Updates in Molecular Classification of Breast Cancer

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Disclosure

• There are no conflicts of interest.
Objectives

- Describe standard of care testing in breast cancer.
- Discuss the most common molecular tests available for invasive breast cancer testing.
- Provide general overview on new emerging tests.
Background

• Several non-molecular tests are part of the standard of care of breast cancer.
  – Diagnosis
  – Classification
  – Prognosis and prediction of response to therapy.

• It is essential for the pathologist to have a working knowledge of molecular techniques used in routine practice.

• It is valuable to have some knowledge of emerging techniques.
Hormone Receptor Testing

- Estrogen receptor (ER) and progesterone receptor (PR) + tumors may be stimulated by circulating estrogen or progesterone.
  - Inhibiting the stimulatory effect is an important component of therapy.
Hormone Receptor Testing

• Predicts response to endocrine therapy including tamoxifen and aromatase inhibitors.

• National Comprehensive Cancer Network (NCCN) guidelines recommend that endocrine therapy be considered for any patient with ER/PR+ breast cancer.
  – Test all new and recurrent breast cancers.
ERBB2 (HER2) Testing

- Primary, recurrent, and metastatic breast cancers are tested for HER2 using a validated test.
  - Either by immunohistochemistry (IHC) or in situ hybridization (ISH).
Clinical Implications

• HER2 amplification was shown to be an independent risk factor for recurrence and death in studies conducted before the advent of targeted therapy.

• Trastuzumab
  – Humanized monoclonal antibody against HER2.
  – Improves progression-free survival and overall survival.

• Pertuzumab and Lapatinib.
Ki-67 Proliferation Index

• Investigated as a predictive/prognostic factor in various settings.

• Analytic validity has not been well established
  – Formal interlaboratory standardization.

• NCCN guidelines do not currently recommend routine clinical workup.
Hierarchical Clustering Using Intrinsic Gene Sets

- 115 tumor tissues
- 7 non-malignant tissues

Subclassification of Invasive Breast Cancer

<table>
<thead>
<tr>
<th></th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>ERBB2</th>
<th>Basal-like (Triple Neg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ER</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>PR</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>HER2</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Kaplan-Meier Analysis

A van't Veer data set

Probability

Time to distant metastasis (months)

B Norway/Stanford data set

Probability

Overall Survival (months)

× Censored, Luminal A, Luminal B, Basal, ERBB2+

p<0.01

# Triple Negative Subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Basal-like 1</th>
<th>Basal-like 2</th>
<th>Immunosubmodulatory</th>
<th>Mesenchymal</th>
<th>Mesenchymal Stem-like</th>
<th>Luminal Androgen Receptor</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Key Features</strong></td>
<td>High proliferation rate High pCR rate</td>
<td>High proliferation rate High pCR rate</td>
<td>Medullary carcinoma</td>
<td>Metaplastic carcinoma</td>
<td>Low proliferation rate Low claudin expression Stem cell enrichment</td>
<td>High AR and luminal CKs expression</td>
</tr>
<tr>
<td><strong>Aberrant Pathways</strong></td>
<td>Cell cycle DNA replication</td>
<td>EGF/NGF/MET/WNT/IGF1R</td>
<td>Immune signaling</td>
<td>EMT Pathway involved in cell motility</td>
<td>EMT Pathway involved in cell motility</td>
<td>AR pathway</td>
</tr>
<tr>
<td><strong>Potential Targeted Therapy</strong></td>
<td>Antimitotic agents: taxanes DNA-PK inhibitors TORC inhibitors</td>
<td>Antimitotic agents: taxanes Growth factor receptor inhibitors</td>
<td>PAPP inhibitors</td>
<td>PI3K/mTOR inhibitors</td>
<td>PI3K/mTOR inhibitors</td>
<td>Androgen antagonists</td>
</tr>
</tbody>
</table>

587 Triple negative Cases

Distribution of 767 TNBC samples
Multi-analyte Assays

• In vitro diagnostic test.

• Combines measurements of multiple genes/analytes to derive predictive or prognostic information.
  – Integrated using a specific, closed-form, often proprietary algorithm.

