Beyond H&E: What is the Role for Pathologists in the Future?

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Disclosures

- No disclosures relevant to the topic of this presentation
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- Scientific Advisory Board member for Otsuka Pharmaceuticals
Tissue Biomarkers

- Oncologists have multiple goals when incorporating new biomarkers into clinical practice:
  - Individualize therapy for patients
  - Improve cancer outcomes

- How can pathologists help us accomplish that?
  - Do things well that oncologists need
  - Don’t do things that oncologists don’t want
    - Dialogue with oncologists prior to implementing new methodology and technology
symposium article

Pathology: is it still necessary?

G. Viale

Department of Pathology, European Institute of Oncology, University of Milan School of Medicine, Milan, Italy

Presented in the ESMO Conference Lugano, 3-6 July 2008: Lugano, Switzerland “Hot Topics in Breast Cancer” session
What Do Pathologists Do Currently?

- Diagnose cancer
  - Distinguish malignant vs normal tissue
  - Identify tissue type/differential diagnosis (breast, lung, colon, etc)
  - Further characterize cancer (tumor grade, margin status, etc)
- Evaluate for presence of regional metastases
- Measure biomarkers
Current Pathology Assessment: H&E and IHC

- Typical pathology assessment of a breast tumor includes:
  - Size, tumor grade, margin status, angiolympathic invasion
  - Lymph node involvement
  - ER, PR, Her2/neu expression

- Oncologists use this information for:
  - Estimation of prognosis
  - Individualization of treatment-decision making
What Do Pathologists Do Currently?

- **Diagnose cancer**
  - Distinguish malignant vs normal tissue
  - Identify tissue type/differential diagnosis (breast, lung, colon, etc)
  - Further characterize cancer (tumor grade, margin status, etc)
  - Evaluate for presence of regional metastases
  - Measure biomarkers
Cancer Biomarkers

- Standard tissue-based biomarkers currently assessed in solid tumors
  - Breast: ER, PgR, Her2/neu, multigene assays
  - Colon: Kras
  - Gastric: ?Her2 (possibly coming soon)

Allegra et al JCO 2009
Harris et al JCO 2007
Locker et al JCO 2006
When is a New Marker Clinically Useful?

- It is either **prognostic** or **predictive**
- It provides **additional information** to that already available to the clinician
- The **magnitude** of effect is sufficiently large that clinical decisions based on the data result in outcomes that are acceptable
  - *Greater chance for benefit*
  - *Smaller toxicity risk*
- The estimate of magnitude of effect is **reliable**
  - *Assay is accurate and reproducible*
  - *Clinical trial/marker study design is appropriate*
  - *Results are validated in subsequent well-designed studies*

Henry and Hayes Oncologist 2006
PURE PROGNOSTIC FACTOR (Unfavorable)

From DF Hayes, MD
PURE PREDICTIVE FACTOR

(For Sensitivity to Therapy)

100% cure
50% cure
10% cure

No Therapy
Factor 1 Neg
Factor 2 Neg

Therapy
Factor 1 Pos
Factor 2 Pos

P = <0.05

From DF Hayes, MD
MIXED PREDICTIVE FACTOR

100% cure

50% cure

10% cure

HER-2 +

No Rx

Rx 1

Rx 2

Rx 3

Poor Prognostic

Moderately Favorable for A Second

Strongly Favorable for A Third

Unfavorable Predictive for One Therapy

Factor 1 Neg

Factor 1 Pos

Rx 1 Pos

Rx 1 Neg for SERM Rx

Rx 2 Neut or Pos for Dox ChemoRx

Absolute Pos for Trastuzumab

Bad
Where is Pathology Going in the Future?

OLD

H&E

ER

PgR

Her2

NEW

Van de Vijver et al. NEJM 2002
What do Oncologists Want?

