequencing of circulating tumor DNA may be a better tool to capture tumor heterogeneity.
TAKE HOME MESSAGE

• Genomic signatures are only informative in ER+/HER2. They distinguish the luminal-A tumors (i.e. low risk, no need for chemo) from luminal-B (i.e. high risk, need for adjuvant chemo)

• Genomic signatures could refine the choice of patients who require adjuvant chemotherapy

• PIK3CA mutations are common in primary BC mostly in ER+BC and associated with good prognosis. Increasing data suggest possible role in predicting outcomes to novel targeted agents in ER+ MBC.

• ESR1 mutation is rather unique to ER+ MBC, and associated with resistance to Al. Novel approaches are worthy exploring in these patients.

• Circulating tumor DNA has the potential to render genomic sequencing easier, and faster. With advancement of discovery of novel agents, this could open the door to embracing genomic sequencing in routine practice in the few years to come.
Points of Discussion

• Pattern and relevance of somatic mutations in primary breast tumors

• Sequencing primary vs. metastatic tumors: is there a difference?

• Does sequencing improve treatment selection?
  – PI3K and mTOR inhibitors
  – CDK4/6 inhibitors
  – Managing expectations from genome sequencing in MBC
Feasibility of sequencing 70%

Matching for targeted therapy 13%!
Points of Discussion

• Pattern and relevance of somatic mutations in primary breast tumors

• Sequencing primary vs. metastatic tumors: is there a difference?

• Does sequencing improve treatment selection?

• Sequencing and disease heterogeneity
Pattern of somatic mutations could differ in the metastatic setting
**ESR1 mutations → Resistance to ET**

- 10-30% of metastatic BC that are resistant to AI
- Ligand-independent activation of the ER
- Potential RX application:
  - Selective drugs?
  - Higher doses of ET?
Potential of circulating tumor DNA
Identify driver mutations in real time
Monitor tumor progression “tumor markers”
Can ctDNA to replace tissue biopsy?

**SOLAR-1 phase 3 trial**

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<thead>
<tr>
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<th>ALP + FUL</th>
<th>PBO + FUL</th>
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<td>Media n PFS</td>
<td>Event n/N (%)</td>
</tr>
<tr>
<td>Patients with PIK3CA mutation: tissue</td>
<td>103/169 (60.9)</td>
<td>11.0</td>
</tr>
<tr>
<td>Patients with PIK3CA mutation: plasma</td>
<td>57/92 (62.0)</td>
<td>10.9</td>
</tr>
<tr>
<td>Patients without PIK3CA mutation: tissue</td>
<td>49/115 (42.6)</td>
<td>7.4</td>
</tr>
<tr>
<td>Patients without PIK3CA mutation: plasma</td>
<td>92/181 (50.8)</td>
<td>8.8</td>
</tr>
</tbody>
</table>

Juric D et al; SABCS 2018

Do not duplicate or distribute without permission of ESO and the author.
CONCLUSIONS
70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer


Relapse estimated (AOL) at 10y with no treatment (<12% ER+, <8% ER-)

Chemo + ET vs. ET alone

Do not duplicate or distribute without permission of ESO and the author
70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer

8.8% of patients with pN0 and G3 have 97.5% survival without distant metastasis.

23% of patients with high clinical risk, low genomic risk have 95.9% survival without distant metastasis.

Do not duplicate or distribute without permission of ESO and the author.
In advanced gBRCAmut disease platinums are more active than taxanes but taxanes still work.
All histological grade 2 treated with endocrine therapy alone (N=280)
How much breast cancer is inherited?

- Hereditary: 70-80%
- Sporadic: 15-20%
- Familial: 5-10%

85% BRCA 1/2

Slide adopted from Banu Arun
Ki67 Index, HER2 Status, and Prognosis of Patients With Luminal B Breast Cancer

Maggie C. U. Cheang, Stephen K. Chia, David Voduc, Dongxia Gao, Samuel Leung, Jacqueline Snider, Mark Watson, Sherri Davies, Philip S. Bernard, Joel S. Parker, Charles M. Perou, Matthew J. Ellis, Torsten O. Nielsen

ROC curve
Gene signature = gene set = Group of genes analyzed together

Improve prognostic - ation?

