Molecular Testing in the Management of Patients with Breast Cancer
Current Status and Future Directions

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Boston, MA
Molecular Testing in the Management of Patients with Breast Cancer

- Many tumor types in which mutation testing is now required at initial diagnosis for therapeutic decision making (e.g., lung, colon, melanoma, glioma)
- Not the case for breast cancer
Molecular Testing in the Management of Patients with Breast Cancer

- Targeted therapy used in patients with breast cancer for decades
  - Hormone receptors (ER/PR): 1970s
  - HER2: 1998
Molecular Testing in the Management of Patients with Breast Cancer

Hormone receptor (ER/PR) and HER2 status remain the major drivers of clinical decision making, even in 2016.
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**NCCN Breast Cancer Panel Members**  
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Ductal Carcinoma in Situ (DCIS) Workup and Primary Treatment (DCIS-1)  
DCIS Postsurgical Treatment and Surveillance/Follow-up (DCIS-2)  
Margin Status in DCIS (DCIS-A)

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**Clinical Trials:** NCCN believes that the best management for any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.  
To find clinical trials online at NCCN Member Institutions, click here: nccn.org/clinical_trials/physician.html.

**NCCN Categories of Evidence and Consensus:** All recommendations are category 2A unless otherwise specified.  
See NCCN Categories of Evidence and Consensus.

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Molecular Testing in the Management of Patients with Breast Cancer


JCO, 2010


Antonio C. Wolff,* M. Elizabeth H. Hammond,* David G. Hicks,* Mitch Dowsett,* Lisa M. McShane,* Kimberly H. Allison, Donald C. Allred, John M.S. Bartlett, Michael Bilous, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Pamela B. Mangu, Soonmyung Paik, Edith A. Perez, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, and Daniel F. Hayes*

JCO, 2013

ER/PR and HER2 Assays According to ASCO/CAP Guidelines
Molecular Testing of Breast Cancers
Beyond Hormone Receptor and HER2 Status

• Intrinsic (molecular) subtypes
• Multigene prognostic tests
• Genomic analysis
Molecular Testing of Breast Cancers
Beyond Hormone Receptor and HER2 Status

• Intrinsic (molecular) subtypes
• Multigene prognostic tests
• Genomic analysis
Breast Cancer Subtypes Determined by Gene Expression Profiling

Sorlie, 2001
Breast Cancer

Estrogen Receptor Positive
- Luminal A (~70%)

Estrogen Receptor Negative
- Luminal B (~15%)
- HER2-E (~15%)
- Basal-like (~15%)

Other: Normal breast-like, claudin low, molecular apocrine
Prognostic Significance of Molecular Subtypes

Sorlie, 2003
Supervised Risk Predictor of Breast Cancer Based on Intrinsic Subtypes

Joel S. Parker, Michael Mullins, Maggie C.U. Cheang, Samuel Leung, David Voduc, Tammi Vickery, Sherri Davies, Christiane Fauron, Xiaping He, Zhiyuan Hu, John F. Quackenbush, Inge J. Stijleman, Juan Palazzo, J.S. Marron, Andrew B. Nobel, Elaine Mardis, Torsten O. Nielsen, Matthew J. Ellis, Charles M. Perou, and Philip S. Bernard

J Clin Oncol 2009

--RT-PCR-based PAM50 assay

--Prognostic value independent of:
  • Nodal status
  • Size
  • Grade
  • ER status
There is heterogeneity within the molecular subtypes:

EVEN THE SUBTYPES HAVE SUBTYPES
Comprehensive molecular portraits of human breast tumours

