Gene Signatures in Breast Cancer: Moving Beyond ER, PR, and HER2?

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When Are Biomarkers Ready To Use? Same Rules for Gene Expression Panels

Key elements of assay evaluation:

1. Analytical validity
   (reproducible and accurate?)

2. Clinical validity
   (differentiate cancers?)

3. Clinically useful
   (testing = better decisions)

Adapted from Simon R, JNCI 2009; Harris LN, JCO 2016
Clinical Questions in HR+ HER2

We want to know:

• Who needs chemotherapy
• Who needs extended adjuvant endocrine therapy (> 5 years)
• Who needs nothing
## What Are These Assays?

“top-down” = tailor from large unselected gene lists
“bottom-up” = tailor from known gene lists

<table>
<thead>
<tr>
<th>Assay</th>
<th>Provenance of RNA-based assays</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oncotype Dx®</strong> Recurrence Score</td>
<td>250 genes from literature tested for relapse in mixed population (dominated by NSABP B-20 HR+ N0 Rx tamoxifen) to derive 16 most relevant genes</td>
</tr>
<tr>
<td><strong>Prosigna® ROR-PT</strong></td>
<td>50 intrinsic subtype genes + proliferation genes + tumor size modeled for relapse (ROR-PT) in N0 untreated population</td>
</tr>
<tr>
<td><strong>Mammaprint®</strong></td>
<td>Top-down to 70 genes from case/control study of relapse within 5y (all N0, mostly HR+)</td>
</tr>
<tr>
<td><strong>EndoPredict®</strong></td>
<td>Top-down to 8 genes + T + N, predictive of distant mets from HR+ HER2- Rx tamoxifen.</td>
</tr>
<tr>
<td><strong>BCI®</strong></td>
<td>Top-down to 2-gene ratio (HOXB13:IL17BR), then bottom-up for Molecular Grade Index combined is predictive of distant mets</td>
</tr>
</tbody>
</table>

Mostly dominated by genes relevant for HR+ disease
Oncotype Dx Recurrence Score®

**Prognosis N0/N+**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Group</th>
<th>N</th>
<th>5-Year Recurrence Rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RS for 1 node</td>
<td>Low, 1 node</td>
<td>68</td>
<td>10.1% (95% CI: 7.6-12.7)</td>
</tr>
<tr>
<td></td>
<td>Inter, 1 node</td>
<td>32</td>
<td>11.0% (95% CI: 8.5-13.5)</td>
</tr>
<tr>
<td></td>
<td>High, 1 node</td>
<td>26</td>
<td>12.7% (95% CI: 10.2-15.2)</td>
</tr>
<tr>
<td>RS for 2-3 nodes</td>
<td>Low, 2-3 node</td>
<td>68</td>
<td>11.0% (95% CI: 8.5-13.5)</td>
</tr>
<tr>
<td></td>
<td>Inter, 2-3 node</td>
<td>37</td>
<td>12.0% (95% CI: 9.5-14.5)</td>
</tr>
<tr>
<td></td>
<td>High, 2-3 node</td>
<td>34</td>
<td>13.7% (95% CI: 11.2-16.2)</td>
</tr>
</tbody>
</table>

**Prediction chemotherapy benefit**

- **Year 5-10 after dx**
  - **Node-negative**
    - RS: 1.01 (ns)
    - ROR: 8.93 (p=0.003)
  - **Node-positive**
    - RS: 5.17 (p=0.02)
    - ROR: 8.37 (p=0.004)

Not as good at late relapse (>5y)

Multivariable HR of late distant relapse with ET only:

- **Year 5-10 after dx**
  - **Node-negative**
    - RS: 1.01 (ns)
    - ROR: 8.93 (p=0.003)
  - **Node-positive**
    - RS: 5.17 (p=0.02)
    - ROR: 8.37 (p=0.004)
More Recent Oncotype Data...

Very low RS strongly prognostic in N0
TailoRx Low RS cohort Rx ET only x 5y
> 99% free from distant mets
However...
~70% T1N0, < 10% high grade
– underlying good px regardless of RS

Prediction of late relapse may work in subset with high ESR1 expression
Late distant relapse if ESR1 > 9.1

Sparano J, NEJM 2015; Wolmark N, JCO 2016
Prosigna® (ROR-PT)

Prognostic for relapse in N0 HR+ Endocrine Rx only

Prognostic for distant mets in N+ with endocrine Rx only.
1 LN+ (A), 2-3 LN+ (B) HR+ ET only (ATAC+ABCSG8)

Prognostic for late distant mets with ET only (ATAC+ABCSG8)

Nielsen, CCR 2010; Gnant, Ann Oncol 2015; Sestak, JCO 2015
EndoPredict®

Prognostic for distant mets
Early relapse
Late relapse
N0/N+ treated with Endocrine Rx x 5y only (ABCSG6+8; ATAC)
Outperformed RS especially in years 5-10

Dubsky, Br J Ca 2013; Buus, JNCI 2016
Breast Cancer Index

Prognostic for relapse in mixed HR, N-/-+ heterogeneous Rx (MA.14)...

