CURRENT ISSUES IN HER2 TESTING IN BREAST CANCER

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PhenoPath Laboratories
Seattle, Washington
Clinical Professor of Pathology
University of British Columbia, Vancouver BC

HER2 Network and Anti-HER2

Key Randomized Adjuvant Trastuzumab Trials

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>DFS HR</th>
<th>OS HR</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-31/N9831</td>
<td>3,55</td>
<td>0.60 (p &lt; 0.0001)</td>
<td>0.63 (p &lt; 0.0001)</td>
</tr>
<tr>
<td>HERA</td>
<td>5,10</td>
<td>0.76 (p &lt; 0.0001)</td>
<td>0.76 (p &lt; 0.0005)</td>
</tr>
<tr>
<td>BCIRG</td>
<td>3,22</td>
<td>0.64 (p &lt; 0.0001)</td>
<td>0.63 (p &lt; 0.001)</td>
</tr>
</tbody>
</table>
Accurate and standardized testing algorithms, which adequately measure HER2 expression in newly diagnosed patients with breast cancer, are crucial for determining which patients may benefit from HER2-targeted therapy.

HER2 Overexpression in Breast Cancer

Normal
~20-50,000 receptors

HER2 Overexpressed
Up to ~2,000,000 receptors

HER2 Gene Amplification and Protein Overexpression

HER2 gene  HER2 mRNA  HER2 protein

Normal Breast Epithelial Cell  Breast Cancer Cell (with HER2 alterations)
Potential Problems with Immunohistochemistry

**PRE-ANALYTICAL**
Specimen type, ischemia time, fixative content and duration, processing, etc.

**ANALYTICAL**
Use of nonvalidated assay, choice of antibody, epitope retrieval, detection system, etc.

**POST-ANALYTICAL**
Scoring system, dichotomization cutoff, etc.

Cold ischemia time (recommendation):
**LESS THAN ONE HOUR**

Type of specimen (recommendation):
**CORE or RESECTION**

Nature and duration of fixation:
**10% NBF for 6-72 HOURS**

*HER2 represents a change from 2007 Guidelines*
How Are We Doing?

Has Control of Pre-Analytic Factors Significantly Improved Diagnostic Accuracy?

Human Epidermal Growth Factor Receptor 2 Testing in Primary Breast Cancer in the Era of Standardized Testing: A Canadian Prospective Study


- N = 711 primary breast cancers analyzed by IHC and ISH
- Using FISH as ‘gold standard’, “false negative” IHC rate was 0.84% (6/711)
- N = 1,212 TMA based cases analyzed by IHC and ISH
- Using FISH as a ‘gold standard’, “false negative” IHC rate was 1.6% (16/978)

Central pathology laboratory review of HER2 and ER in early breast cancer: an ALTTO trial (BIG 2-06/NCCITG N063D (Alliance)) ring study

Ann F. McCallough · Patricia dell’Orto · Monica M. Reidah · Richard D. Gels · Amy Lee C. Dauch · Lilia Russo · Robert B. Jenkins · Stefania Andrejshetty · Binyan Chen · Christian Jakobs · Michael Utzsch · Edith A. Perez · Marilyn J. Piccart-Gebhart · Giuseppe Viale

- Phase III Adjuvant Lapatinib and/or Trastuzumab Treatment Optimisation trial for HER2 positive breast cancer
- ER and HER2 reviewed at Mayo for North America and European Institute of Oncology for rest of world (except China)
57th Annual HSCP Spring Symposium

How Accurate and Reproducible is HER2 Assessment (2015)?