The Cancer Genome Atlas Network*

Comprehensive molecular portraits of human breast tumours

The Cancer Genome Atlas Network*

*Only 3 genes mutated in >10% of tumors

<table>
<thead>
<tr>
<th>Subtype</th>
<th>PIK3CA</th>
<th>TP53</th>
<th>MAP3K1</th>
<th>MAP2K4</th>
<th>MLL3</th>
<th>CDH1</th>
<th>PTEN</th>
<th>PIK3R1</th>
<th>AKT1</th>
<th>RUNX1</th>
<th>CBB</th>
<th>TBX3</th>
<th>NCOR1</th>
<th>CTCF</th>
<th>FOXA1</th>
<th>SF3B1</th>
<th>CDK4</th>
<th>RB1</th>
<th>AFF2</th>
<th>N1</th>
<th>PTPN2</th>
<th>PTPRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>45%</td>
<td>12%</td>
<td>13%</td>
<td>7%</td>
<td>14%</td>
<td>8%</td>
<td>9%</td>
<td>4%</td>
<td>0.4%</td>
<td>4%</td>
<td>5%</td>
<td>2%</td>
<td>3%</td>
<td>5%</td>
<td>4%</td>
<td>2%</td>
<td>3%</td>
<td>1%</td>
<td>0.4%</td>
<td>1%</td>
<td>2%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Luminal B</td>
<td>29%</td>
<td>29%</td>
<td>5%</td>
<td>2%</td>
<td>5%</td>
<td>6%</td>
<td>5%</td>
<td>4%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>4%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>0%</td>
<td>1%</td>
<td>3%</td>
<td>2%</td>
<td>4%</td>
</tr>
<tr>
<td>HER2-enriched</td>
<td>39%</td>
<td>72%</td>
<td>4%</td>
<td>2%</td>
<td>2%</td>
<td>7%</td>
<td>5%</td>
<td>2%</td>
<td>4%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>4%</td>
<td>2%</td>
<td>2%</td>
<td>2%</td>
<td>5%</td>
<td>0%</td>
<td>5%</td>
<td>0%</td>
<td>5%</td>
</tr>
<tr>
<td>Basal-like</td>
<td>9%</td>
<td>80%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>2%</td>
<td>5%</td>
<td>0%</td>
<td>1%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>0%</td>
<td>1%</td>
<td>0%</td>
<td>1%</td>
<td>0%</td>
<td>4%</td>
<td>4%</td>
<td>2%</td>
<td>0%</td>
</tr>
</tbody>
</table>

Percentages of cases with mutation by expression subtype

Only 3 genes mutated in >10% of tumors

- **p53** 37%
- **PIK3CA** 36%
- **GATA3** 11%
Molecular Subtypes Largely Defined by ER, HER2 and Proliferation Genes
Classifier using 3 genes (ER, HER2, Aurora Kinase A) compared with five other multi-gene classifiers (50-726 genes) on 36 publicly available datasets to determine molecular subtypes

3-gene classifier most robust for molecular subtyping
# Immunophenotyping to Approximate Molecular Subtype Using Three Markers

Brenton, 2005

<table>
<thead>
<tr>
<th></th>
<th>Luminal A</th>
<th>Luminal B</th>
<th>HER2-E</th>
<th>Basal-like</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ER</strong></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>PR</strong></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>HER2</strong></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Limitations to Using IHC for Receptors as Surrogates for Molecular Subtype

Prat, 2011

Luminal A
2% 5% 7%

87%

Luminal B
1% 7% 20%

72%

Legend:
- ER+/HER2+
- ER+/HER2-
- ER-/HER2+
- ER-/HER2-
# Defining Breast Cancer Intrinsic Subtypes by Quantitative Receptor Expression


<table>
<thead>
<tr>
<th>Subtype</th>
<th>HER2− (n = 976)</th>
<th>HER2+ (n = 127)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal-like</td>
<td>8 (0.8)</td>
<td>0</td>
</tr>
<tr>
<td>HER2-E</td>
<td>102 (10.5)</td>
<td>69 (54.3)</td>
</tr>
<tr>
<td>Luminal A</td>
<td>477 (48.9)</td>
<td>16 (12.6)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>346 (35.5)</td>
<td>39 (30.7)</td>
</tr>
<tr>
<td>Normal-like</td>
<td>43 (4.4)</td>
<td>3 (2.4)</td>
</tr>
</tbody>
</table>

