PROGNOSTIC GENE EXPRESSION TESTS FOR EARLY STAGE BREAST CANCER

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Conflict of Interest

• Dr. Bernard is an inventor of the PAM50 signature and a stakeholder in Bioclassifer LLC, a company that licensed the PAM50 know-how to Nanostring Inc for commercialization of Prosigna.
Learning Objectives

• Review differences between prognostic and predictive tests

• Review gene expression tests for prognosis in early stage breast cancer clinically available in 2014

• Review levels of evidence for using different tests based on clinical indications

• Review differences between research assays, Laboratory Developed Tests (LDT), and FDA-cleared tests
Prognostic vs Predictive

**Prognostic** biomarkers provide information about the probability of survival (relapse or overall outcome) when patients are given the standard of care for their stage of disease.

- In early stage breast cancer, prognostic factors are used to determine who will have long-term survival without chemotherapy.
  - Standard histopathologic staging: tumor size, node involvement, grade
  - Molecular: ER, PR, HER2, Ki67, and **gene expression tests**

**Predictive** biomarkers provide information about who will respond to a *particular* therapy (e.g. HER2+ breast cancer predicts response to Trastuzumab).
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<th>Main Challenge</th>
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<tr>
<td>Ia</td>
<td>Prospective clinical trial designed to test biomarker</td>
<td>Cost and time of running trial</td>
</tr>
<tr>
<td>Ib</td>
<td>Two or more clinical trials of similar design, well-annotated samples, long-term patient follow-up, retrospective sample collection, prospective statistical plan</td>
<td>Overcoming technical challenges of working with FFPE and identifying independent trials of similar design</td>
</tr>
<tr>
<td>II</td>
<td>Prospective trial(s), collected under clinical SOPs, and designed for therapeutic response</td>
<td>Trials powered for therapeutic response but not biomarker</td>
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<td>III</td>
<td>Clinical trials run using research assays or observational studies using tissues collected under generic tissue banking protocols</td>
<td>Accuracy of data input and lack of targeted population</td>
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*Adapted from Simon R.M. et al, JNCI, 2009*
Onctype Dx: 21-gene Test to Determine Risk of Recurrence in Tamoxifen-Treated, Node-Negative Breast Cancer (NSABP-B14)

**Figure 1. Panel of 21 Genes and the Recurrence-Score Algorithm.**

The recurrence score on a scale from 0 to 100 is derived from the reference-normalized expression measurements in four steps. First, expression for each gene is normalized relative to the expression of the five reference genes (ACTB [the gene encoding β-actin], GAPDH, GUS, RPLPO, and TFRG). Reference-normalized expression measurements range from 0 to 15, with a 1-unit increase reflecting approximately a doubling of RNA. Genes are grouped on the basis of function, correlated expression, or both. Second, the GRB7, ER, proliferation, and invasion group scores are calculated from individual gene expression measurements, as follows: GRB7 group score = 0.9 × GRB7 + 0.1 × HER2 (if the result is less than 8, then the GRB7 group score is considered 8); ER group score = (0.8 × ER + 1.2 × PGR + BCL2 + SCUBE2) + 4; proliferation group score = Survivin + Ki67 + MYBL2 + CCNB1 [the gene encoding cyclin B1] + STK15 + 5 (if the result is less than 6.5, then the proliferation group score is considered 6.5); and invasion group score = (CTSL2 [the gene encoding cathepsin L2] + MMP11 [the gene encoding stromelysin 3]) + 2. The unscaled recurrence score (RSU) is calculated with the use of coefficients that are predefined on the basis of regression analysis of gene expression and recurrence in the three training studies. RSU = 0.47 × GRB7 group score – 0.34 × ER group score + 1.04 × proliferation group score + 0.10 × invasion group score + 0.05 × CD68 – 0.08 × GSTM1 – 0.07 × BAG1. A plus sign indicates that increased expression is associated with an increased risk of recurrence, and a minus sign indicates that increased expression is associated with a decreased risk of recurrence. Fourth, the recurrence score (RS) is rescaled from the unscaled recurrence score, as follows: RS = 0 if RSU < 0; RS = 20 × (RSU – 6.7) if 0 < RSU < 100; and RS = 100 if RSU > 100.