- Oncologists would like the following:
  - More accurate and reliable predictive information
  - More detailed prognostic information
  - Information about host and tumor influences on response and toxicity
Assay Accuracy, Reliability, and Reproducibility

Example: Her2 Testing
Assay Reproducibility

Several Technical and Biological issues

- The same assay gives the same readout results in the same hands
- The same assay gives the same readout results in different hands
- A different assay gives the same outcome results as the first assay
  - Response
  - Progression-free Survival
  - Overall Survival
Adjuvant Trastuzumab Trials

Study Designs

### NSABP B-31
- 4 cycles
- Dox/Cyc
- Paclitaxel q 3 wk
- Trastuzumab
- 4 cycles
- Paclitaxel q 3 wk

### NCCTG 9831
- 4 cycles
- Dox/Cyc
- Paclitaxel q wk
- Trastuzumab weekly
- 12 wks
- 52 wks

### HERA
- 1 Yr
- No therapy
- Standard ChemoRx
- Trastuzumab
- Trastuzumab
- 2 Yr

### BCIRG 006
- 4 cycles
- Dox/Cyc
- Docetaxel x 4
- Trastuzumab x 1 yr
- 6 cycles
- Dox/Cyc
- Carboplatin
- Trastuzumab x 1 yr
**Combined B31/9831 Analysis**

**Survival Data**

**Disease-free Survival**
- Trastuzumab: 87% at 4 years, 85% at 5 years
- Control: 75% at 4 years, 67% at 5 years
- HR = 0.48 (95% CI, 0.39, 0.59)  
  *p < 0.0001*

**Overall Survival**
- Trastuzumab: 94% at 4 years, 91% at 5 years
- Control: 92% at 4 years, 87% at 5 years
- HR = 0.67 (95% CI, 0.48, 0.93)  
  *p = 0.015*

Data from Romond et al. *N Engl J Med.* 2005
## Adjuvant Trastuzumab Trials

### HER2 Determination

<table>
<thead>
<tr>
<th>Trial</th>
<th>IHC</th>
<th>FISH</th>
<th>Where?</th>
</tr>
</thead>
<tbody>
<tr>
<td>B31/ N9831</td>
<td>HercepTest (Dako)</td>
<td>3+ (2+ if FISH+)</td>
<td>Vysis</td>
</tr>
<tr>
<td>HERA</td>
<td>HercepTest (Dako)</td>
<td>3+ (2+ if FISH+)</td>
<td>Vysis</td>
</tr>
<tr>
<td>BCIRG</td>
<td>N.A.</td>
<td>N.A.</td>
<td>Vysis</td>
</tr>
</tbody>
</table>
## Discordance in B-31 Trial: First 104 Enrolled Subjects

<table>
<thead>
<tr>
<th></th>
<th>PathVysion FISH (Central lab)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Not amplified</td>
</tr>
<tr>
<td>Herceptest™ (central lab)</td>
<td>3+</td>
</tr>
<tr>
<td></td>
<td>0-2+</td>
</tr>
<tr>
<td>FISH (NSABP)</td>
<td>Not amplified</td>
</tr>
<tr>
<td></td>
<td>Amplified</td>
</tr>
</tbody>
</table>

19 cases were negative by both methods centrally

Paik et al JNCI 2002
## Discordance in B-31 Trial

<table>
<thead>
<tr>
<th>Test used for eligibility</th>
<th>Type of lab</th>
<th>HercepTest</th>
<th>PathVysion FISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>HercepTest 3+ (n=80)</td>
<td>Small volume (&lt;100 tests/mo)</td>
<td>10/52</td>
<td>12/52</td>
</tr>
<tr>
<td></td>
<td>Large volume (≥100 tests/mo)</td>
<td>1/28</td>
<td>1/28</td>
</tr>
<tr>
<td>Other IHC assays (n=24)</td>
<td>Small volume</td>
<td>11/23</td>
<td>9/23</td>
</tr>
<tr>
<td></td>
<td>Large volume</td>
<td>0/1</td>
<td>0/1</td>
</tr>
</tbody>
</table>

Local and central agreed in only 79% of cases by IHC or FISH
Her2 Testing

**Clinical issues:**

- Are the IHC 2+, FISH negative results really false negatives? Would patients benefit from trastuzumab?

- Are the IHC 3+, FISH negative results really false positives? Are patients being exposed to unnecessary toxicity?
Importance of Testing for Her2 (and other Predictive Markers)

- 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
  - We hope to accurately identify who:
    - Should receive a potentially beneficial/curative therapy
    - Should not receive a costly and potentially toxic therapy

- Pathologists are now “wearing the stethoscope”
  - Key role: perform and interpret results that will dictate therapy
  - Share responsibility for treatment decisions made in the clinic …

Adapted from A. Wolff, MD
Her2 Testing: Sources of Variation

- **Preanalytic**
  - Time to fixation, time of fixation, type of fixation
  - Method of tissue processing

- **Analytic**
  - Assay validation, equipment validation, test reagents
  - Use of standardized control materials
  - Use of automated laboratory methods