**84.4%**
### Defining Breast Cancer Intrinsic Subtypes by Quantitative Receptor Expression

<table>
<thead>
<tr>
<th>Subtype</th>
<th>ER/PR+ (≥10%)</th>
<th>ER/PR borderline (1%–9%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HER2− (n = 976)</td>
<td>HER2+ (n = 127)</td>
</tr>
<tr>
<td>Basal-like</td>
<td>8 (0.8)</td>
<td>0</td>
</tr>
<tr>
<td>HER2-E</td>
<td>102 (10.5)</td>
<td>69 (54.3)</td>
</tr>
<tr>
<td><strong>Luminal A</strong></td>
<td><strong>477 (48.9)</strong></td>
<td><strong>16 (12.6)</strong></td>
</tr>
<tr>
<td><strong>Luminal B</strong></td>
<td><strong>346 (35.5)</strong></td>
<td><strong>39 (30.7)</strong></td>
</tr>
<tr>
<td>Normal-like</td>
<td>43 (4.4)</td>
<td>3 (2.4)</td>
</tr>
</tbody>
</table>

**84.4%** for Luminal A, **43.5%** for Luminal B.
### Molecular subtype by PAM50

<table>
<thead>
<tr>
<th>ER Expression</th>
<th>#</th>
<th>% Lum A</th>
<th>% Lum B</th>
<th>% HER2-E</th>
<th>% Basal</th>
<th>% Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>183</td>
<td>1</td>
<td>0.5</td>
<td>28</td>
<td>61</td>
<td>10</td>
</tr>
<tr>
<td>1-9%</td>
<td>25</td>
<td>0</td>
<td>8</td>
<td>32</td>
<td>48</td>
<td>12</td>
</tr>
<tr>
<td>10%</td>
<td>6</td>
<td>33</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>&gt;10%</td>
<td>251</td>
<td>48</td>
<td>24</td>
<td>15</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
Limitations to Using IHC for Receptors as Surrogates for Molecular Subtype
Prat, 2011
Limitations to Using IHC for Receptors as Surrogates for Molecular Subtype

Prat, 2014
Limitations to Using IHC for Receptors as Surrogates for Molecular Subtype
Prat, 2011
# Defining Breast Cancer Intrinsic Subtypes by Quantitative Receptor Expression


<table>
<thead>
<tr>
<th>Subtype</th>
<th>HER2− (n = 283)</th>
<th>HER2+ (n = 106)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal-like</td>
<td>207 (73.1)</td>
<td>6 (5.7)</td>
</tr>
<tr>
<td>HER2-E</td>
<td>48 (17)</td>
<td>87 (82.1)</td>
</tr>
<tr>
<td>Luminal A</td>
<td>6 (2.1)</td>
<td>3 (2.8)</td>
</tr>
<tr>
<td>Luminal B</td>
<td>15 (5.3)</td>
<td>8 (7.5)</td>
</tr>
<tr>
<td>Normal-like</td>
<td>7 (2.5)</td>
<td>2 (1.9)</td>
</tr>
</tbody>
</table>

The Oncologist 2015
BASAL-LIKE

~70-80%

TRIPLE NEGATIVE
Four stable subtypes defined by RNA and DNA profiling

– Luminal AR
– Mesenchymal
– Basal-like immune-suppressed
– Basal-like immune-activated
Four stable subtypes defined by RNA and DNA profiling

- Luminal AR
- Mesenchymal
- Basal-like immune-suppressed
- Basal-like immune-activated
### Comprehensive Genomic Analysis Identifies Novel Subtypes and Targets of Triple-Negative Breast Cancer

Matthew D. Burstein¹, Anna Tsimelzon², Graham M. Poage³, Kyle R. Covington², Alejandro Contreras², Suzan A.W. Fuqua⁶, Michelle I. Savage³, C. Kent Osborne², Susan G. Hilsenbeck², Jenny C. Chang⁵, Gordon B. Mills⁶, Ching C. Lau⁷, and Powel H. Brown³

<table>
<thead>
<tr>
<th>TNBC Subtype</th>
<th>Potential Therapeutic Targets</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal AR</td>
<td>AR; MUC1</td>
</tr>
<tr>
<td>Mesenchymal</td>
<td>Growth factor receptors (PDGFR, c-Kit)</td>
</tr>
<tr>
<td>BL-Immune suppressed</td>
<td>PD1, VTCN1 (immune suppressing molecule)</td>
</tr>
<tr>
<td>BL-Immune activated</td>
<td>STAT signal transduction molecules and cytokines</td>
</tr>
</tbody>
</table>
Do the intrinsic subtypes have value in current clinical practice?
Table 3. Systemic treatment recommendations for subtypes