- Unstained sections on 35 breast cancer cases sent to each of 12 laboratories in Tuscany
- Cases known to be FISH+ (19) or FISH- (16)
- No false positive IHCs (3+ but FISH-negative)
- False negative IHC rate 24.6% (0 or 1+ but FISH-positive)

IHC-FISH Concordance
PhenoPath Laboratories 2008-2012

N = 9,022 cases analyzed

<table>
<thead>
<tr>
<th></th>
<th>Negative (0, 1+)</th>
<th>Positive (3+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-amp</td>
<td>3903 (98.6%)</td>
<td>13</td>
</tr>
<tr>
<td>Amplified</td>
<td>57 (97.2%)</td>
<td>450</td>
</tr>
<tr>
<td>TOTALS</td>
<td>3960</td>
<td>463</td>
</tr>
</tbody>
</table>
Labratory Compliance With the American Society of Clinical Oncology/College of American Pathologists Human Epidermal Growth Factor Receptor 2 Testing Guidelines
A 3-Year Comparison of Validation Procedures

- Sent out 1150 surveys, 85% returned
- 95% negative concordance between IHC and FISH achieved by 76.5% of laboratories
- 95% positive concordance between IHC and FISH achieved by 70.4%

### 2013 ASCO-CAP HER2 TESTING GUIDELINES

Comparison of 2007 v. 2013 Concordance Requirements

<table>
<thead>
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<tr>
<td>At least 95% concordance (for both positives and negatives) compared with a validated assay to which it is compared</td>
<td>Laboratories are responsible for ensuring the reliability and accuracy of their testing results, by compliance with accreditation and proficiency testing requirements for HER2 testing assays. Specific concordance requirements are not required</td>
</tr>
<tr>
<td>25–100 cases required for validation</td>
<td>20 positive and 20 negative cases (FDA approved assay); 40 positive and 40 negative cases (LDT)</td>
</tr>
</tbody>
</table>

Factors Affecting HER2 Test Accuracy by IHC

- Different primary antibodies, kits
- Sub optimized epitope retrieval methods
- Technical artifacts on slides (edge, crush)
- Inaccuracies inherent in ‘binning’
- Tendency to “up score” to avoid missing a positive case
- Suboptimal positive controls
Comparison of 2007 v. 2013

IHC Definitions

<table>
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<th>2013 Guidelines</th>
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<tr>
<td>3+ IHC</td>
<td>&gt;30% strong membranous signal</td>
</tr>
<tr>
<td>2+ IHC</td>
<td>Complete membranous signal either nonuniform or weak but circumferential in ≥15% of cells OR strong membranous signal &lt;30%</td>
</tr>
<tr>
<td>1+ IHC</td>
<td>Weak, incomplete membranous signal in any proportion of cells</td>
</tr>
<tr>
<td>0 IHC</td>
<td>No signal</td>
</tr>
</tbody>
</table>

2013 ASCO-CAP HER2 TESTING GUIDELINES
Positive (3+)
Intense, uniform membranous ("chicken wire") signal with ≥10% of tumor cells showing circumferential signal.

IHC 3+

2007 or 2013

Comparison of 2007 v. 2013 IHC Definitions

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<td>&gt;30% strong membranous signal</td>
<td>&gt;10% strong membranous signal</td>
</tr>
<tr>
<td>2+ IHC</td>
<td>Complete membranous signal either nonuniform or weak but circumferential in ≥10% of cells OR strong membranous signal &lt;30%</td>
<td>Weak to moderate complete membranous signal within ≥10% tumor cells OR strong membranous in ≤10% of cells</td>
</tr>
<tr>
<td>1+ IHC</td>
<td>Weak, incomplete membranous signal in any proportion of cells</td>
<td>Incomplete membranous signal faint/barely perceptible within ≥10% cells</td>
</tr>
<tr>
<td>0 IHC</td>
<td>No signal</td>
<td>No signal OR incomplete membranous signal faint/barely perceptible within ≤10% cells</td>
</tr>
</tbody>
</table>
The Phenomenon of "Binning"

Continuous Variable

1 2 3 4
4 Bins for HER2 IHC

<table>
<thead>
<tr>
<th>Negative</th>
<th>Equivocal</th>
<th>Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1+</td>
<td>2+</td>
</tr>
</tbody>
</table>

A and B Same Shade of Gray?