Recurrences in Study:
28 Low Risk
25 Intermediate Risk
56 High risk

- Proliferation genes are incorporated in all prognostic tests for recurrence in early stage, ER+ breast cancer

Prognosis in ER+ Breast Cancer (NSABP-B20)

Fig 2. Kaplan-Meier plots for distant recurrence comparing treatment with tamoxifen (Tam) alone versus treatment with tamoxifen plus chemotherapy (Tam + chemo). (A) All patients; (B) low risk recurrence score (RS < 18); (C) intermediate risk (RS 18-30); (D) high risk (RS ≥ 31). The number of patients at risk and the number of distant recurrences (in parentheses) are provided below each part of the figure.

Gene expression profiling predicts clinical outcome of breast cancer
L. J. van 't Veer et al.

Sporadic breast tumours
patients <55 years
tumour size <5 cm
lymph node negative (LN0)
No chemotherapy given

5,000 genes differentially expressed across 78 tumors

supervised correlation analysis to identify genes that correlated with Bad versus Good prognosis
(230 genes)

Distant metastases <5 years (2.5)
Bad prognosis

No distant metastases >5 years (8.7)
Good Prognosis

Gene set optimized for determining which patients with early stage breast cancer do not require chemotherapy
**Gene Profile Test Result**

The breast cancer tissue sample submitted was analyzed by MammaPrint, an IVDMA 70 - Gene Profile of Breast Cancer for Metastatic Risk that has been validated to correlate with high or low outcome risk for distant metastases in patients with invasive breast cancer. In the reference group as published, “Low Risk” means that a lymph node negative breast cancer patient has a 10% chance (95% CI 4-15) that their cancer will recur within 10 years without any additional adjuvant treatment, either hormonal therapy or chemotherapy.

**Molecular Subtyping Test Result**

Luminal-type breast cancers are characterized by gene expression of luminal epithelial cells that line the breast ducts and glands. The Luminal-type cancers are typically hormone receptor positive tumors and therefore responsive to hormonal therapy. A Luminal-type molecular subtyping result means that the tumor phenotype most closely resembles the Luminal-type intrinsic subtype. Patients classified as MammaPrint® 70-gene signature “Low Risk” and Luminal-type can be expected to have a clinical course similar to luminal A, usually treated with hormonal therapy, whereas those with a MammaPrint “High Risk” and Luminal-type, a clinical course similar to luminal B patients who usually benefit from more aggressive treatment which may include chemotherapy.
Discovery of the PAM50: Standardized Gene Set for Identifying Intrinsic/Biologic Subtypes of Breast Cancer

1. Expression profiled 218 breast samples by full-genome microarrays (>25,000 genes) using RNA from fresh frozen tissues

2. Statistically identified 9 significant groups of invasive breast cancer and selected the common subtypes (LumA, LumB, HER2-enriched, Basal-like, and Normal-like) for training

3. Expression profiled the same tumors analyzed by microarray but using the corresponding FFPE blocks and RT-qPCR assays for 200 genes

4. Selected minimal gene set from RT-qPCR data that had the highest concordance to the microarray subtype assignment (i.e. PAM50)

Parker et al., J Clin Oncol. 2009 Mar 10;27(8):1160-7
Supervised Predictor of PAM50 Subtypes

New Patient

Dx: Luminal B
Developing a Risk Score Based on Correlation to Subtypes and Clinical Variables

Distance to each centroid as a genomic summary
**Prognostic Risk Classification Strategy: Risk Of Relapse (ROR)**

- Similarity to the subtypes are used as variables in the prognostic model where the outcome is **Risk of Relapse (ROR):**

  (Model 1) \( ROR-S = \beta_1 \cdot \text{Basal} + \beta_2 \cdot \text{HER2} + \beta_3 \cdot \text{LumA} + \beta_4 \cdot \text{LumB} \)

