High False-Negative Rate of HER2 Quantitative Reverse Transcription Polymerase Chain Reaction of the Oncotype DX Test: An Independent Quality Assurance Study

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SPECIFIC AIMS

• Perform a quality assurance study to ascertain if the Her2 qRT-PCR of the Oncotype dx (Odx) test meets the ASCO/CAP standards for Her2 testing, by comparing Odx qRT-PCR results with IHC/FISH testing at Magee-Women’s Pathology department.

• Determine patient impact of the above.
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
Oncotype DX Genes

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

**HER2**
- GRB7
- HER2

**GST-M1**

**CD68**

**INVASION**
- Stromelysin 3
- Cathepsin L2

**ESTROGEN**
- ER
- PGR
- BCL2
- SCUBE2

**REFERENCE**
- Beta-actin
- GAPDH
- RPLPO
- GUS
- TFRC
Unscaled Recurrence Score equation

- RSU = +0.47 x GRB7 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 GSTM1 – 0.07 x BAG1
**oncotype DX™**

- Reported as distant disease recurrence score (RS)
  - Low-risk RS: 0-17
    - Risk of recurrence 7%
  - Intermediate risk RS: 18-30
    - Risk of recurrence 14%
  - High risk RS: 31-100
    - Risk of recurrence 31%
- Test validated for ER+, lymph node negative tumors: 651 patients on NSABP B-20
  - *Validation study of 668 patients in the NSABP trial B-14 (N Engl J Med 2004;351:2817-2826)*
Oncotype dx Recurrence Score Curve
Table 1. Kaplan–Meier Estimates of the Rate of Distant Recurrence at 10 Years, According to Recurrence-Score Risk Categories.*

| Risk Category | Percentage of Patients | Rate of Distant Recurrence at 10 Yr (95% CI) †
|---------------|------------------------|---------------------------------------------------
| Low           | 51                     | 6.8 (4.0–9.6)                                     |
| Intermediate  | 22                     | 14.3 (8.3–20.3)                                   |
| High          | 27                     | 30.5 (23.6–37.4)‡                                 |

* A low risk was defined as a recurrence score of less than 18, an intermediate risk as a score of 18 or higher but less than 31, and a high risk as a score of 31 or higher.
† CI denotes confidence interval.
‡ P<0.001 for the comparison with the low-risk category.
**oncotype DX™**

- 2004-2008, reported RS only
- 2008-because of “oncologists demand”- started reporting ER, PR, Her2 by qRT-PCR.
- No outcomes studies for qRT-PCR-(technical validation against IHC.)
- Laboratory developed test..Not FDA approved.
In 2008, when GHI began to report the separate quantitative q-RT-PCR raw values for ER, PR and Her2 this permitted a “peek” into the “black box” proprietary GHI test, which is a laboratory developed, non-FDA approved test.
qRT-PCR scores ER, PR, Her2
qRT-PCR ranges for ER, PR, Her2
Initial Results at Magee Pathology-QA
Her2 Study

• Less than 40% positive agreement between IHC/FISH and the oncotype Her2 qRT-PCR
• More than a 60% FALSE NEGATIVE RATE.
• 19 other labs were canvassed for results.
• Two labs had data, were willing to participate.
Methods

• All patients at three participating laboratories (Magee-Womens Hospital [Pittsburgh, PA], Cleveland Clinic [Cleveland, OH], and Riverside Methodist Hospital [Columbus, OH]) with available HER2 RT-PCR results from GHI were included in this study. All IHC-positive and equivocal patient cases were further evaluated and classified by FISH at respective laboratories (same antibodies & FISH).
Results

• Of the total 843 patient cases, 784 (93%) were classified as negative, 36 (4%) as positive, and 23 (3%) as equivocal at the three institutions using IHC/FISH.

• Of the 784 negative patient cases, 779 (99%) were also classified as negative by GHI RT-PCR assay.
Results

• All 23 equivocal patient cases were reported as negative by GHI.

• Of the 36 positive cases, only 10 (28%; 95% CI, 14% to 45%) were reported as positive, 12 (33%) as equivocal, and 14 (39%) as negative.
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<th>Institution</th>
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<th>Total</th>
<th>Cohen Kappa</th>
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<th>PPA (%)</th>
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*PPA and PNA percentages are italicized and bolded for emphasis.