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Type of therapy</th>
<th>Notes on therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>Endocrine therapy alone</td>
<td>Few require cytotoxics (e.g. high nodal status or other indicator of risk; see text).</td>
</tr>
<tr>
<td>Luminal B (HER2 negative)</td>
<td>Endocrine ± cytotoxic therapy</td>
<td>Inclusion and type of cytotoxics may depend on level of endocrine receptor expression, perceived risk and patient preference.</td>
</tr>
<tr>
<td>Luminal B (HER2 positive)</td>
<td>Cytotoxics + anti-HER2 + endocrine therapy</td>
<td>No data are available to support the omission of cytotoxics in this group.</td>
</tr>
<tr>
<td>HER2 positive (non luminal)</td>
<td>Cytotoxics + anti-HER2</td>
<td></td>
</tr>
<tr>
<td>Triple negative (ductal)</td>
<td>Cytotoxics</td>
<td></td>
</tr>
<tr>
<td>Special histological types*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. Endocrine responsive</td>
<td>Endocrine therapy</td>
<td></td>
</tr>
<tr>
<td>B. Endocrine nonresponsive</td>
<td>Cytotoxics</td>
<td>Medullary and adenoid cystic carcinomas may not require any adjuvant cytotoxics (if node negative).</td>
</tr>
</tbody>
</table>

*Special histological types: Endocrine responsive (cribriform, tubular, and mucinous); Endocrine nonresponsive (apocrine, medullary, adenoid cystic and metaplastic).
• “...in clinical practice the key question is not the separation of the molecularly-defined intrinsic subtypes, but the discrimination between patients who will or will not benefit from particular therapies.”
### Clinically Useful Groups

<table>
<thead>
<tr>
<th>ER/PR</th>
<th>HER2</th>
<th>Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>positive</td>
<td>negative</td>
<td>Endocrine +/--chemo (depending on level of risk)</td>
</tr>
<tr>
<td>positive</td>
<td>positive</td>
<td>HER-2 targeted, chemo, endocrine</td>
</tr>
<tr>
<td>negative</td>
<td>positive</td>
<td>HER2-targeted, chemo</td>
</tr>
<tr>
<td>negative</td>
<td>negative</td>
<td>Chemo</td>
</tr>
</tbody>
</table>

Coates, 2015
• Strongly endocrine responsive, low proliferation, good prognosis “luminal A-like” can be distinguished from less endocrine responsive, higher proliferation, poorer prognosis “luminal B-like” tumors using IHC assays for ER, PR, and Ki67
• But, use of Ki67 requires knowledge of local laboratory values
# Immunophenotyping to Approximate Molecular Subtype, 2016

<table>
<thead>
<tr>
<th>Subtype</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Luminal A</td>
<td>ER+, HER2-, Ki-67&lt;14% or Ki-67 intermediate (14-19%) and PR≥20%</td>
</tr>
<tr>
<td>Luminal B</td>
<td>ER+, HER2- Ki-67 intermediate (14-19%*) and PR- or low (&lt;20%) or Ki-67≥20%; ER+, HER2+</td>
</tr>
<tr>
<td>HER2-E</td>
<td>ER-, PR-, HER2+</td>
</tr>
<tr>
<td>Basal-like</td>
<td>ER-, PR-, HER2-, [CK5/6+ and/or EGFR+]</td>
</tr>
</tbody>
</table>

*St. Gallen, 2015 considers PR 20-29% to be intermediate*
Current Status of Molecular Subtyping for Pathologists

- Intrinsic subtype terminology generally not included in pathology reports
- Subtypes often inferred by clinicians from combination of ER/PR and HER2 status (and grade)
Current Status of Molecular Subtyping for Pathologists

• Inferred subtypes based on receptor IHC not always the same as molecular subtypes defined by GEP or PAM50

• Conceptual value > practical value since treatment driven by the presence or absence of targetable features such as hormone receptors and HER2
Molecular Testing of Breast Cancers
Beyond Hormone Receptor and HER2 Status