  (Model 2) \( ROR-C = \beta_1 \cdot \text{Basal} + \beta_2 \cdot \text{HER2} + \beta_3 \cdot \text{LumA} + \beta_4 \cdot \text{LumB} + \beta_5 \cdot \text{Tumor Size} \)

  (Model 3) \( ROR-X = \beta_1 \cdot \text{Basal} + \beta_2 \cdot \text{HER2} + \beta_3 \cdot \text{LumA} + \beta_4 \cdot \text{LumB} + \beta_5 \cdot \text{Size} + \beta_6 \cdot \text{Node} \)

- Weights for each term are learned from a training data set using a Cox model with Ridge Regression

- The weighted sum is assigned as the ROR score for a test case and a threshold may be applied for class assignment

Ridge regression with Cox model: Tibshirani, Statistics in Medicine 1997
Comparative study: Bovelstad et al. Bioinformatics 2007
Prognosis in no Adjuvant Systemic Therapy (no AST): PAM50 ROR

The c-index is the proportion of all pairs of subjects whose survival time can be ordered such that the subject with the higher predicted survival is the one who survived longer” (taken from Harrell, Regression Modeling Strategies, Springer Series in Statistics).

ROR Model in Tamoxifen Series from
University of British Columbia

Subtype predictions weighted for tumor size and proliferation identifies a patient subset that could forego chemotherapy!

Nielsen, et al, CCR, 2010 Nov 1;16(21):5222-32
Intrinsic Subtypes: Discovery and Research

- Perou et al., Nature, 2000
- Sorlie et al., PNAS, 2003
- Perreard et al., Breast Cancer Res, 2006
- Parker et al., JCO, 2009
- Nielsen et al., CCR, 2010
- Cheang et al., CCR, 2012
- Bastien et al., BMC Med Genomics, 2012
- Martin et al., Breast Cancer Res Treat, 2013
- Sweeney et al., CEBP, 2014
- Caan, et al, CEBP, 2014
- Kroenke et al, Breast Cancer Res Treat, 2014

- Microarray discovery
- Prognostic significance of subtypes

- Technical feasibility using RT-qPCR assays on FFPE tissues

- Discovery of PAM50 for subtyping and ROR score
- ROR score for prognosis in ER+ disease
- Prognosis in chemo treated patients
- Anthracycline benefit in HER2-E disease
- Correlation of subtypes with standard markers

- Association of subtype with race and age
- Prognosis of subtypes in population-based study
Transfer of RT-qPCR PAM50 Research Assay to nCounter Platform for FDA-clearance and Decentralization

6 Fluorescent spots are labeled RNA molecules complementary to ssDNA backbone

- ~50 fluorophores / spot generate very bright signal allowing for digital detection
- 4 colors, no consecutive spots of same color = 972 possible codes

Single molecule fluorescent barcodes, each attached to an individual nucleic acid
Prosigna Multi-site Analytical Validation

- Forty-three specimens shared across 3 sites
- All subtypes represented with large range of ROR scores
- Block processing, macrodissection, RNA extraction
- No samples changed from low-high risk

Nielsen TO, BMC Cancer, 2014
Generating a Prosigna Score

Determine intrinsic subtype through Pearson’s correlation to centroids

Calculate ROR (Prosigna Score)

\[
ROR = \frac{aR_{LumA} + bR_{LumB} + cR_{Her2e} + dR_{Basal}}{eP + fT}
\]

- Pearson’s correlation to centroids
- Proliferation score
- Tumor size
Prosigna Validation Studies

- **TransATAC Study**
  - ATAC study
    - Postmenopausal women with invasive, ER+, BC (N=9,366)
  - Tamoxifen alone (N=3,116)
  - Tam + Anastrozole (N=3,125)
  - Anastrozole alone (N=3,125)
  - TransATAC (Prosigna) (N=1,007 patients)

- **ABCSG-8 study**
  - ABCSG-8 study
    - Postmenopausal women with invasive, ER+, BC (N=3,714)
    - 2 yrs Tam
    - 3 yrs Tamoxifen (N=1,849)
    - 3 yrs Anastrozole (N=1,865)
    - ABCSG-8 (Prosigna) (N=1,478 patients)