• Largely based on measuring mRNA levels for selected genes.
# Multi-analyte Assays

<table>
<thead>
<tr>
<th></th>
<th>MammaPrint</th>
<th>OncotypeDx</th>
<th>Prosigna</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analysis</strong></td>
<td>Microarray</td>
<td>qRT-PCR</td>
<td>qRT-PCR</td>
</tr>
<tr>
<td><strong>Provider</strong></td>
<td>Agendia (Amsterdam, Netherlands)</td>
<td>Genomic Health (Redwood City, CA)</td>
<td>Nanostring Technologies (Seattle, WA)</td>
</tr>
<tr>
<td><strong>Assay</strong></td>
<td>70-Gene signature</td>
<td>21-gene recurrence score</td>
<td>50-Gene signature</td>
</tr>
<tr>
<td><strong>Tissue Type</strong></td>
<td>Fresh, frozen, FFPE (10 USS, 5 um, 30% TC)</td>
<td>FFPE (15 USS, 5 um, highest grade &amp; cross-sectional area)</td>
<td>FFPE (variable USS, 10 um, 4 mm² (10% tumor cellularity))</td>
</tr>
<tr>
<td><strong>Clinical Indications</strong></td>
<td>All ages Stage I-II Node 0-3 ≤5 cm ER +/- HER2 +/-</td>
<td>All ages ER + Stage I-IIa HER2 -</td>
<td>Post Menopausal Stage I-III Node 0-3 ER +/-</td>
</tr>
<tr>
<td><strong>Prognostic/Predictive Value</strong></td>
<td>Prognostic for early distance recurrence w/in 5 years Predictive for chemoresponse in poor prognostic groups</td>
<td>Prognostic for distant recurrence in 10 years Predictive of chemoresponse in high RS group</td>
<td>Prognostic based on assigned intrinsic molecular subtypes Predictive for tamoxifen in luminal subtype</td>
</tr>
</tbody>
</table>
MammaPrint

- Cleared by the FDA in 2007.
- Initially limited by its requirement for fresh tissue.
  - Now validated and cleared for FFPE tissue.
- MammaPrint measures the expression of 70 genes using a microarray platform.

10 Year Recurrence w/o Chemotherapy

High Risk

Low Risk
Clinical Implications

- **RASTER trial**
  - 5 year prospective outcome based
  - 427 subjects
  - MammaPrint testing could accurately stratify breast cancer patients as low or high risk.

Molecular subtyping of early-stage breast cancer identifies a group of patients who do not benefit from neoadjuvant chemotherapy.
OncotypeDx

• qRT–PCR analysis of 21 genes.
• Initially validated for ER+, HER2-, node-invasive cancer.
  – Extended to node + disease (1–3 nodes).
• Marketed under the form of a laboratory-developed test.
OncotypeDx

**Proliferation**
- Ki-67
- STK15
- Survivin
- Cyclin B1
- MYBL2

**HER2**
- GRB7
- HER2
- GSTM1

**Estrogen**
- ER
- PGR
- Bcl2
- SCUBE2

**Invasion**
- Stromelysin 3
- Cathepsin L2
- CD68
- BAG1

**Reference**
- Beta-actin
- GAPDH
- RPLPO
- GUS
- TFRC

**Formula**

\[ RS_U = + 0.47 \times \text{HER2 Group Thresholded Score} - 0.34 \times \text{ER Group Score} + 1.04 \times \text{Proliferation Group Thresholded Score} + 0.10 \times \text{Invasion Group Score} + 0.05 \times \text{CD68} - 0.08 \times \text{GSTM1} - 0.07 \times \text{BAG1} \]

