Measurement of HER2

Daniel F. Hayes, MD
Clinical Director, Breast Oncology Program
University of Michigan Comprehensive Cancer Center
Why Test HER2?

• Trastuzumab
  – Metastatic
  – Adjuvant
• Lapatinib
  – Metastatic
  – Trials
• ? Selection of best or any chemotherapy
  – Adjuvant
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Adjuvant Trastuzumab: Combined Analysis
NSABP B-31 / NCCTG N9831

AC→TH 1672 96
AC→T 1679 194

HR=0.47, 2P=8x10^{-10}

DDFS

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- Lapatinib
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- ? Selection of best or any chemotherapy
  - Adjuvant
Chemotherapy ± Lapatinib in MBC

Reproduced with permission from Press. ASCO. 2007 (abstr 51).
Primary surgery: locally-determined HER2-positive invasive breast cancer

Centrally-determined HER2 positive

Complete adjuvant chemotherapy
Complete adjuvant radiation therapy (if given)

LVEF ≥50%

Randomization

Trastuzumab for 1 year

Lapatinib for 1 year

Trastuzumab for 3 months → (washout)
Lapatinib for 3 months (total 1 year)

Trastuzumab plus lapatinib for 1 year

Patients with ER- or PgR-positive tumors receive endocrine therapy
Selected according to menopausal status; administered concurrently with biologics and continuing for at least 5 years

OPEN IN NORTH AMERICA, WINTER 2008, WE HOPE
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- Selection of best or any chemotherapy
  - Adjuvant
HER2 Is Predictive of Paclitaxel Benefit by Estrogen Receptor Disease-Free Survival

Full text: HER2 Negative
- N=390 (29%)
- Paclitaxel vs. No paclitaxel

HER2 Positive
- N=144 (11%)
- Paclitaxel vs. No paclitaxel

ER Negative
- N=1322
- Paclitaxel vs. No paclitaxel

ER Positive
- N=703 (53%)
- Paclitaxel vs. No paclitaxel

What Is Different About HER2 (and Predictive Markers in General)?

• HER2 test is not a simple adjunct to anatomic pathology to confirm a tissue diagnosis
  – Assays are being used as the sole determinant of treatment selection
    • Although HER2 predicts benefit, the big issue is NO BENEFIT:
  • Should we withhold therapy from a group of patients in whom it might improve survival?
Assays Used for HER2 Testing

• Immunohistochemistry assays
  – CTA CB11 and 4D5 MoAbs (not commercial)
  – HercepTest A085 polyclonal Ab
  – Pathway CB11 MoAb

• Fluorescence in situ hybridization assays
  – PathVysion \( \textit{HER2}:\text{CEP17} \) ratio
  – pharmDx kits \( \textit{HER2}:\text{CEP17} \) ratio
  – Inform kit \( \textit{HER2} \) gene copy number

• ISH assays without fluorescence (not approved in the U.S.)
  – SPoT-Light Chromogenic ISH
  – EnzMet GenePro Silver enhanced ISH
A Recipe for Problems

• HER2 testing has been done in a decentralized fashion

• US FDA allows individual anatomic path labs to develop and use their own “home brew” assays if:
  – Use FDA-approved analyte specific reagents
  – Lab is CLIA approved

• Assay validation is not the norm

• Ongoing proficiency testing is not the norm
Problem?

• Would you give (or take!) a drug that:
  – Was made in a laboratory next to your clinic?
  – You were not sure of the dose?
  – You were not sure of what it was mixed in?
  – It seems close to the drug that has been tested, but the laboratory that made the one you are going to use has never validated that their drug works as well (or at all)?
## HER2 Testing Concordance in N9831

### Concordance Central vs Local Lab

<table>
<thead>
<tr>
<th></th>
<th>JNCI 2002 (total N=119)</th>
<th>ASCO 2004 (total N=976)</th>
<th>JCO 2006 (total N=2535)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC 3+ (HercepTest)</td>
<td>74%</td>
<td>79.5%</td>
<td>82%</td>
</tr>
<tr>
<td>FISH + (PathVysion)</td>
<td>67%</td>
<td>85%</td>
<td>88%</td>
</tr>
</tbody>
</table>
ASCO-CAP HER2 Initiative