Improve treatment allocation?
High sensitivity

= Low probability of falsely classifying a patient as LOW RISK among those who die from breast cancer

Good performance for identifying the "bad tumors"

Courtesy of M. Piccart
Key questions

• Can we use gene signatures to inform on need for adjuvant chemo?

• Can we use gene signatures to inform on need for extended endocrine therapy?

• Do they provide equal benefit in pN0 vs pN+ disease?

• Are they all the same? Relevant differences?
SPECIFICITY (WITH 95% CI) OF CLINICO-PATHOLOGICAL RISK ASSESSMENTS FOR BC DEATH WITHIN 10 YEARS

<table>
<thead>
<tr>
<th></th>
<th>Adjuvant software</th>
<th>St-Gallen</th>
<th>Nottingham prognostic index</th>
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<tbody>
<tr>
<td>Low specificity</td>
<td>0.29</td>
<td>0.10</td>
<td>0.47</td>
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<tr>
<td></td>
<td>0.23</td>
<td>0.07</td>
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<td>0.35</td>
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<td>0.42</td>
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<td>0.48</td>
<td>0.29</td>
<td>0.53</td>
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</table>

Low specificity = High probability of falsely classifying a patient HIGH RISK among those who do not die from breast cancer.

Poor performance of identifying the "good" tumors.

Courtesy of M. Piccart
MINDACT Trial Design (n = 6,000)
Node negative & 1-3 positive nodes

55% Clinical-pathological and MammaPrint both HIGH risk
n = 3,300

32% Discordant cases
n = 1,920

Clin-Path HIGH MammaPrint LOW
Clin-Path LOW MammaPrint HIGH

13% Clinical-pathological and MammaPrint both LOW risk
n = 780

REGISTRATION

RANDOMIZE

Use Clin-Path risk to determine Chemo use

Use 70-gene risk to determine Chemo use

Chemotherapy
Endocrine therapy

All ER+ Patients Receive Endocrine Therapy
Potential to spare chemotherapy in 10-15% patients

Supported by the EU framework VI programme
Key questions

• Can we use gene signatures to inform on need for **adjuvant chemo**?

• Can we use gene signatures to inform on need for **extended endocrine therapy**?

• **Are they all the same?** Relevant differences?
Objective

• Understanding genomics from an oncologist perspective
  – Basic understanding of the tests / technology
  – Potential clinical relevance
Molecular Origins of Cancer

Gene-Expression Signatures in Breast Cancer

Christos Sotiriou, M.D., D.Phil., and Lajos Pusztai, M.D., D.Phil.
**All patients - Invasive DFS at 9 years**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Recurrence Score of ≤10</th>
<th>Recurrence Score of 11–25</th>
<th>Recurrence Score of ≥26</th>
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<tr>
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<td>Endocrine Therapy (N = 1618)</td>
<td>Endocrine Therapy (N = 3139)</td>
<td>Chemoendocrine Therapy (N = 1312)</td>
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<tr>
<td>Median age (range) — yr</td>
<td>56 (25–75)</td>
<td>55 (23–75)</td>
<td>56 (23–75)</td>
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<tr>
<td>Age ≤50 yr — no. (%)</td>
<td>429 (26)</td>
<td>1339 (44)</td>
<td>1077 (33)</td>
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<tr>
<td>Menopausal status — no. (%)†</td>
<td>478 (30)</td>
<td>1212 (36)</td>
<td>1203 (36)</td>
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<tr>
<td>Premenopausal</td>
<td>84%</td>
<td>83.3%</td>
<td>84.3%</td>
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Could ctDNA replace tissue biopsy?

**SOLAR-1 phase 3 trial**

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Juric D et al; SABCS 2018