Markers and Regulatory Issues in Breast Cancer Diagnosis

Ann Thor M.D.

International Society of Breast Pathology
The primary purpose of the ASCO/CAP guidelines for ER, PgR and Her2 testing in invasive breast cancer is:

A. To educate practitioners about the biology underlying breast cancer and the role these antigens play in breast carcinogenesis
B. To improve the accuracy of ER, PgR and Her2 testing in clinical practice and their utility as prognostic and predictive markers
C. To teach pathologists how to generate quantitative data from immunohistochemical assays
D. To reconcile guideline inconsistencies in test evaluation
There is considerable variance in the sensitivity and specificity of immunohistochemical assays for ER and PgR. Which of the following factors will not influence the test results?

A. Time of fixation in 10% buffered formalin
B. Cold ischemia time
C. Cut point (percent positive cells with nuclear staining) used by the pathologist to determine positivity
D. Vendor source for FDA approved IHC antibodies against ER and PgR
Many breast biopsies are currently performed in surgical clinics or radiology. In this setting, cold ischemia time may be quite variable. Which of the following statements is not true?

A. The ASCO/CAP Guidelines from 2007 and 2011 do not provide recommendations about remotely derived specimens.
B. Cold ischemia time appears to affect immunohistochemical assays more than in situ hybridization assays.
C. Cold ischemia time is the time from tissue biopsy or excision to appropriate fixation.
D. Cooling of the tissue sample after removal from the patient may reduce the influence of prolonged cold ischemia time on in situ hybridization data.
Breast Cancer Biomarkers

• **Prognostic**
  – outcome independent of treatment

• **Predictive**
  – outcome in context of treatment

• **Molecular**
  – Critical molecular pathways
  – Genetic alterations that predict risk or therapeutic response
  – Molecular subtyping to facilitate *Personalized Medicine* approaches *including* expression arrays, sequencing
Regulatory Framework Applies to Breast Cancer Marker Testing

- **CLIA ’88’**
  - Stringent quality standards
  - External quality controls including proficiency standards for all highly complex tests.
  - Predictive assays by definition highly complex

- **FDA**
  - Regulation of medical devices since 1976.
  - Marker reagents and kits recognized to have high impact on patient mortality and morbidity, therefore, requires guideline recommendation adoption without modification
“Testing Scandal Shines Spotlight on Black Box of Clinical Laboratory Testing” JNCI 2008

• “public inquiry into botched estrogen receptor tests has shaken public confidence in cancer care…”

• “between 1997 and 2005 nearly 400 of 1,000 breast cancer patients received incorrect test results of the ER status…of these patients, more than 100 have died”
And in other headlines...........

Up to 20% of IHC data for ER and PR are inaccurate (false negative or false positive)

Discrepancies between labs, and assays are significant in HER2 testing
Marker testing in an era of scrutiny…
a difficult but important journey
NCCN IHC Guidelines: 2009

- Hormone receptor data: a strong predictor of response to endocrine therapy

- ER and PR assays: for all new invasive and in situ breast cancers

- All labs performing ER and PR by IHC should undergo validation studies (technical and clinical)

- All labs must follow ASCO/CAP guidelines

ASCO/CAP Guidelines: ER and PR Testing

J Clin Oncol, April 2011  www.asco.org/guidelines/erpr

• Primary goal is to improve the accuracy

• Most of the inaccuracy is secondary to
  – pre-analytic variables
  – thresholds for positivity and interpretive criteria

• Resolve discrepancies in prior guidelines for
  – cold ischemia time
  – handling of remote specimens
  – fixation time
  – selection of optimal sample for testing
Cold Ischemia Time: < 1 hour, must record and report

Only 10% NBF

Fixation time 6-72 hours, must record and report

Multiple large core biopsies preferable to resection specimen

Use cut unstained slides in < 6 weeks
Interpretive Guidelines 2011

Positive: \( \geq 1\% \) of tumor nuclei are positive
   Report percentage of positive
   Report intensity (weak, moderate or strong)
   May also use a composite score

Negative: if < 1\% of tumor nuclei are positive

Equivocal

Uninterpretable:
   Internal or external controls are not as expected
   (positive and/or negative), must repeat
CAP Lab Accreditation Elements