Dowsett M, JCO, 2013
Filipits M, Clin Cancer Res, 2014
Oncotype Dx vs Prosigna: Head-to-Head Comparison in TransATAC

Node negative only; N=739

Dowsett M, JCO, 2013
Risk Stratification by Prosigna (ROR) Score in ABCSG-8: HR+/HER2-, N0 and N1, Adjuvant Endocrine Therapy Alone

10-year probability of distant recurrence of < 10% is considered **low risk**

10-year probability of distant recurrence of > 20% is considered **high risk**
Risk Interpretation by Nodal Status

10-Year Probability of Distant Recurrence

ROR: 0-100

- 4+ positive nodes
- 1-3 Positive nodes
- Node-negative
- 95% CI
Risk Stratification by Luminal Subtypes (A/B) in ABCSG-8: HR+/HER2-, N0 and N1, Adjuvant Endocrine Therapy Alone

Luminal A and luminal B have different prognoses

12-15% greater probability of distant relapse at 10 years if Luminal B compared to Luminal A

Adapted from Prosigna Package Insert, 2013.
FDA Clearance Allows Decentralization of Prosigna

- Medicine is practiced close to the patient
- Pathologists remain integral to the decision-making
- Local clinical lab remains the service provider
MA.17 and ATLAS trials demonstrate the benefit of extending endocrine therapy beyond 5 years... but only a small percentage of patients benefit!

Breast Cancer Index (BCI): Early Stage ER+ Breast Cancer

- BCI is a laboratory developed test using RT-qPCR for measuring 7 genes and housekeepers
- Developed by combining biomarkers from two complementary gene expression signatures:

  HoxB13/IL17BR (H/I)
  - Gene expression ratio
  - Estrogen signaling-related

  Molecular Grade Index (MGI)
  - 5 cell cycle genes
  - Assesses tumor proliferation

- BCI Test Report provides two key pieces of information:
  1) Risk of recurrence over 10 yrs from diagnosis
  2) Risk of recurrence after 5 yrs endocrine therapy
BCI Validation in MA.17:
Who Benefits from Extended Endocrine Therapy

MA.17 Study Data

- Nested case-control design of 83 recurrences matched to 166 nonrecurrences
- Patients with high BCI (H/I) had a 5yr absolute benefit of 16.5% from extended endocrine therapy with letrozole (p=0.007)
- Patients with low BCI (H/I) had no significant benefit from extended endocrine therapy with letrozole (p = 0.35)

Sgroi DC, JNCI, 2013
BCI Validation in TransATAC: Key Results

- BCI significantly predicted recurrence beyond clinical and pathologic factors for both early and late recurrence
  - At diagnosis: Low/Intermediate and High Risk
  - At 5 years and recurrence free: Low and Intermediate/High Risk
- In comparison with Oncotype Dx and IHC4, BCI was the only biomarker able to predict late recurrences

Sgroi DC, Lancer Oncol, 2013
Late Recurrence: Population Evaluated by Prosigna

**ATAC**
N=9366

Excluded:
- Combination arm
- Chemotherapy
- No blocks received
- Insufficient tumour material

**transATAC***
N=1125

**PAM50**
N=1007

Excluded:
- Insufficient residual RNA
- Failed PAM50 QC
- Not recurrence free at 5 years (N=145)

**ABCSDG-8**
N=3714

Excluded:
- No tissue specimen
- No consent

**Tissue database**
N=1620

**PAM50**
N=1478

Excluded:
- Insufficient residual RNA
- Failed PAM50 QC
- Not recurrence free at 5 years (N=203)

