HER2 Testing in Breast Cancer

- No Conflict of Interest to Report

Human Epidermal Growth Factor Receptor 2-HER2

- Human epidermal growth factor receptor 2, is also known as ERBB2.
- Tyrosine kinase receptor.
- Member of family of transmembrane receptor proteins:
  - EGFR (HER1)
  - HER2, HER3, HER4
HER Family of Proteins

- As tyrosine kinases, proteins involved in:
  - Cell growth
  - Cell survival
  - Cell differentiation
  - Critical role in oncogenesis

HER Family of Proteins

- Activated by ligand-induced dimerization or receptor pairing.
- Leads to phosphorylation of tyrosine residues initiating signal transduction via the RAS/MAP kinase and PI3K/ pathways.

http://www.biooncology.com/biological-pathways/her-signaling
HER2
- HER2 (ERBB2) is a 185-kd membrane receptor protein with tyrosine kinase activity.
- Gene is located at 17q21.
- Over-expression in 15-25% of breast cancers is usually associated with gene amplification.

HER2 Testing
- HER2 positivity correlates with poor prognosis and:
  - Aggressive clinical course
  - Lymph node positivity
  - High nuclear grade
  - High proliferative activity

Anti-HER2 Therapy
- Goal: block TK signaling.
- First developed by Genentech 1990’s.
- Humanized monoclonal antibody inhibits dimerization via domain IV of HER2-Trastuzumab.
- FDA approved following safety and efficacy studies.
Anti-HER2 Therapy-Dual Blockade

- Pertuzumab—recombinant, humanized monoclonal antibody exerts effect thru extracellular domain II.
- Lapatinib—dual (EGFR & HER2) TK inhibitor exerting effect intracellular Ser/Tyr protein kinases.

HER2 Testing

- Laboratory analysis:
  - Immunohistochemistry—measuring the amount of protein receptor on the cell membrane.
  - FISH—determining presence of gene amplification.
  - SISH, CISH.
Methods for HER2 Detection (IHC and FISH)

HER2 Testing

- Accurate determination important because:
  - Availability of anti-HER2 therapy for metastatic disease or adjuvant therapy.
  - Predicts response to adjuvant anthracycline-based chemotherapy /taxane therapy.
  - Risk benefit ratio of anti-HER2 therapy.

HER2 Testing

- Laboratory evaluation performed on formalin-fixed, paraffin-embedded tissue.
- Tissue should be fixed in formalin for 6-72 hours before processing.
- Non-formalin-based fixatives are discouraged.
**HER2 Testing**

- FISH is morphologically-driven.
- Uses fluorescent probes to enumerate HER2 and centromere 17 copy number.
- Single HER2 probe assays.
- Dual probe, dual color HER2 probe and centromere 17 probe assays determine ratio (HER2 nominator/CEP17 denominator).

**HER2 Testing**

- Standardization of assay necessary and include:
  - Pre-analytic variables
  - Analytic variables
  - Post-analytic variables

**HER2 Testing**

- Pre-analytic variables:
  - Invasive breast cancer
  - Formalin-fixed tissue (10% neutral buffered formalin)
  - 4-5 um sections on positively-charged slides
  - Validation of method:
    - Familiarization
    - Pilot study
    - Clinical study
**Probe Validation Procedure – IU Cytogenetics Lab**

- **New Probe Enters the Lab**
  - Familiarization
  - Analytical Validation:
    - 20 sets of 50 nuclei (karyotypically normal male controls)
  - Pilot Study
  - Clinical Study (Blinded)
  - Analytical Validation:
    - 20 sets of 50 nuclei
    - 10 abnormal and 10 normal patients
  - Interpretation:
    - 2 sets of 200 nuclei
    - 10 abnormal and 10 normal patients

**HER2 Testing**

- **Analytic variables:**
  - Trained-technologists
  - Proper equipment/filters
  - Scoring criteria including:
    - non-overlapping nuclei;
    - two different areas;
    - two different readers;
    - all cells must have one HER2/one centromere signal

- **Post-analytical variables:**
  - Control Review (performed as expected)
  - Result interpretation
  - Result reporting-FDA Disclaimer
  - Experience, PT, CME
ASCO/CAP Guidelines For HER2 Testing

- Variability in results between local and central labs for both IHC and FISH led to ASCO/CAP guidelines published in 2007:
  - Archives of Laboratory Medicine and Pathology. 2007; 131:18-43.

Why Guidelines? HER2 Testing Often Inaccurate

- Three studies documented high frequency of false positive IHC tests among laboratories (range 3–50%; average 18%).
- FISH testing also associated with inter-laboratory variability, disagreement levels ranging from 5–23%, (average 13%).

Source: Comparative studies of National Clinical Trial Groups with Institutional Pathologist Performance (NSABP and Intergroup Studies)

Highlights of ASCO/CAP 2007 Guidelines for HER2

- "Equivocal" result category
- New (more stringent) Scoring Criteria for IHC.
- Testing Algorithm established.
- Test validation requirements.
- QA/Proficiency testing requirements.
- Reporting elements.
- Interpretation and Rejection Criteria.
2007 IHC Scoring Criteria

- **Score 3+:**
  - Uniform intense membrane staining of more than 30% of invasive tumor cells.