†Combined† refers to the overall comparison.
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<th>Discordant Patient Case No.</th>
<th>Discordant Category</th>
<th>GHI Results</th>
<th>Percentage Invasive Tumor Within Block Sent for Oncotype DX</th>
<th>Repeat FISH on Same Block Sent for Oncotype DX</th>
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<td>18</td>
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<td>9.4</td>
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Conclusion

• There was an unacceptable false-negative rate for HER2 status with GHI HER2 assay in this independent study. This could create confusion in the decision-making process for targeted treatment and potentially lead to mismanagement of patients with breast cancer if only GHI HER2 information is used.
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. Bochner, Ninah Achacoso, Tara Maaddala, Steve Shak, Charles P. Quesenberry Jr, Lynn C. Goldstein, Allen M. Gown, and Laurel A. Habel
<table>
<thead>
<tr>
<th>Oncotype DX Status</th>
<th>Central FISH Positive</th>
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<th>Total No. of Patients</th>
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<tr>
<td></td>
<td>No. of Patients</td>
<td>%</td>
<td>No. of Patients</td>
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<tr>
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<td>55</td>
<td>98</td>
<td>11</td>
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<tr>
<td>Total</td>
<td>56</td>
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NOTE. Percentages are column percentages. FISH HER2 status is used as the denominator. Concordance is 97% (95% CI, 96% to 99%); \( \kappa \) is 89% (95% CI, 83% to 95%).

Abbreviations: HER2, human epidermal growth factor receptor 2; ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; FISH, fluorescence in situ hybridization.
WHY THE DIFFERENCE?

• GHI CORPORATE STUDY - a case-controlled research study where variables are in a controlled environment.
Disturbing Results

• 1) in direct conflict with the GHI corporate publication of Baehner et al-Baehner et al J Clin Oncol 28:4300-6, 2010 showed 98% agreement between FISH and qRT-PCR for Her2.

• 2) Our data suggests poor microdissection as the root cause of false negative GHI qRT-PCR Her2, which implies that other gene components on the platform could also be compromised, yielding a spurious “recurrence score”

• 3) Importantly, 100% of Her2 FISH equivocal cases are called NEGATIVE by GHI ODX
Disturbing Results

4) Clinicians may be confused about the reliability of Her2 testing which will lead to poor treatment decisions—either on the basis of the ODX recurrence score, or inaccurate Her2 results.

5) Some clinicians rely on Odx as a “tiebreaker” for patients with FISH equivocal results = guaranteed mis-management.
Take Home Message #1
Of 36 UNEQUIVOCAL POSITIVE 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%
Take Home Message #2
23 FISH EQUIVOCAL CASES

• 100% NEGATIVE by Odx Her2 qRT-PCR
• In addition, the equivocal qRT-PCR range DOES NOT specify a gene ratio.
Take Home Message #3 - ruminations

• If Her2 is unreliable...
• Other 15 genes unreliable?
• Proliferation gene set?
• ?Recurrence score affected
• I am just back from vacation and need to read your paper and the editorial in detail but what I can say is this is very important and backs up my own perspective from both our own RNA work on HER2 and that in TransATAC with GHI. As it happens one of my colleagues contacted me today about how one can explain a neg GHI HER2 in a patient already diagnosed as HER2+; I was able to refer him to your paper. This is already very timely as the HER2 guidelines committee of ASCO/CAP is just reconvening and will be able to take a stance on this.

Well done and best wishes
RMH+/GHI equivocal
HER2
MWH+/GHI equivocal case
MWH+/GHI− case
Questions?
“Clinician Quotes”

• Your’re only one lab
“Clinician Quotes”

• Your’re only one lab.
• We don’t pay attention to er/pr/her2.
“Clinician Quotes”

• Your’re only one lab.
• We don’t pay attention to er/pr/her2.
• There is no gold standard for Her2 testing.
“Clinician Quotes”

- Your’re only one lab.
- We don’t pay attention to er/pr/her2.
- There is no gold standard for Her2 testing.
- No Her2 test measures accurate clinical response
“Clinician Quotes”

• Your’re only one lab.
• We don’t pay attention to er/pr/her2.
• There is no gold standard for Her2 testing.
• No Her2 test measures accurate clinical response.
• I send equivocal FISH for Oncotype.
Resolution?

• Met with Drs. Shak and Baehner at MWH July 25, 2011.
• Dr David Hicks as an observer.
• GHI made no claim there was a problem.
• Made no attempt to address or resolve this testing issue.