Breast Cancer Molecular Profiling: Promises and Limitations

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“grading” the tumor by morphology

Grade 1

Low risk

Grade 2

“Intermediate”

Grade 3

High risk
IVDMIAss (In Vitro Diagnostic Multivariate Index Assays) Definition (FDA)

1) “Combines the values of multiple variables using an interpretation function to yield a single, patient-specific result (e.g., a “classification,” “score,” “index,” etc.), that is intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment or prevention of disease, and

2) “Provides a result whose derivation is non-transparent and cannot be independently derived or verified by the end user.”
IVDMIA

- Mammaprint-FDA cleared
- A laboratory-developed IVDMIA (Oncotype Dx) is a specific subset of LDTs and is *not* FDA cleared/approved.
- LDT-laboratory developed test whose performance is determined by the lab.
• IVDMIAs are inherently different from other LDTs in that they have...
  • – Complex unique interpretation functions
  • -LDT IVDMIAs “high risk, high impact assays that raise significant issues of safety and effectiveness”
• There is a need for FDA to regulate these devices to ensure that the IVDMIA is safe* and effective for its intended use.
Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group

- EGAPP is a project developed by the National Office of Public Health Genomics at the CDC to support a rigorous, evidence-based process for evaluating genetic tests and other genomic applications that are in transition from research to clinical and public health practice in the U.S.

- A key goal of the EWG is to develop conclusions and recommendations regarding clinical genomic applications, and to establish clear linkage to the supporting scientific evidence.
Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group

Genetics in Medicine • Volume 11, Number 1, January 2009

Summary of Findings on Gene Expression Profiling To Predict Risk for Breast Cancer Recurrence

there was not enough evidence to state whether breast cancer GEP should or should not be used for early stage breast cancer treatment decision making. The balance of benefits and harms of using breast cancer GEP could not be determined from the available evidence.
EWG Summary...

- no direct or indirect evidence linking tumor gene expression profiling of women with breast cancer to improved health outcomes.

- For one test, the EWG found preliminary evidence of potential benefit of testing results to some women who face decisions about treatment options (reduced adverse events due to low risk women avoiding chemotherapy), but could not rule out the potential for harm for others (breast cancer recurrence that might have been prevented).

- further development and evaluation of these technologies encouraged.
Agendia..privately held

- MammaPrint-FDA cleared-to compare gene activity of a sample to an GEP template of tumors with good prognosis. 4200. USD
- Original shareholders include the founders of Agendia and Stichting Fondsen Nederland that originated from the Netherlands Cancer Institute.
• Mammaprint
• Excellent data set provides for stratification of patients into low risk and high risk types—but technically a prognostic test-Low risk vs High Risk.
• Untreated population/unsupervised genes.
• BluePrint-molecular classification
• TargetPrint-ER/PR/Her2
• MammaPrint: No direct evidence links use of the MammaPrint test to clinical outcomes.
• No studies evaluated MammaPrint for ability to predict benefit from other treatments (e.g., chemotherapy).
• No studies determined whether use of MammaPrint in place of, or in addition to, current clinicopathologic markers (e.g., Adjuvant! Online, St. Gallen) changes management, and/or improves outcomes based on management with clinicopathologic markers alone.
MammaPrint genes were selected by the biology of breast cancer itself

Whole-genome Analysis
~25,000 genes

70 genes
55 known function
15 unknown function
70 significant prognosis genes

van't Veer et al., Nature 415, p. 530-536, 2002
MammaPrint

• **MINDACT (Microarray In Node-negative and 1 to 3 positive lymph node Disease may Avoid ChemoTherapy)**
  
• A prospective randomized study comparing the 70-gene signature with the common clinical-pathological criteria in selecting patients for adjuvant chemotherapy in breast cancer with 0-3 positive nodes. *(EWG*)
• In the MINDACT trial, women with breast cancer who are assessed as “High Risk” by both MammaPrint and Adjuvant! Online are advised to have chemotherapy whereas for women with “Low Risk” concordance, hormonal therapy alone is recommended. However, discordant cases are randomized to receive either chemotherapy or hormonal therapy based on clinical-pathological risk assessment or MammaPrint and the patients are followed.
MammaPrint interrogates critical genomic pathways

1. IGFBP5, TGFβ3, FGF18, ESM1, RARRES3, PITRM1, EXT1, EXT1L3, SCUBE2, EBF4, CDC42BPA, CDCA7L, CDCA7L, GMPS, MELK, RFC4, WISP1, HRASLS, BBC3, DTL, FBXO31, EGLN1, GNAZ, MT DH, FLT1, ECT2, DIAPH3, NUSAP1, AKAP2, NDC80, PRC1, ORC6L, CENPA, DCK, CCNE2, MCM6, QSOX2, STK32B