- Intrinsic (molecular) subtypes
- Multigene prognostic tests
- Genomic analysis
## Commercially Available Multigene Prognostic Tests

<table>
<thead>
<tr>
<th>Gene expression test</th>
<th>Oncotype DX®</th>
<th>MammaPrint®</th>
<th>“Intrinsic gene molecular classification/PAM50/Prosigna™”</th>
<th>MapQuant DX®</th>
<th>EndoPredict®</th>
<th>Breast Cancer Index℠ (HoxB13:IL17BR/MGI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Provider</strong></td>
<td>Genomic Health</td>
<td>Agenda BV</td>
<td>NanoString Technologies Inc.</td>
<td>Qiagen (formerly Ipsogen Inc.); still available?</td>
<td>Sividon Diagnostics</td>
<td>bioTheranostics</td>
</tr>
<tr>
<td><strong>Assay</strong></td>
<td>21-gene recurrence score</td>
<td>70-gene signature</td>
<td>“Intrinsic gene” list or 50-gene PCR</td>
<td>97-gene signature or 8-gene qRT-PCR</td>
<td>qRT-PCR 8 prognostic genes, 3 normalization gene</td>
<td></td>
</tr>
<tr>
<td><strong>RNA isolated from</strong></td>
<td>Formalin-fixed, paraffin-embedded tumor tissue</td>
<td>Frozen or formalin-fixed, paraffin-embedded tumor tissue</td>
<td>Frozen or formalin-fixed, paraffin-embedded tumor tissue</td>
<td>Frozen or formalin-fixed, paraffin-embedded tumor tissue</td>
<td>Formalin-fixed, paraffin-embedded tumor tissue</td>
<td></td>
</tr>
<tr>
<td><strong>Outcome</strong></td>
<td>Disease-free relapse at 10 years</td>
<td>Distant metastasis at 5 years</td>
<td>Disease-free, distant metastasis-free and overall survival</td>
<td>Good (GGI I) or poor (GGI III) prognosis</td>
<td>Distant metastasis at 10 years</td>
<td>Relapse-free and overall survival</td>
</tr>
<tr>
<td><strong>Clinical Application</strong></td>
<td>Prediction of recurrence risk in ER+ BC treated with tamoxifen</td>
<td>Prognosis of N0 BC, &lt;5 cm diameter</td>
<td>Classification of invasive breast cancers</td>
<td>Molecular grading, for ER+, histological grade II BC</td>
<td>Prognosis of endocrine-treated BC</td>
<td>Prognostic in ER+ BC, prediction of response to tamoxifen</td>
</tr>
<tr>
<td><strong>Risk groups identified</strong></td>
<td>Three risk groups based on recurrence score</td>
<td>Dichotomous; good or poor prognosis</td>
<td>Classification of tumors into luminal A, luminal B, HER2, and basal-like subtypes</td>
<td>Dichotomous; GGI I or GGI III</td>
<td>Dichotomous; low risk or high risk</td>
<td>Continuous variable; risk of recurrence score</td>
</tr>
</tbody>
</table>

*Van de Vijver, 2014*
SYSTEMIC ADJUVANT TREATMENT - HORMONE RECEPTOR-POSITIVE - HER2-NEGATIVE DISEASE

- Tumor ≤0.5 cm or Microinvasive
  - pT0 or pT1mi (≤2 mm axillary node metastasis)
- Tumor >0.5 cm
  - RT-PCR assay

Histology:
- Ductal
- Lobular
- Mixed
- Metaplastic

Node positive (one or more metastases ≥2 mm to one or more ipsilateral axillary lymph nodes)

Consider 21-gene RT-PCR assay

- pN0
- pN1mi

Adjuvant endocrine therapy (category 2B)

Adjunct chemotherapy (category 2B)

Low recurrence score (≤18)

Intermediate recurrence score (18-30)

High recurrence score (≥31)

See Follow-Up (BINV-16)

Note: All recommendations are category 2A unless otherwise indicated.