<table>
<thead>
<tr>
<th>Score</th>
<th>Formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>HER2 Group Score</td>
<td>((0.9 \times \text{GRB7}) + (0.1 \times \text{HER2}))</td>
</tr>
<tr>
<td></td>
<td>If HER2 Group Score is less than 8 then the HER2 Group Score is considered equal to 8</td>
</tr>
<tr>
<td>ER Group Score</td>
<td>(\left(0.8 \times \text{ER} + [1.2 \times \text{PGR}] + \text{Bcl2} + \text{SCUBE2}\right)/4)</td>
</tr>
<tr>
<td>Proliferation Group Score</td>
<td>((\text{SURV} + \text{KI-67} + \text{MYBL2} + \text{Cyclin B1} + \text{STK15})/5)</td>
</tr>
<tr>
<td></td>
<td>If the Proliferation Group Score is under 6.5 then the Proliferation Group Score is considered equal to 6.5</td>
</tr>
<tr>
<td>Invasion Group Score</td>
<td>((\text{Cathepsin L2} + \text{Stromelysin 3})/2)</td>
</tr>
</tbody>
</table>
NSABP B-14 Study

![Graph showing rate of distant recurrence at 10 years for low, intermediate, and high risk groups.]

- **Low Risk**: Recurrence score result <18, rate of distant recurrence at 10 years: 6.8% (95% CI: 4.0%-9.6%).
- **Intermediate Risk**: Recurrence score result 18-30, rate of distant recurrence at 10 years: 14.3% (95% CI: 8.3%-20.3%).
- **High Risk**: Recurrence score result ≥31, rate of distant recurrence at 10 years: 30.5% (95% CI: 23.6%-37.4%).

**Low recurrence score result** = **Lower rate** of distant recurrence

**High recurrence score result** = **Higher rate** of distant recurrence

Genomic Health
LIFE, CHANGING.

Moffitt Cancer Center
NSBP B-20 Study

**Low Recurrence Score Result (<18)**

- Little to no chemotherapy benefit
- 97% vs. 96%

**Intermediate Recurrence Score Result (18-30)**

- No substantial chemotherapy benefit
- 91% vs. 89%

**High Recurrence Score Result (≥31)**

- Large chemotherapy benefit
- 88% vs. 60%

- Absolute benefit from TAM + chemotherapy

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**Genomic Health**

*LIFE, CHANGING.*
OncotypeDx DCIS

- Predicts recurrence in patients who have had a local excision for DCIS.
- Low-, intermediate- and high-risk groups to facilitate clinical decision making.
Multi-analyte Assays

153,782/476,128 (30.2%) Mammaprint or OncotypeDx were performed.

↑ assay use over time (24.6%) ➔ ↓ rate of chemotherapy administration (37.2%).

Adjuvant chemotherapy administration:
- Mammaprint (41.3%)
- OncotypeDx (23.4%).
Prosigna

- Clinical implementation of the intrinsic subtype concept.
- Based on the expression of a smaller set of genes.
  - Prediction Analysis of Microarray-50 (PAM50) classifier.
- Recurrence score provides prognostic information and predicts the effectiveness of chemotherapy.
Prosigna

Extract RNA from FFPE tumor sample

Run RNA and Prosigna CodeSet on nCounter Analysis System

Patient specific expression profile

Prosigna

- Estimates risk of distant recurrence from 5 to 10 years after diagnosis and hormonal therapy.

**DRFS 10 year**
- >95%  
- 90.4%  
- <85%

**Node-negative**
- Estimated 10 year risk of distant recurrence

**Node-positive (1-3 nodes)**
- Estimated 10 year risk of distant recurrence

Moffitt Cancer Center
Germline Testing

• Fraction of breast cancer cases are caused by inherited mutations.
• Predisposition is typically inherited in an autosomal dominant manner.
• Genes associated with hereditary breast cancer are predominantly classical tumor suppressors.
  – Mutations at numerous amino acid positions.
Germline Testing

- Hereditary breast-ovarian cancer syndrome is relatively common.
  - ~2% Ashkenazi Jews carriers of a BRCA1/BRCA2 mutation
  - ~0.3% US population.
Germline Testing

- Test most likely genes
  - Comprehensive sequencing of entire coding region

Negative Results

Reflex for less likely genes
Next Generation Sequencing

- NGS panel sequencing approach:
  - Cover a large # of genes.
  - < procurements of tissue from the paraffin block.
  - Can be both cost- and time-effective.