**Combined dataset**
N=2137

Sestak I., JNCI, 2013
## Patients Characteristics

<table>
<thead>
<tr>
<th></th>
<th>transATAC (N=862)</th>
<th>ABCSG-8 (N=1275)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median follow-up, years (IQR)</td>
<td>10.0 (9.1-10.1)</td>
<td>10.3 (8.8-12.4)</td>
</tr>
<tr>
<td>Age &gt; 65 years</td>
<td>41.5%</td>
<td>39.3%</td>
</tr>
<tr>
<td>Node positive</td>
<td>24.9%</td>
<td>26.8%</td>
</tr>
<tr>
<td>Tumour size, mm (mean, SD)</td>
<td>19.0 (10.1)</td>
<td>16.7 (8.3)</td>
</tr>
<tr>
<td>Grade</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Well</td>
<td>195 (22.6%)</td>
<td>242 (19.0%)</td>
</tr>
<tr>
<td>Moderate</td>
<td>519 (60.2%)</td>
<td>1033 (81.0%)</td>
</tr>
<tr>
<td>Poor</td>
<td>148 (17.2%)</td>
<td>-</td>
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<tr>
<td>Distant recurrence</td>
<td>80 (9.3%)</td>
<td>68 (5.3%)</td>
</tr>
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</table>
Risk groups – ROR score

<table>
<thead>
<tr>
<th>Risk Group</th>
<th>N</th>
<th>Percentage</th>
<th>HR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>1183</td>
<td>55.4%</td>
<td>-</td>
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<tr>
<td>Intermediate</td>
<td>538</td>
<td>25.2%</td>
<td>3.26 (2.07-5.13)</td>
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<tr>
<td>High</td>
<td>416</td>
<td>19.5%</td>
<td>6.90 (4.54-10.47)</td>
</tr>
</tbody>
</table>

Distant recurrence (%)

Follow-up time [years]

Sestak I., SABCS, 2013
Luminal A vs Luminal B

Luminal A (N=1530 (71.6%))
Luminal B (N=542 (25.4%))

HR (95% CI)  P-value
2.89 (2.07-4.02) <0.0001

Follow-up time [years]

Distant recurrence (%)
Luminal B (12.9%)  Luminal A (4.1%)
ROR (Prosigna) vs RS (Oncotype) for Late Recurrence in TransATAC

Multivariate Analysis

ROR provides additional prognostic information in multivariate analyses
## Gene Expression Tests for Recurrence in ER+ Breast Cancer Receiving Endocrine Therapy Alone

<table>
<thead>
<tr>
<th>Test Name</th>
<th>Number Classifiers</th>
<th>Platform</th>
<th>FDA-cleared</th>
<th>Decentralized Testing</th>
<th>Recommended for HER2+</th>
<th>Validated in N0 and N1</th>
<th>Utility in Late Recurrence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oncotype Dx</td>
<td>16 genes</td>
<td>qPCR</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>EndoPredict</td>
<td>8 genes</td>
<td>qPCR</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>Yes</td>
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<td>Breast Cancer Index</td>
<td>7 genes</td>
<td>qPCR</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
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<tr>
<td>Mammaprint</td>
<td>70 genes</td>
<td>Microarray</td>
<td>Yes</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
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<tr>
<td>Prosigna*</td>
<td>50 genes</td>
<td>nCounter</td>
<td>Yes</td>
<td>Yes</td>
<td>No (US only)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Used for prognosis in ER+ breast cancer in US under FDA regulations but all cancers outside the US
<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
<th>Examples in Clinical Breast Cancer Tests</th>
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MINDACT  
RxPONDER (node positive, 1-3 nodes) |
| Ib       | Two or more clinical trials of similar design, well-annotated samples, long-term patient follow-up, retrospective sample collection, prospective statistical plan | Prosigna (TransATAC, ABCSG-8)  
Oncotype (NSABP-B14, NSABP-B20)  
EndoPredict (ABCSG-6, ABCSG-8)  
BCI (MA.17, TransATAC) |
| II       | Prospective trial(s), collected under clinical SOPs, and designed for therapeutic response | Oncotype (NSABP20) - CMF vs CEF  
Oncotype (SWOG8814) - CAF |
| III      | Clinical trials run using research assays or observational studies using tissues collected under generic tissue banking protocols | Primarily research assays and training sets |

*Adapted from Simon R.M. et al, JNCI, 2009*