Clinical Trials: NCCN believes that the best management of any cancer patient is in a clinical trial. Participation in clinical trials is especially encouraged.
A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer

Soonmyung Paik, M.D., Steven Shak, M.D., Gong Tang, Ph.D., Chungyeul Kim, M.D., Joffre Baker, Ph.D., Maureen Cronin, Ph.D., Frederick L. Baehner, M.D., Michael G. Walker, Ph.D., Drew Watson, Ph.D., Taesung Park, Ph.D., William Hiller, H.T., Edwin R. Fisher, M.D., D. Lawrence Wickerham, M.D., John Bryant, Ph.D., and Norman Wolmark, M.D.

RS = +0.47 x HER2 group score -0.34 x ER group score +1.04 x proliferation group score +0.10 x invasion group score +0.05 x CD68 -0.08 x GSMT1 -0.07 x BAG1

<18 Low
18-31 Intermediate
>31 High

NEJM, 2004
Recurrence Score in ER+, N- Breast Cancer

Prognostic

Predictive of chemo benefit

10yr Distant Recurrence Rates
Low risk  7%
Int. risk  14%
High risk  30%

Figure 2. Likelihood of Distant Recurrence, According to Recurrence-Score Categories.  
Paik, 2004
Recurrence Score also:

• Prognostic in ER+, N- and N+ patients treated with anastrazole (TransATAC study)*

• Prognostic for LRR (NSABP B-14 and B-20)*

• Predictive of chemotherapy benefit in ER+, N+ patients (SWOG 8814)*

*Dowsett, JCO 2010  
*Mamounas, JCO 2010  
**Albain, Lancet Oncol 2010
What is the impact of RS on clinical decision making?
The impact of the Oncotype Dx breast cancer assay in clinical practice: a systematic review and meta-analysis

Josh J. Carlson · Joshua A. Roth

Table 5 Pooled mean proportion of physicians changing adjuvant chemotherapy recommendation after ODX testing (vs. clinical-pathological factors only)

<table>
<thead>
<tr>
<th>Study</th>
<th>% Changing ACT recommendation after ODX test</th>
<th>Study weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ademuyiwa et al. [23]</td>
<td>38.0</td>
<td>0.19</td>
</tr>
<tr>
<td>de Boer et al. [42]</td>
<td>22.8</td>
<td>0.07</td>
</tr>
<tr>
<td>Holt [43]</td>
<td>26.8</td>
<td>0.10</td>
</tr>
<tr>
<td>Joh et al. [28]</td>
<td>24.9</td>
<td>0.11</td>
</tr>
<tr>
<td>Klang et al. [30]</td>
<td>40.0</td>
<td>0.26</td>
</tr>
<tr>
<td>Lo et al. [31]</td>
<td>31.5</td>
<td>0.06</td>
</tr>
<tr>
<td>Rezai et al. [44]</td>
<td>30.3</td>
<td>0.17</td>
</tr>
<tr>
<td>Tatarian et al. [35]</td>
<td>42.9</td>
<td>0.04</td>
</tr>
<tr>
<td><strong>Pooled mean</strong></td>
<td><strong>33.4</strong></td>
<td>–</td>
</tr>
</tbody>
</table>

• 8 studies
• 1437 pts
• RS integrated with pathologic and clinical factors (Recurrence Score-Pathology-Clinical)
• RSPC showed significantly improved prognostic value compared with:
  -- RS alone
  -- Pathologic and clinical factors alone (tumor size, grade and patient age)
• RSPC classified fewer patients as intermediate risk and more as lower risk
## Commercially Available Multigene Prognostic Signatures