...and in ER+ N0 endocrine Rx only (ATAC)

May outperform RS in 10-year distant mets rates with ET only (ATAC)

Sestak, CCR 2016; Sgroi, Lancet Oncol 2013; Sestak, CCR 2016
Primary endpoint: Distant metastasis-free survival @ 5 y in discordant clinical (high) : genomic (low) risk without chemo of no less than 92%
### MINDACT

**Clinical: Genomic risk**

<table>
<thead>
<tr>
<th>Comparison</th>
<th>Low:Low</th>
<th>Low:High</th>
<th>High:Low</th>
<th>High:High</th>
</tr>
</thead>
<tbody>
<tr>
<td>2745 pts</td>
<td>2745</td>
<td>592</td>
<td>1550</td>
<td>1806</td>
</tr>
</tbody>
</table>

- **6693 pts** N-/-+, mixed receptors
- Randomized Chemo vs not

#### Randomized Chemo vs not

<table>
<thead>
<tr>
<th>Stage</th>
<th>Low:High</th>
<th>High:Low</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>98%</td>
<td>41%</td>
</tr>
<tr>
<td>gr 1/2</td>
<td>86%</td>
<td>71%</td>
</tr>
<tr>
<td>N0</td>
<td>97%</td>
<td>90%</td>
</tr>
<tr>
<td>HR+</td>
<td>90%</td>
<td>98%</td>
</tr>
<tr>
<td>HER2+</td>
<td>12%</td>
<td>8%</td>
</tr>
<tr>
<td>TNBC</td>
<td>9%</td>
<td>1%</td>
</tr>
</tbody>
</table>

**5y DMFS = 94.7% (92.5-96.2%) = success!**

**Caveats:**
- Entire population did well (H:H DMFS=91%)
- H:L population was 98% HR+ and mostly N0 (comfortable using genomics in these)
- Chemotherapy Δ 22% proportional, would this be meaningful in a higher risk population?

Cardoso, NEJM 2016
How and Which Assay to Use?

- They all work.
- Comparative studies to date:
  - Relapse on ET alone: BCI, Prosigna > RS
  - Late relapse: RS less prognostic of late relapse. Possible that stratifying by ESR1 expression will help?
  - Multiple assays identify < 10% risk of late relapse, but when is omitting ET ok?
- Chemotherapy benefit data = RS > Mammaprint, Prosigna (from nonrandomized studies).
What Does This Statement Mean?

“(assay name) provided significant additional information on distant relapse after endocrine therapy”

1. The assay is prognostic (or predictive of endocrine benefit)
2. The clinical variables are prognostic
3. Both

In all of the assay validation studies, the clinical variables remained highly prognostic.
New Applications?
Metastatic Disease

Metastatic HR+ HER2- disease categorized by Recurrence Score
• High – chemotherapy = endocrine therapy
• Low – endocrine therapy better

HR+ HER2- MBC treated with endocrine Rx...

Low Recurrence Score
High Recurrence Score

King T et al, CCR 2016
Hormone receptor-positive breast cancer: Challenge of too many good biomarkers

HER2-positive and triple negative disease do not have this problem.
Triple Negative Breast Cancer: Molecular Entities Within

Potentially targetable:
- DNA damage repair
- Growth factor pathways
  - AR pathways
- Immune tolerance....
In chemotherapy-treated TNBC, good prognosis:

- Less Luminal A-like
- More proliferative
TNBC Molecular Phenotypes by Molecular Profiling

4 clusters
“LAR”, luminal AR: AR, ER, prolactin, HER4 (no ERα)
“MES”, mesenchymal: overlaps claudin-low
“BLIS”, basal-like immunosuppressed
“BLIA”, basal-like immune activated (best prognosis!)

Overlap with PAM50
Some overlap with “TNBCtype”

All characterized by basal, immune, and stromal signatures

*omits Claudin-Low
Targeting AR in Triple Negative Disease

Background

- Phase II bicalutamide in 26 AR+ TNBC patients: CBR 19%, mPFS 12 weeks
- Enzalutamide > bicalutamide in metastatic prostate Ca

Endpoints:
1st CBR@16wks
2nd:
- CBR@24wks, PFS, OS etc
- AR biomarker

Enzalutamide 160 mg/d po

MDV3100-11

165 screened patients
89 AR ≥ 10%
75 evaluable
61% 1st / 2nd line

<table>
<thead>
<tr>
<th>Endpoint</th>
<th>CBR16</th>
<th>35% (24-46%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CBR24</td>
<td>29% (20-41%)</td>
</tr>
<tr>
<td></td>
<td>RR</td>
<td>8%</td>
</tr>
<tr>
<td></td>
<td>SAE</td>
<td>29%</td>
</tr>
</tbody>
</table>

How much is natural history, how much is drug?
What About Microenvironmental Signatures?