• Convened during Fall 2005 through Summer 2006
• Multi-disciplinary expertise
• Assumed that the answer to “Should you measure HER2?” is Yes
• Developed guidelines for:
  – Clinical algorithm
  – Pre-analytical handling
  – Accreditation


HER2 Testing Algorithm
Adjuvant Trials and Clinical Practice

IHC

3+

2+

0 and 1+

Reflex FISH

Amp

No Amp

Trastuzumab
ASCO/CAP Testing Algorithms

**Immunohistochemistry**

ASCO/CAP Testing Algorithms

Fluorescent In Situ Hybridization

Breast cancer specimen (invasive component)

- HER2 testing by validated FISH assay for HER2 gene amplification
  - Positive for HER2 gene amplification (FISH ratio > 2.2 or HER2 gene copy > 6.0)
  - Equivocal for HER2 gene amplification (FISH ratio 1.8-2.2 or HER2 gene copy 4.0-6.0)
  - Negative for HER2 gene amplification (FISH ratio < 1.8 or HER2 gene copy < 4.0)

Count additional cells for FISH or retest, or test with HER2 IHC

Equivocal HER2 gene amplification result (Patients with HER2/CEP17 ratio ≥ 2.0 were eligible for the adjuvant trastuzumab trials)

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HER2 Antigen Becomes “Hidden” With Formalin Fixation/Paraffin Embedding

HER2 specimen amplified 2- to 5-fold by Southern Hybridization

HER2 “negative” status is a fixation artifact that requires antigen retrieval for “correction”

Photos courtesy of Michael Press.
FISH Can Also Be Affected by Formalin Overfixation

FISH not interpretable
(sample fixed in formalin over the weekend…)

Photo courtesy of Dr. Elizabeth Hammond.
Sources of HER2 Testing Variation

<table>
<thead>
<tr>
<th>Preanalytic</th>
<th>Guideline Recommendation: “…samples for HER2 testing are fixed in neutral buffered formalin for 6-48 hours.”</th>
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</thead>
<tbody>
<tr>
<td>Time to fixation</td>
<td></td>
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<tr>
<td>Method of tissue processing</td>
<td></td>
</tr>
<tr>
<td>Time of fixation</td>
<td></td>
</tr>
<tr>
<td>Type of fixation</td>
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<table>
<thead>
<tr>
<th>Analytic</th>
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<tbody>
<tr>
<td>Assay validation</td>
<td></td>
</tr>
<tr>
<td>Equipment calibration</td>
<td></td>
</tr>
<tr>
<td>Use of standardized laboratory procedures</td>
<td></td>
</tr>
<tr>
<td>Training and competency assessment of staff</td>
<td></td>
</tr>
<tr>
<td>Type of antigen retrieval</td>
<td></td>
</tr>
<tr>
<td>Test reagents</td>
<td></td>
</tr>
<tr>
<td>Use of standardized control materials</td>
<td></td>
</tr>
<tr>
<td>Use of automated laboratory methods</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Postanalytic</th>
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</thead>
<tbody>
<tr>
<td>Interpretation criteria</td>
<td></td>
</tr>
<tr>
<td>Use of image analysis</td>
<td></td>
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<tr>
<td>Reporting elements</td>
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<tr>
<td>Quality assurance procedures</td>
<td></td>
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<tr>
<td>Laboratory accreditation</td>
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<tr>
<td>Proficiency testing</td>
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ASCO-CAP HER2 Initiative

The panel recommends that HER2 testing be done in a CAP-accredited laboratory or in a laboratory that meets the accreditation and proficiency testing requirements set out by this document.
“The Panel recommends that HER2 testing be done in a CAP-accredited laboratory or in a laboratory that meets the accreditation and proficiency requirements set out by this document”
HER2 Testing

So... now we have solved everything, right?