<table>
<thead>
<tr>
<th>Gene expression test</th>
<th>Oncotype DX®</th>
<th>MammaPrint®</th>
<th>MapQuant DX®</th>
<th>EndoPredict®</th>
<th>Breast Cancer Index&lt;sup&gt;SM&lt;/sup&gt; (HoxB13:IL17BR/MGI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Provider</td>
<td>Genomic Health</td>
<td>Agendia BV</td>
<td>NanoString Technologies Inc.</td>
<td>Qiagen (formerly Ipsogen Inc.); still available?</td>
<td>Sividon Diagnostics</td>
</tr>
<tr>
<td>Assay</td>
<td>21-gene recurrence score</td>
<td>70-gene signature</td>
<td>“Intrinsic gene” list or 50-gene PCR or 97-gene signature or 8-gene qRT-PCR</td>
<td>qRT-PCR 8 prognostic genes, 3 normalization gene</td>
<td>2-gene HOXB13:IL17R/molecular-grade index</td>
</tr>
<tr>
<td>RNA isolated from</td>
<td>Formalin-fixed, paraffin-embedded tumor tissue</td>
<td>Frozen or formalin-fixed, paraffin-embedded tumor tissue</td>
<td>Frozen or formalin-fixed, paraffin-embedded tumor tissue</td>
<td>Frozen or formalin-fixed, paraffin-embedded tumor tissue</td>
<td>Formalin-fixed, paraffin-embedded tumor tissue</td>
</tr>
<tr>
<td>Outcome</td>
<td>Disease-free relapse at 10 years</td>
<td>Distant metastasis at 5 years</td>
<td>Disease-free, distant metastasis-free and overall survival</td>
<td>Good (GGI I) or poor (GGI III) prognosis</td>
<td>Distant metastasis at 10 years</td>
</tr>
<tr>
<td>Clinical Application</td>
<td>Prediction of recurrence risk in ER+ BC treated with tamoxifen</td>
<td>Prognosis of N0 BC, &lt;5 cm diameter</td>
<td>Classification of invasive breast cancers</td>
<td>Molecular grading, for ER+, histological grade II BC</td>
<td>Prognosis of endocrine-treated BC</td>
</tr>
<tr>
<td>Risk groups identified</td>
<td>Three risk groups based on recurrence score</td>
<td>Dichotomous; good or poor prognosis</td>
<td>Classification of tumors into luminal A, luminal B, HER2, and basal-like subtypes</td>
<td>Dichotomous; GGI I or GGI III</td>
<td>Dichotomous; low risk or high risk</td>
</tr>
</tbody>
</table>

Van de Vijver, 2014
• PAM50 Risk of Recurrence (ROR) Score provided more prognostic information in endocrine-treated, ER+, node- patients than Onco\textit{type}Dx recurrence score, especially in HER2-negative group
  – More patients scored as high risk and fewer as intermediate risk
Prediction of Late Distant Recurrence After 5 Years of Endocrine Treatment: A Combined Analysis of Patients From the Austrian Breast and Colorectal Cancer Study Group 8 and Arimidex, Tamoxifen Alone or in Combination Randomized Trials Using the PAM50 Risk of Recurrence Score

Ivana Sestak, Jack Cuzick, Mitch Dowsett, Elena Lopez-Knowles, Martin Filipits, Peter Dubsky, John Wayne Cowens, Sean Ferree, Carl Schaper, Christian Fesl, and Michael Gnant

Recurrence in years 5-10
Which Expression Signature is Best?
Fan, NEJM, 2006

• Five different gene-expression based models compared among a single set of 295 samples
• High level of concordance in outcome prediction
• But, very little overlap in genes
• **Proliferation genes** are the common driving force in all prognostic signatures

• Factors associated with tumor burden (size, nodal status) remain independently associated with prognosis
Multigene Prognostic Tests: Unresolved Issues

- Almost all available data from retrospective analyses
- Prognostic value greatest in first 5 years for most
  - PAM50 ROR prognostic for late recurrence (beyond 5 years)
- Cost
- Which test?
- Which patients?
  - Of value only for ER+ (luminal) cancers
Multigene Prognostic Tests: Unresolved Issues

• Is this approach really better than using a combination of clinical and pathologic factors supplemented by appropriate biomarkers detected by IHC (e.g., ER, PR, HER2 and Ki67)?
• 1125 pts with ER+ breast cancer in the TransATAC trial
• ER, PR, HER2 and Ki67 assessed by IHC
• Combined “IHC4 Score” provided similar prognostic information as Onco
typeDX Recurrence Score
Prospective Clinical Trials to Assess Role of Multigene Prognostic Tests

• **TAILORx (Onco
typeDX)**
  - *Trial Assigning Individualized Options for Treatment*

• **RxPONDER (Onco
typeDX)**
  - *Rx for Positive Node Endocrine Responsive Breast Cancer*