2. COL4A2, FLT1, FGF18, MMP9

3. FLT1, TGFβ3, IGFBP5, FGF18, RARRES3, CDCA7L, WISP1, DIAPH3, AKAP2, CDC42BPA, PALM2, DCLK2, NMU, NMUR1, NMUR2

4. COL4A2, FLT1, MMP9, TGFβ3, MT DH, DIAPH3, PALM2, DCLK2, NMU, NMUR1, NMUR2

5. COL4A2, FLT1, MMP9, TGFβ3, DIAPH3, PALM2, DCLK2, NMU, NMUR1, NMUR2

6. COL4A2, FLT1, MMP9, TGFβ3, MT DH, DIAPH3, PALM2, DCLK2, NMU, NMUR1, NMUR2

7. MMP9, COL4A2
Genomic Health Inc

- Oncotype Dx
  (4600.USD)
- (NASDAQ:GHDX)
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.
Oncotype DX Selection Process
RT-PCR Technology

250 candidate genes derived from the available literature (the knowledge of the year 2000)

250 cancer-related genes based on objectives of clinical study

21 genes:
16 known functions
5 reference genes
0 unknown functions
Oncotype DX: 21 Gene Recurrence Score (RS) Assay

Validation set as a Prognostic Test: 668 patients NSABP B-14 Paik et al NEJM 2004 351:2817

16 Cancer and 5 Reference Genes From 3 Studies

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

**Estrogen**
- ER
- PR
- Bcl2
- SCUBE2

**Invasion**
- Stromolysin 3
- Cathepsin L2

**HER2**
- GRB7
- HER2

**Reference**
- Beta-actin
- GAPDH
- RPLP0
- GUS
- TFRC

**Coefficient x Expression Level**

The recurrence score defined as:

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

Scaled – 0 to 100

**Category** | **RS (0 – 100)**
--- | ---
Low risk | RS < 18
Int risk | RS ≥ 18 and < 31
High risk | RS ≥ 31
Derivation of the Recurrence Score

• RS (U) = + $0.47 \times \text{HER2}$ group score $-0.34 \times \text{ER}$ group score $+ 1.04 \times \text{proliferation group score}$ $+ 0.1 \times \text{invasion group score}$ $+ 0.05 \times \text{CD68} - 0.08 \times \text{GSTM1} - 0.07 \times \text{BAG1}$.

• Predict tumor recurrence at 10 years for patients on 5 years of tamoxifen.
Oncotype dx Recurrence Score Curve

Recurrence Score vs Distant Recurrence in **Node Negative, ER-Positive Breast Cancer Prognosis**

- **Low Risk**
  - Group Average: 7%
  - 95% CI: 4%-10%

- **Intermediate Risk**
  - Group Average: 14%
  - 95% CI: 8%-20%

- **High Risk**
  - Group Average: 31%
  - 95% CI: 24%-37%

Average Rate of Distant Recurrence at 10 Years vs Breast Cancer Recurrence Score after 5 Years of Tamoxifen Treatment

*For Recurrence Scores > 50, group average*
NSABP Clinical Trials Overview
1/4/82-1/25/88

• A Clinical Trial to Assess Tamoxifen in Patients With Primary Breast Cancer and Negative Axillary Nodes Whose Tumors are Positive for Estrogen Receptors
• 1998-Her2 therapy approved for metastatic breast cancer
• 2005-Her2 therapy in adjuvant setting (5 clinical trials)
A Clinical Trial to Determine the Worth of Chemotherapy and Tamoxifen over Tamoxifen Alone in the Management of Patients with Primary Invasive Breast Cancer, Negative Axillary Nodes and Estrogen-Receptor-Positive Tumors
Ioannidis J. *The Oncologist* 2007;12:301–311

- Mixing training and test datasets: a failed validation presented as a successful validation with extension of the clinical indications. The development of the 21-gene recurrence score (Oncotype DX®) involved training in data from the patients of the tamoxifen arm of (NSABP)-20 trial.
As shown, these data suggest, in fact, that the 21-gene recurrence score fails to discriminate the risk of recurrence in the true test dataset (chemotherapy).
onco\textit{type} DX\textsuperscript{TM}

- 2004-2008, reported RS only
- 2008-because of “oncologists demand”- started reporting ER, PR, Her2 by qRT-PCR.
- No Her2 clinical outcomes studies for qRT-PCR-(technical validation against IHC only.)
- Laboratory developed test..Not FDA approved.
- How about use in \textbf{Her2+ patients}?
qRT-PCR ranges for ER, PR, Her2
Quantitative HER2

oncotyPte DX
Breast Cancer Assay

Accurate, Precise, Reproducible.
The “GOOD”

• **Concordance between semiquantitative immunohistochemical assay and oncotype DX RT-PCR assay for estrogen and progesterone receptors.** (80 cases)
  

• **97% ER percent positive agreement with IHC; 93% PR.**

• **Semi-quantitative immunohistochemical assay versus oncotype DX(®) qRT-PCR assay for estrogen and progesterone receptors: an independent quality assurance study.** (464 cases)
  