NO
What About HER2 Subsets?

• HER2 test discordants? (~4% of specimens)
  – IHC positive / FISH negative
  – IHC negative / FISH positive

• HER2 test negative? (80% of specimens)

• HER2 polysomy (~8% of specimens)
  – Polysomy chromosome 17
  – Low levels of protein expression
## Mortality by FISH Positive vs Negative

*(relative risk, 95% CI)*

<table>
<thead>
<tr>
<th>H648g</th>
<th>2+ and 3+</th>
<th>3+</th>
<th>2+</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td>0.80 (0.64-1.00)</td>
<td>0.70 (0.51-0.90)</td>
<td>1.26 (0.82-1.94)</td>
</tr>
<tr>
<td></td>
<td>N=469</td>
<td>N=349</td>
<td>N=120</td>
</tr>
<tr>
<td>FISH positive</td>
<td>0.70 (0.53-0.91)</td>
<td>0.67 (0.51-0.89)</td>
<td>1.31 (0.53-3.27)</td>
</tr>
<tr>
<td></td>
<td>N=325</td>
<td>N=293</td>
<td>N=32</td>
</tr>
<tr>
<td>FISH negative</td>
<td>1.06 (0.70-1.63)</td>
<td>0.88 (0.39-1.98)</td>
<td>1.11 (0.68-1.82)</td>
</tr>
<tr>
<td></td>
<td>N=126</td>
<td>N=43</td>
<td>N=83</td>
</tr>
</tbody>
</table>

Retrospective, unplanned.

**Treatment Outcome in N9831 as a Function of HER2 Overexpression or Amplification**

<table>
<thead>
<tr>
<th>HER2 Assay Result*</th>
<th>Number of Patients</th>
<th>HR for DFS† (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IHC 3+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FISH Positive</td>
<td>1170</td>
<td>0.42 (0.27-0.64)</td>
</tr>
<tr>
<td>FISH Negative</td>
<td>51</td>
<td>0.71 (0.04-11.79)</td>
</tr>
<tr>
<td>FISH Unknown</td>
<td>51</td>
<td>0.69 (0.09-5.14)</td>
</tr>
<tr>
<td>IHC 0, 1+, or 2+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FISH Positive</td>
<td>174</td>
<td>1.01 (0.18-5.65)</td>
</tr>
</tbody>
</table>

* IHC by Herceptest, FISH by PathVysion as performed at a central laboratory.
†Hazard ratio: risk of recurrence, second primary malignancy, or death in the trastuzumab plus chemotherapy vs the chemotherapy arm; estimated by Cox regression stratified by number of positive nodes and hormone receptor status.

ASCO 2007 Oral Presentations

• Updated results of the combined analysis of NCCTG N9831 and NSABP B-31 adjuvant chemotherapy with/without trastuzumab in patients with HER2-positive breast cancer (Perez, Abstract 512)

• Benefit from adjuvant trastuzumab may not be confined to patients with IHC 3+ and/or FISH-positive tumors: Central testing results from NSABP B-31 (Paik, Abstract 511)

• CALGB 150002: Correlation of HER2 and chromosome 17 copy number with trastuzumab efficacy in CALGB 9840, paclitaxel with or without T in HER2-positive and HER2-negative metastatic breast cancer (Kaufman, Abstract 1009)
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## HER2 Central Testing: N9831

**Results of IHC/FISH and DFS**

<table>
<thead>
<tr>
<th>IHC Result</th>
<th>FISH Ratio</th>
<th>N=1842</th>
<th>Events (N)</th>
<th>HR</th>
<th>95% CI</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>A</td>
<td>C</td>
<td>A</td>
<td>C</td>
<td></td>
</tr>
<tr>
<td>3+</td>
<td>≥2.0</td>
<td>715</td>
<td>690</td>
<td>116</td>
<td>58</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td>&lt;2.0</td>
<td>30</td>
<td>23</td>
<td>5</td>
<td>3</td>
<td>0.61</td>
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<tr>
<td>0, 1, 2+</td>
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<td>95</td>
<td>123</td>
<td>7</td>
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<td>9</td>
<td>0.51</td>
</tr>
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</table>

RR of ACTH/ACT for RFI (NSABP B-31)

- FISH+ (1588)
- FISH- (207)
- IHC 3+ (1488)
- IHC <3 (299)
- FISH- and IHC <3 (174)

Reproduced with permission from Paik. ASCO. 2007.
Does Trastuzumab Work in HER2 Low or Negative Patients??