Tumor-infiltrating lymphocytes prognostic in TNBC and HER2+

CALGB 40603 Neoadjuvant TNBC

Stage II-III TNBC 2x2 Randomization

Pacilitaxel 80 mg/m² wkly x 12 ddAC x 4

Pacilitaxel 80 mg/m² wkly x 12 ddAC x 4

Pacilitaxel 80 mg/m² wkly x 12 ddAC x 4

Pacilitaxel 80 mg/m² wkly x 12 ddAC x 4

Both carboplatin and bevacizumab significantly increased pCR

Regardless of treatment arm or subtype, so did activated immune cells

<table>
<thead>
<tr>
<th>Signature</th>
<th>pCR Predictive Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All samples</td>
</tr>
<tr>
<td>B Cell cluster</td>
<td>0.0367</td>
</tr>
<tr>
<td>CD8 cluster</td>
<td>0.0099</td>
</tr>
<tr>
<td>T Cell cluster</td>
<td>0.0074</td>
</tr>
<tr>
<td>IGG Cluster</td>
<td>0.0013</td>
</tr>
<tr>
<td>Immune cell</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Sikov, JCO 2015; Sikov, AACR-SABCS 2014
HER2-Positive Disease and Intrinsic Subtype

Analysis TCGA + METABRIC: Clinical HER2 and molecular HER2 are overlapping but different.
HER2-Positive Disease and Intrinsic Subtype

N9831: Immune signature associated with RFS only in trastuzumab-treated arms

CALGB 40601: Randomized Phase 3 Trial (Dual and Single HER2-Targeting)
100% of ~300 patients underwent dedicated research biopsy.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dual vs single</td>
<td>1.38</td>
<td>0.003</td>
</tr>
<tr>
<td>Trastuzumab vs lapatinib</td>
<td>0.36</td>
<td></td>
</tr>
<tr>
<td>Intrinsic subtype</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG signature</td>
<td>2.96</td>
<td>0.002</td>
</tr>
<tr>
<td>11-gene proliferation signature</td>
<td>1.56</td>
<td>0.002</td>
</tr>
<tr>
<td>P53 mutation signature</td>
<td>3.32</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Perez, JCO 2015; Carey, JCO 2016
HER2-Positive Disease, Response, and Outcome

<table>
<thead>
<tr>
<th>Gene</th>
<th>OR</th>
<th>CI</th>
<th>p</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESR1</td>
<td>0.56</td>
<td>0.35 to 0.9</td>
<td>0.015</td>
<td>0.058</td>
</tr>
<tr>
<td>EPBB2</td>
<td>2.7</td>
<td>1.7 to 4.3</td>
<td>5.2 × 10⁻⁶</td>
<td>6.2 × 10⁻⁶</td>
</tr>
<tr>
<td>Her2 enriched (PAM50)</td>
<td>2.8</td>
<td>1.2 to 6.3</td>
<td>0.011</td>
<td>0.063</td>
</tr>
<tr>
<td>Immune1</td>
<td>1.4</td>
<td>1.1 to 1.9</td>
<td>0.041</td>
<td>0.081</td>
</tr>
<tr>
<td>Immune2</td>
<td>1.3</td>
<td>0.93 to 1.7</td>
<td>0.13</td>
<td>0.19</td>
</tr>
<tr>
<td>Immune3</td>
<td>1.4</td>
<td>1.1 to 1.9</td>
<td>0.025</td>
<td>0.06</td>
</tr>
<tr>
<td>GGI</td>
<td>1.5</td>
<td>1.1 to 2.1</td>
<td>0.018</td>
<td>0.054</td>
</tr>
<tr>
<td>aurka</td>
<td>1.3</td>
<td>0.94 to 1.8</td>
<td>0.11</td>
<td>0.19</td>
</tr>
<tr>
<td>AKTmTOR</td>
<td>1.2</td>
<td>0.91 to 1.7</td>
<td>0.17</td>
<td>0.23</td>
</tr>
<tr>
<td>Stroma1</td>
<td>0.95</td>
<td>0.7 to 1.3</td>
<td>0.74</td>
<td>0.74</td>
</tr>
<tr>
<td>Stroma2</td>
<td>1.1</td>
<td>0.81 to 1.5</td>
<td>0.55</td>
<td>0.56</td>
</tr>
<tr>
<td>AR</td>
<td>0.89</td>
<td>0.66 to 1.2</td>
<td>0.45</td>
<td>0.54</td>
</tr>
</tbody>
</table>

• Very similar to C40601

Signatures and EFS (?):
• pCR genes – HER2-E, HER2, immune
• ER and proliferation genes

Response and outcome in HER2 are driven by multiple tumor and microenvironmental factors, as well as treatment
HER2 Clinical:Molecular Discordance – An Opportunity?

EGF30008 Stage IV HR+ Letrozole + lapatinib

Prat, JAMA Onc 2016
Summary – What Do We Know About Signatures in Clinical Use?

- Clinical variables are consistently prognostic – do not ignore them!
- In HR+ HER2- breast cancer, multiple signature-based assays can help guide decision-making
- Unvalidated molecular assays should not override validated clinical assays
  - Would you recommend against endocrine therapy if the tumor is ER+ by IHC but ER-negative by mRNA?
- Triple negative breast cancer – no validated genomic assays.
  - Is help on the way with immune signatures?
- HER2+ disease is molecularly heterogeneous in ways that may let us tailor therapy