- **Score 2+:**
  - Complete membrane staining that is non-uniform or weak but with obvious circumferential distribution in at least 10% of cells, or intense complete membrane staining in less than 30% of tumor cells.

- **Score 1+:**
  - Weak, incomplete membrane staining in any proportion of invasive tumor cells, or weak, complete membrane staining in less than 10% of cells.

- **Score 0:**
  - No staining is observed in invasive tumor cells.

Assessing HER2 with IHC

Herceptin Treatment Indicated for 3+ and some 2+

New “Equivocal” Result

- Three categories of test results for each test:
  - **Positive:**
    - FISH HER2 gene/CEP17 ratio of 2.2 or greater or
    - FISH HER2 gene copy number of >6.0
  - **Equivocal:**
    - FISH HER2 gene/CEP17 ratio of 1.8-2.2 or
    - FISH HER2 gene copy number of 4.0-6.0
  - **Negative:**
    - FISH HER2 gene/CEP17 <1.8 or
    - FISH HER2 gene copy number of <4.0
Method Validation Requirements

- Testing must be initially validated using 25-100 formalin-fixed samples using standard operating procedure.
  - Parallel testing by an alternative method (e.g., FISH) or parallel testing by same method in another lab with validated assay.
- Concordance between methods or labs must be:
  - 95% for positive HER2 test, and
  - 95% for negative HER2 test.
  - Equivocal cases not included.
ASCO-CAP 2007 HER2 Guidelines

- Considered a “living document”.
- All guidelines subject to review and revision.
- Discussions on genetic heterogeneity, polysomy, binary result for treatment.

New Expert Panel Convened in 2012

Steering Committee Members:
- Antonio Wolfe
- Elizabeth Hammond
- David Hicks
- Mitch Dowsett
- Lisa McShane

Goal:
- Update 2007 ASCO/CAP Guidelines to improve the accuracy of HER2 testing and its utility as a predictive marker in invasive breast cancer.

ASCO/CAP HER2 Guidelines 2013

- HER2 testing performed on all pts with invasive breast cancer.
- HER2-positive interpretation (IHC or ISH) when >10% of contiguous cells demonstrate overexpression or gene amplification.
- Repeat testing should be considered if results discordant with histology.
ASCO/CAP HER2 Guidelines 2013

- Reviewed more than 70 new publications.
- Reviewed data from clinical trials:
  - Ex. Initial data from ALTTO trial suggested decrease in false positive calls, <6% comparison of local to central lab.
- Concerns by individuals or groups:
  - Regarding false negative rate
  - Equivocal calls
- Account for new technology.

Comparison of HER2 Guideline 2007 and 2013
Recommendations – What’s Changed?

<table>
<thead>
<tr>
<th>Specimens to be Tested</th>
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<tbody>
<tr>
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<tr>
<td>All primary breast cancer specimens and metastases should have at least one HER2 test performed</td>
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<tr>
<td>2013 Guideline Update</td>
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<tr>
<td>Most request HER2 testing on every primary invasive breast cancer and metastatic sites (if tissue specimens available)</td>
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Comparison of HER2 Guideline 2007 and 2013
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The ASCO/CAP HER2 Guideline and ER/PgR Guideline now have the same parameters for the length of fixation.
### Comparison of HER2 Guideline 2007 and 2013 Recommendations – What’s Changed?

#### Interpretation Criteria: HER2 Positive

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#### Interpretation Criteria: HER2 Negative

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<td>IHC Negative</td>
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**Note:** Criteria have been clarified because Bright Field ISH is now approved for testing.

**Example:**
New Test Interpretation Category

- **Indeterminate**

Clinical Consequence of New Guideline?

- One institutional retrospective study to evaluate test interpretation differences of 2007 when compared to 2013 ASCO/CAP guidelines.
- Study population: 2364 patients referred for HER2 testing from 2008 to 2013.
Clinical Consequence of New Guideline

- All cases scored for 60-90 cells by two or three readers.
- Data first analyzed using 2007 ASCO/CAP guideline and then re-analyzed using 2013 ASCO/CAP guideline.

Study Findings

- Range for **positive** calls with 2007 guideline was 12%-19%.
- Range for **negative** calls with 2007 guideline was 77-86%.
- Range for **equivocal** calls with 2007 guideline was 2-5%.

Study Findings (continued)

- Range for **positive** calls with 2013 guideline increased by 1.63% (p=0.0001).
- Range for **negative** calls with 2013 guideline decreased by 2.33% (p=0.0018).
- Range for **equivocal** calls with 2013 guideline increased by 0.65% (not statistically significant).
Study Conclusions

- The number of negative test results by FISH analysis significantly decreased while the number of positive test results significantly increased when the same patient data was re-analyzed by ASCO/CAP 2013 HER2 Guideline.
- A shift in test interpretation will likely result in increased numbers of patients treated with anti-HER2 therapy.

Study Conclusions (continued)

- Comparative studies from other institutions
- Follow-up of clinical consequences for these patients for improved treatment and treatment outcomes

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  - Matthew Swecker
- Sujata Chakraborty, PhD
THANK YOU