• Possible answers:
  – Yes
  – No
  • Retrospective, unplanned, partial subset analysis
  • All of these patients were “POS” somewhere
  • Biological plausibility?
Tumor Marker Development: The Problems and Pitfalls of Translating Laboratory Observations to Clinical Utility: It Isn't Easy!

2007 ASCO Extended Educational Session

“If you torture the data long enough it will confess to anything”

Lisa McShane, PhD

Explanations: chance, technical, biological?
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HER2 FISH Patterns

FISH Amplified
FISH Not amplified
Aneusomy Not amplified

Photos courtesy of Dr. Ken Bloom.
CALGB 9840: Paclitaxel Every 1 vs 3 Weeks; Trastuzumab vs Nil in “HER2 Negative”

- Weekly vs 3-weekly paclitaxel
- 288 patients with HER2 negative randomized: trastuzumab vs not

Possible explanations:
1) Not real?
2) False negative HER2 assay?
3) Change in HER2 status?


N (events/pts) =

(Adjusted HR=1.45, $P=0.0008$)
# CALGB 9840: Central Testing

| HER2-negative subjects in C9840 | 585 |
| Tissue blocks available for C150002 | 303 |
| HER2:CEP17 FISH ratio <2 | 192 |
| CEP17 copy number > 2.2 (polysomy) | 38 |
| IHC 0-2+ / IHC 3+ | 34 / 3 |

<table>
<thead>
<tr>
<th>RESPONSE in FISH Ratio &lt;2</th>
<th>PAC</th>
<th>PAC + Trastuzumab</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No polysomy</td>
<td>18/50 (36%)</td>
<td>19/53 (36%)</td>
<td>NS</td>
</tr>
<tr>
<td>Polysomy</td>
<td>5/19 (26%)</td>
<td>12/19 (63%)</td>
<td>P=0.048</td>
</tr>
</tbody>
</table>

Kaufman. ASCO. 2007 (abstr 1009).
Questions Raised by These Data

• Do patients with HER2-negative disease benefit from trastuzumab? Hypothesis
  – Does it represent undetected HER2 heterogeneity in primary tumor? Clonal evolution in the metastasis?

• Is polysomy associated with protein expression? Yes

• Does polysomy predict treatment benefit? Hypothesis

• Is one measure of HER2 (gene or protein) superior to the other? No (a few disagree)

• What about discordant results (~4% of all specimens)? Larger numbers needed, may not matter
Next Steps

• Retrospective subset analyses regarding benefit from trastuzumab in patients with HER2-negative disease (including those with HER2 polysomy) are hypotheses-generating

• Data from individual adjuvant trials should be pooled to improve precision and test reproducibility of these initial observations

• If confirmed, prospective randomized trials targeting well-defined patient subgroups should be considered to test these intriguing hypotheses

For now, status quo (ie, high-quality testing/reporting)
Cancer Therapy: Take Aim…

The Economist, June 7, 2007.
Acknowledgements:
Joint CAP/ASCO HER2 Panel

- Antonio C. Wolff (co-Chair)
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- Jared Schwartz (co-Chair)
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- Richard J. Cote
- Mitchell Dowsett
- Patrick L. Fitzgibbons
- Wedad M. Hanna
- Amy Langer
- Lisa McShane

- Soonmyung Paik
- Mark D. Pegram
- Edith A. Perez
- Michael F. Press
- Anthony Rhodes
- Catharine Sturgeon
- Sheila Taube
- Raymond Tubbs
- Gail H. Vance
- Marc van de Vijver
- Thomas M. Wheeler