A and B Same Shade of Gray?
Factors Affecting HER2 Test Accuracy by IHC

- Different primary antibodies, kits
- Sub optimized epitope retrieval methods
- Technical artifacts on slides (edge, crush)
- Inaccuracies inherent in ‘binning’
- Tendency to “up score” to avoid missing a positive case
- Suboptimal positive controls

Which part do you score?

Case 1442
Case 1442

- Appears heterogeneous, but this is largely related to partially crushed tissue
- In areas where tumor is uncrushed, signal is 1+
- In areas where tumor crushed, signal is 2+ - 3+
- Always score uncrushed tumor

Factors Affecting HER2 Test Accuracy by IHC

- Different primary antibodies, kits
- Sub optimized epitope retrieval methods
- Technical artifacts on slides (edge, crush)
- Inaccuracies inherent in ‘binning’
- Tendency to “up score” to avoid missing a positive case
- Suboptimal positive controls
Suboptimal HER2 IHC Controls

Optimal HER2 IHC Controls

0
1+
2+
3+

So Why Don’t We Just Use In Situ Hybridization Instead of IHC?
(F)ISH Also Has Accuracy Problems

Assessment Run H7 2015
HER2 ISH (CISH or FISH)

<table>
<thead>
<tr>
<th>Optimal</th>
<th>Good</th>
<th>Borderline</th>
<th>Poor</th>
<th>Suff.¹</th>
<th>Suff. OPS²</th>
</tr>
</thead>
<tbody>
<tr>
<td>28</td>
<td>25</td>
<td>15</td>
<td>10</td>
<td>68%</td>
<td>75%</td>
</tr>
</tbody>
</table>

Potential Sources of Error In Situ Hybridization

- Weak signal leading to undercounting
- Sampling error and bias in selecting cells ("cherry picking")
- Counting of overlapping cells
- Misidentification of invasive v. in situ carcinoma
- Reliance on CEP17 as reference gene

Signal Truncation
Case 3348

- 1+ by immunohistochemistry
- Metasystems count (390 tiles)
  - HER2 = 5.7
  - CEP17 = 4.1
  - HER2:CEP17 = 1.4 (Not amplified, score equivocal)
- Manual count (60 cells)
  - HER2 = 3.92
  - CEP17 = 2.17
  - HER2:CEP17 = 1.8 (Not amplified*)

*Scattered positive cells, less than 5% of population
Potential Sources of Error
In Situ Hybridization

- Weak signal leading to undercounting
- Sampling error and bias in selecting cells ("cherry picking")
- Counting of overlapping cells
- Misidentification of invasive v. in situ carcinoma
- Reliance on CEP17 as reference gene
SPREAD OF THE AMPLICONS ON CHROMOSOME 17

Different Results with Different Reference Genes

Change in HER2 Gene Status With Use of Alternative Chromosome 17 Reference Genes

Table 1. Comparison of HER2 Status Based on Chromosome 17 Reference Genes

<table>
<thead>
<tr>
<th>HER2 Status Based on CEP17</th>
<th>No. Nonamplified</th>
<th>No. Equivocal</th>
<th>Amplified No.</th>
<th>Amplified %</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonamplified</td>
<td>45</td>
<td>29</td>
<td>88</td>
<td>43.9</td>
<td>132</td>
</tr>
<tr>
<td>Equivocal</td>
<td>0</td>
<td>1</td>
<td>13</td>
<td>92.9</td>
<td>14</td>
</tr>
<tr>
<td>Amplified</td>
<td>0</td>
<td>0</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>30</td>
<td>96</td>
<td>171</td>
<td></td>
</tr>
</tbody>
</table>
Clinical validation of an array CGH test for HER2 status in breast cancer reveals that polysomy 17 is a rare event

Mod Pathol 22:1169-75, 2009

‘Pseudoamplification’ Owing to Loss of CEP17

- N = 6274 cases with IHC and FISH from 2014
- 40/6274 (0.64%) were IHC-negative but FISH-positive (discordant)
- Of the 40 cases, 15 (37.5%) are FISH “positive” only because their CEP17 ratio was below 1.8, not because of elevated HER2 signals