Validation:
before test is offered
With changes in the testing system

QA and QC:
Quality control and equipment maintenance
Initial and ongoing lab personnel training and competency
External controls
Ongoing competency and education for pathologists
External proficiency testing (2 tests/year)
Biannual on site inspections and annual self-inspection
New CAP Checklist Items
2011 (ANP.22970)

• Monitor and record institutional/lab incidence of ER negative breast cancers…should not exceed 30% (invasive and in situ)

• Must be annual competency assessment and education of pathologists; enroll in PT testing by 2011, monitor performance by 2012

– See CAP Reference Resources
Image Analysis Aided Interpretation of Hormone Receptors

- FDA approved equipment, software, protocols

- Must follow recommended protocols, report any discrepancies

- More expensive, billing codes unique
HER2

The most highly regulated marker in Anatomic Pathology
HER-2 Testing

• There is no GOLD STANDARD for prediction!

• Primary and metastatic lesions by IHC or FISH demonstrate up to 20% variance.

• Wide variation in lab to lab performance of HER2 tests
  • NSABP studies indicate 18% of community-based assays were not confirmed
HER2 and Treatment

• HER2 overexpressed/amplified tumors are eligible for Trastuzumab (Herceptin) therapy

• Cost for 1 year of trastuzumab exceeds $100,000
HER2 Assays

New Algorithm:

IHC or FISH

Lab must document:

- Proof of initial test validation, revalidate if change in procedure or reagent
- Ongoing Internal QA
- External proficiency testing
- Current accreditation by valid agency
CAP Algorithm for HER2 Test

**HER2 positive**
- a. HER2 IHC 3+ (>30% of tumor cells are circumferentially intensely stained)
- b. FISH amplified (ratio of HER2 to Cep17 > 2.2 or HER2 > 6 copies)

**HER2 equivocal**
- a. HER2 IHC 2+
- b. HER2 IHC 3+ ≤ 30%
- c. FISH (ratio of HER2 to Cep 17 1.8 – 2.2 or HER2 4 – 6 copies)

**HER2 negative**
- a. HER2 IHC 0 or 1+
- b. FISH (ratio of HER2 to Cep 17 < 1.8 or HER2 copies < 4)
## Reconciliation Guide for HER2 Testing

April 2011

<table>
<thead>
<tr>
<th>Requirements</th>
<th>Guidelines</th>
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<tbody>
<tr>
<td>Cold Ischemia Time</td>
<td>≤ 1 hour</td>
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<tr>
<td></td>
<td>*FISH most sensitive to CI</td>
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<tr>
<td>Remote Specimens</td>
<td>Follow ER guidelines</td>
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<tr>
<td>Fixation time 10% NBF</td>
<td>6-48 hours*</td>
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<tr>
<td>Optimal sample</td>
<td>Multiple core bx</td>
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</table>
HER2 FISH/CISH Testing

- Typically performed on IHC indeterminate cases or by choice of physician, patient, clinical trial, institution
- More sensitive to cold ischemia
- Less rapid, more technical, more expensive, may need to be ordered or approved reflex to be reimbursed
- Single or dual probe
CAP Reporting Recommendations

• FDA approved test kits do not require additional reporting information

• FDA approved test kits where changes have been made require validation and reporting language to indicate changes from protocol in the final report

• Non-FDA approved antibodies require validation against an FDA approved test kit and reporting language indicating a non-FDA approved antibody and methodology. Methodology must be included in final report
1. Participation in external proficiency testing program (2 test events/year)
2. Satisfactory performance requires $\geq 90\%$ correct responses for each test
3. Unsatisfactory performance will require laboratory to respond to accreditation agency program requirements
4. Unsatisfactory performance on site visit will result in suspension of the laboratory testing for HER2 by that method
Be aware of Jan 2007 HER2 Data Use Guidelines

• Equivocal data may be sufficient to treat with trastuzumab
  – HER2 IHC 2+ with uniform, intense, circumferential staining of membrane involving > 10% but less than 30% of invasive cancer
  – HER2 FISH ratio > 2
Summary

• ER, PR and HER 2 Testing is highly regulated

• Rules and Regs a shifting target, follow CAP, ASCO, ASCP references and tutorials to keep up to date.
Umbilical cord: Dr. S Lewis