• **98 % ER percent positive agreement with IHC; 94% PR.**
High False-Negative Rate of HER2 Quantitative Reverse Transcription Polymerase Chain Reaction of the Oncotype DX Test: An Independent Quality Assurance Study

David J. Dabbs, Molly E. Klein, Syed K. Mohsin, Raymond R. Tubbs, Yongli Shuai, and Rohit Bhargava
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Results

- All 23 Her2 equivocal patient cases were reported as negative by Oncotype Dx.
- Of the 36 unequivocally positive Her2 cases, only 10 (28%; 95% CI, 14% to 45%) were reported as positive, 12 (33%) as equivocal, and 14 (39%) as negative.
Take Home Message
Of 36 UNEQUIVOCAL POSITIVE IHC/FISH CASES:

• 14/36 GHI called **NEGATIVE** (39%)
• 12/36 GHI called **EQUIVOCAL** (33%)
• 10/36 GHI called **POSITIVE** (28%) PPA
• ASCO/CAP DEMANDS 95% PPA
  COMBINE 28+33=61%

*All results were verified by a member of the ASCO-CAP committee*
GHI has a 98% PPA for Her2 FISH-RT-PCR

Human Epidermal Growth Factor Receptor 2 Assessment in a Case-Control Study: Comparison of Fluorescence In Situ Hybridization and Quantitative Reverse Transcription Polymerase Chain Reaction Performed by Central Laboratories

Frederick L. Baehler, Ninah Achacoso, Tara Maddala, Steve Shak, Charles P. Quesenberry Jr, Lynn C. Goldstein, Allen M. Gown, and Laurel A. Habel
7 Laboratories World-Wide have documented the Her2 single gene flaw in Oncotype Dx


Why the marked discrepancy in Her2 results between GHI Oncotype Dx and the “Real World”? 

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.
Soon, Dave Dabbs and I have had a multi year running argument about the utility of Oncotype Dx in Her2 positive, ER positive, node negative breast cancer, as you can see below.

• > Can you shed any light on this issue? Thanks in advance for your time and thoughts.
• Soon Paik: All HER2 positive cases were mostly high RS or at least intermediate RS. Therefore I simply do not recommend performing OncotypeDx on HER2 positive tumors.
the issue of OncotypeDx being inaccurate about HER2 status - yes it is - since HER2 mRNA levels of HER2 positive and negative tumors overlap. Therefore you will have false negative cases if you rely on HER2 mRNA levels only (even if you use GRB7 or other genes around HER2, same situation). The only time HER2 mRNA levels reported as part of OncotypeDx can be useful is to rule out false negative cases. I guess if the Genomic Health has been marketing otherwise, then they should not do that.

Hope this makes sense.

Sincerely, Soon Paik
Isabel Pinhel, Margaret Hills, Suzanne Drury, Janine Salter, Georges Sumo, Roger A’Hern, Judith M Bliss, Ivana Sestak, Jack Cuzick, Peter Barrett-Lee, Adrian Harris and Mitch Dowsett,* on behalf of the NCRI Adjuvant Breast Cancer Trial Management Group*

**Breast Cancer Research**

This Provisional PDF corresponds to the article as it appeared upon acceptance. Copyedited and fully formatted PDF and full text (HTML) versions will be made available soon.

**ER and HER2 expression are positively correlated in HER2 non-overexpressing breast cancer**

Overlap of mRNA in Her+ve and Her2-ve ER+ Breast Cancers
GHI has a 98% PPA for Her2 FISH-RT-PCR

Human Epidermal Growth Factor Receptor 2 Assessment in a Case-Control Study: Comparison of Fluorescence In Situ Hybridization and Quantitative Reverse Transcription Polymerase Chain Reaction Performed by Central Laboratories

Frederick L. Baehner, Ninah Achacoso, Tara Maddala, Steve Shak, Charles P. Quesenberry Jr, Lynn C. Goldstein, Allen M. Gown, and Laurel A. Habel
21-Gene Assay Profile Limitations

- Assumes 5 years of tamoxifen treatment
  - Nearly half of patients (49%) do not comply with 5 years of Tamoxifen therapy (Owusu et. al., JCO 2008, Glück, Mamounas, Oncology 2010)

- 37% of results are intermediate (Palmer et. al., European Journal of Cancer Supplements 2009)

- Improper validation-not predictive for chemotherapy.
- Her 2 gene flaw. Others?
Foundation Medicine is privately held and backed by top-tier life science investors including founding investor Third Rock Ventures and venture capital backers Google Ventures and Kleiner Perkins Caufield & Byers. Additional investors include public crossover funds Deerfield Management Company, L.P., Casdin Capital and Redmile Group and strategic investors Roche Venture Fund and WuXi Corporate Venture Fund.
Foundation One Issues

- Deep sequencing for mutations.
- Druggable target?
- Drugs @ 80-100k/year in absence of clinical trials - who pays?
Problematic Issues with Technology in the United States

• Corporate “science”
• Pollution of clinical trials groups-NSABP, ECOG, etc
• Competing interests of physicians
• Independently verified?
• Cost