Fulton R and Gown AM, 2015 (unpublished data)
Case 16538
Metasystems Results

<table>
<thead>
<tr>
<th></th>
<th>HER2</th>
<th>CEP17</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3.70 mean signals per nucleus</td>
<td>1.60 mean signals per nucleus</td>
</tr>
<tr>
<td>HER2 / CEP17</td>
<td>2.2</td>
<td></td>
</tr>
</tbody>
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Result (2013 Guidelines): POSITIVE

‘Pseudoamplification’ Owing to Loss of CEP17

- N = 6274 cases with IHC and FISH from 2014
- 40/6274 (0.64%) were IHC-negative but FISH-positive (discordant)
- Of the 40 cases, 15 (37.5%) are FISH “positive” only because their CEP17 ratio was below 1.8, not because of elevated HER2 signals

Fulton R and Gown AM, 2015 (unpublished data)

ISH v. IHC

From 2007 ASCO-CAP Guidelines

“...There is no gold standard at present; no assay currently available is perfectly accurate to identify all patients expected to benefit or not from anti-HER2 therapy.”
Both FISH and IHC are Imperfect Tests

- Both are complex tests requiring highly skilled pathologists and technologists
- Both IHC and FISH can demonstrate high accuracy
- Both IHC and FISH have demonstrated considerable “real world” false negative/positive rates

Both FISH and IHC are Imperfect Tests

- This can result in discordant results on the same case when tested both by FISH and IHC
- This can result in discordant results on the same case when tested by two different laboratories

HER2 Assessment in the Future: RT-PCR?
HER2 by PCR?

Very High Concordance

Oncotype (RT-PCR) v. FISH

FISH Positive | FISH Negative
--- | ---
RT-PCR Positive | 55 (98%) | 11 (3%)
RT-PCR Negative | 1 (2%) | 408 (97%)
The relationship between quantitative human epidermal growth factor receptor 2 gene expression by the 21-gene reverse transcriptase polymerase chain reaction assay and adjuvant trastuzumab benefit in Alliance N9831

Edith A. Perez1, Frederick L. Baehner2, Steven M. Buzdar3, E. Aubrey Thompson4, Amy Liu5, D. J. Blum6, Fard Jamshidian7, Diana Chehabov8, Carl Yoshizawa9, Steven Shah10, Peter A. Kaufman11, Nancy E. Davidson12, Julie Gralow12, Yan W. Axmann13 and Karla V. Balmann1

• N9831 adjuvant trastuzumab for HER2+ tumors (N = 901 with consent and sufficient tissue)
• Does quantitative mRNA expression predict benefit from trastuzumab?

Rt-PCR vs. IHC

Perez E et al., Breast Cancer Res 17:133, 2015

Rt-PCR vs. FISH

Perez E et al., Breast Cancer Res 17:133, 2015
RT-PCR Measure of mRNA as Alternative to HER2 FISH or IHC?

- Using Central IHC as reference, RT-PCR positive concordance = 87.8%, negative concordance = 86.6%
- Using Central FISH as reference, RT-PCR positive concordance = 83.8%, negative concordance = 89.8%

Perez E et al., Breast Cancer Res 17:133, 2015

The relationship between quantitative human epidermal growth factor receptor 2 gene expression by the 21-gene reverse transcriptase polymerase chain reaction assay and adjuvant trastuzumab benefit in Alliance N9831


“We do not recommend HER2 testing by RT-PCR replacing IHC or FISH assays in standard practice.”

How Can We Ensure Accurate HER2 Results?

- Adherence to ASCO-CAP Guidelines
- Use experience of others - eg., NordiQC
- Proficiency testing
- Keep pathologists directly involved in evaluation of FISH and IHC
- Use of image analysis
- Be aware of HER2 testing performance (e.g., concordance rate or other measurement of accuracy and reproducibility)
Thank you for your attention.