- Potential to detect all 4 canonic classes of genetic variation:
  - Single-nucleotide variants
  - Insertions/deletions
  - Copy-number variants
  - Structural variants (rearrangements, translocations).
Next Generation Sequencing

• Sequencing of 46 genes in 415 breast cancer samples revealed somatic nonsynonymous mutations in 220/354 (62.1%) cases.
  – 13/61 pairs (21%) demonstrated additional mutations in the metastasis.
• Systematic multinational study of NGS profiling in breast cancer is underway.
Next Generation Sequencing

• NGS has a limited role in the management of ER/PR+ and/or HER2+ cancers.
• NGS can be used as a means for matching tumors with novel therapies and/or clinical trials.
  – Triple-negative tumors.
Foundation One

- Comprehensive genetic profile for solid tumors.
- Hybrid capture, NGS.

- Reports:
  - Clinically significant alterations
  - Potential targeted therapies
  - Available clinical trials
  - Markers of response to immunotherapy.
Circulating Tumor Cells

- Strong, independent predictor of overall and progression-free survival in metastatic breast cancer.
- Monitor a patient’s status:
  - Prognosis on therapy at any given time.
Circulating Tumor Cells

**Metastatic Breast Cancer**—A Reduction in CTC to Below 5 After the Initiation of Therapy Predicts Longer OS whereas an Increase in CTC Count to 5 or above Predicts Shorter OS in mBC Patients.

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
<th>N (%)</th>
<th>Months (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt;5 CTCs at All Time Points</td>
<td>63 (47%)</td>
<td>22.6 (19.4 to 25.8)</td>
</tr>
<tr>
<td>2</td>
<td>25 at Baseline &amp; &lt;5 CTCs at Last Draw</td>
<td>26 (21%)</td>
<td>19.9 (14.5 to 25.6)</td>
</tr>
<tr>
<td>3</td>
<td>≥5 at Early Draw &amp; ≥5 CTCs at Last Draw</td>
<td>17 (10%)</td>
<td>19.8 (14.2 to 25.2)</td>
</tr>
<tr>
<td>4</td>
<td>≥5 CTCs at All Time Points</td>
<td>39 (22%)</td>
<td>4.1 (2.9 to 5.4)</td>
</tr>
</tbody>
</table>

**Determination of CTC Cutoff Using Median progression-free survival (PFS) in mBC**

- Cox Hazard Ratio
- Median PFS for Patients with < CTC# at 1st Follow-Up Blood Draw
- Median PFS for Patients with ≥ CTC# at 1st Follow Blood Draw

**Table:**

<table>
<thead>
<tr>
<th></th>
<th>mBC</th>
<th>mPC</th>
<th>mCRC</th>
</tr>
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<tbody>
<tr>
<td><strong>Favorable</strong></td>
<td>0-4 CTCs</td>
<td>0-4 CTCs</td>
<td>0-2 CTCs</td>
</tr>
<tr>
<td><strong>Unfavorable</strong></td>
<td>≥5 CTCs</td>
<td>≥5 CTCs</td>
<td>≥3 CTCs</td>
</tr>
</tbody>
</table>
Circulating Tumor DNA

- Detected ctDNA PFS = 4.1 months.
- Non-detected ctDNA PFS = 11.2 months.
- May allow treatment to be adapted prior to progression.

Predicting sensitivity to palbociclib with early circulating tumor DNA dynamics in the PALOMA-3 trial.

Ben O’Leary, Sarah Hrebien, James P Morden, Matthew Beane, Yuan Liu, Cynthia Huang Bartlett,
Summary

• Ancillary workup of invasive breast cancer includes non-molecular testing and the assessment of nucleic acids.
  – ER, PR & HER2
  – Predictive testing via MAAA
  – Germ line testing
  – Next-generation sequencing.

• Optimal breast cancer care requires pathologists and clinicians maintain a working knowledge of all of these rapidly evolving techniques.
References

10. https://www.cellsearchctc.com/clinical-applications/interpretation-of-results#