• **MINDACT (MammaPrint)**
  - *Microarray in Node Negative Disease May Avoid Chemotherapy*
Prospective Validation of a 21-Gene Expression Asssay in Breast Cancer


NEJM, 2015

• 1626 women with ER+, HER2-, N-breast cancer with Oncotype DX recurrence score of 0-10 (enrolled in TAILORx trial)

• Endocrine therapy alone

• Excellent outcome at 5 yrs
**Table 3. Event Rates at 5 Years, According to Histologic Grade.**

<table>
<thead>
<tr>
<th>Tumor Grade</th>
<th>Invasive Disease–free Survival (95% CI)</th>
<th>Freedom from Distant Recurrence (95% CI)</th>
<th>Freedom from Any Recurrence (95% CI)</th>
<th>Overall Survival (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All grades</td>
<td>93.8 (92.4–94.9)</td>
<td>99.3 (98.7–99.6)</td>
<td>98.7 (97.9–99.2)</td>
<td>98.0 (97.1–98.6)</td>
</tr>
<tr>
<td>Low grade</td>
<td>95.8 (93.5–97.3)</td>
<td>99.8 (98.3–100)</td>
<td>99.8 (98.3–100)</td>
<td>98.7 (97.0–99.4)</td>
</tr>
<tr>
<td>Intermediate grade</td>
<td>93.6 (91.7–95.1)</td>
<td>99.0 (98.0–99.5)</td>
<td>98.2 (97.0–99.0)</td>
<td>97.9 (96.8–98.7)</td>
</tr>
<tr>
<td>High grade</td>
<td>91.3 (83.9–95.4)</td>
<td>100 (NC–NC)</td>
<td>98.7 (91.1–99.8)</td>
<td>97.3 (91.9–99.1)</td>
</tr>
</tbody>
</table>
Molecular Testing of Breast Cancers
Beyond Hormone Receptor and HER2 Status

- Intrinsic (molecular) subtypes
- Multigene prognostic tests
- Genomic analysis
Genomic Analysis

• Identification of genomic alterations in tumors, particularly “driver alterations”
  – Mutations
  – Copy number variations
  – Translocations
  – Insertions/deletions
Genomic Analysis

• Variety of techniques (including targeted sequencing, whole genome/exome sequencing)
• Goal: To identify “actionable” targets in order to personalize therapy (“precision oncology”)
• Currently used primarily in patients with advanced disease
Genomic Analysis

- Caveats
  - “Actionable” ≠ Clinically useful
  - Many tumors have multiple genetic alterations (including multiple driver alterations)
The landscape of cancer genes and mutational processes in breast cancer

- 100 breast cancers
- 40 cancer genes
- 73 different combinations of mutated cancer genes!!

Stephens, Nature 2012
Genomic Analysis

• Caveats
  – “Actionable” ≠ Clinically useful
  – Many tumors have multiple genetic alterations (including multiple driver alterations)
  – Intra-tumor heterogeneity
  – Genetic alterations frequently change during the course of tumor progression
Molecular Testing of Breast Cancers

Future Directions

- Standardization of Ki67 IHC
- Prognostic / predictive factors for ER-subtypes (esp. TNBC)
- Immune response / TILs / Immunotherapy
- Non-coding RNAs
- Circulating DNA / Liquid biopsies
- Predictors of therapeutic resistance
  - Endocrine therapy
  - HER2 targeted therapy
- Tumor heterogeneity
Conclusions

- ER, PR, and HER2 testing using ASCO/CAP guidelines remain the most important ancillary tests in the management of patients with breast cancer.
Conclusions

• While molecular subtypes are of conceptual interest, clinically useful subgroups are defined by ER, PR, and HER2 assay results.

• Among patients with ER+/HER2- ("luminal") disease, Ki67 proliferation rate or multigene prognostic tests are of value in further defining risk of recurrence and potential benefit from chemotherapy in addition to endocrine therapy.
  – But Ki67 not standardized.
Conclusions

• The major clinical role of genomic/mutation analysis at this time is to identify patients with advanced disease who may be candidates for targeted therapies based on specific genomic alterations