- **Postanalytic**
  - Interpretation criteria
  - Use of image analysis
  - Quality assurance procedures
This poor concordance led to development of joint CAP/ASCO guidelines for Her2 testing

- Testing algorithms (IHC, FISH)
- Sample handling and exclusion criteria
- Results interpretation criteria
- Reporting elements
Starting in 2007 – every CAP-accredited lab performing Her2 testing is required to participate in guideline concordant proficiency testing

Separate testing for each assay method

Proficiency testing programs distribute specimens at least twice per year (testing events)

Challenges to assess lab performance

- If 10+ challenges, must correctly ID ≥90%
- If <90%, at risk for next event
- If unsatisfactory, suspension of Her2 testing until performance issues corrected
Reliability of Assays

- These guidelines will hopefully lead to more accurate and reliable Her2 results
  - Treatment recommendations should not depend on where a patient’s sample is assayed

- Similar approach for standardization of ER testing is underway
  - Problem recently highlighted in eastern Canada:
    - 40% of more than 2000 patients diagnosed with ER-negative disease were found to be ER positive
    - They didn’t receive a potentially curative therapy
Assays to Refine Prognosis
Prognostic Information in Breast Cancer

- Previously, have relied on information gathered using H&E and IHC

- Now, newer options are available:
  - RT-PCR (eg OncotypeDX®)
  - Genomic assays (eg Mammaprint™)
  - Molecular assays to identify tumor cells in sentinel lymph nodes (GeneSearch™)
Oncotype DX 21 Gene Recurrence Score

16 Cancer and 5 Reference Genes From 3 Studies

RS = + 0.47 \times \text{HER2 Group Score} - 0.34 \times \text{ER Group Score} + 1.04 \times \text{Proliferation Group Score} + 0.10 \times \text{Invasion Group Score} + 0.05 \times \text{CD68} - 0.08 \times \text{GSTM1} - 0.07 \times \text{BAG1}

<table>
<thead>
<tr>
<th>Category</th>
<th>RS (0 – 100)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low risk</td>
<td>RS &lt; 18</td>
</tr>
<tr>
<td>Int risk</td>
<td>RS 18-30</td>
</tr>
<tr>
<td>High risk</td>
<td>RS &gt;30</td>
</tr>
</tbody>
</table>

Paik et al, NEJM 2004
NSABP: Validation Study of OncotypeDX

- **NSABP 20 and other data sets**: Node Neg, ER+, Tam treated patients
- **10 yr Distant Recurrence**

**Recurrence Score/Continuous Variable**

Paik et al, NEJM 2004
Oncotype DX Predictive Value

Chemo Benefit in LN- ER+ (NSABP B-20)

Paik et al JCO 2006
Will the 21 Gene Assay replace pathologists?

Perhaps:

- Gives more information about the “biology” of the tumor than just grade and receptor status
- Provides quantitative data for all three major markers (ER, PgR, and Her2)
- Is possibly a more technically reliable assay
Will OncotypeDX replace pathologists?

- But perhaps not:
  - Still need to confirm diagnosis (invasive breast cancer), margin status
  - Still need to know the basics (ER status) before know if should order the assay
  - Cost-effectiveness issues
Other Prognostic Information: Genetic Mutations

- Inherited and somatic genetic mutations
  - Germline mutations can influence response to or toxicity of therapy (pharmacogenetics)
    - UGT1A1, TPMT, cytochrome P450 enzymes
  - Somatic mutations can affect response to therapy
Somatic Mutations

- Multiple gene mutations have been found that confer worse prognosis
  - P53

- More recently, mutation associated with decreased response to specific therapy in colorectal cancer
  - KRAS → EGFR monoclonal Ab
KRAS Mutations in CRC

ASCO provisional clinical opinion:
“...all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR MoAb therapy should have their tumor tested for KRAS mutations in a CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients...should not receive anti-EGFR Ab therapy”

Example of new assay that oncologists now know how to apply in the clinic
KRAS Mutations in CRC

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“...all patients with metastatic colorectal carcinoma who are candidates for anti-EGFR MoAb therapy should have their tumor tested for KRAS mutations in a CLIA-accredited laboratory. If KRAS mutation in codon 12 or 13 is detected, then patients...should not receive anti-EGFR Ab therapy”

But who will perform these assays?
Cytogeneticists?
Pathologists?
Send-out lab?

Allegra et al JCO 2009
Other Available Prognostic Tests

- Examples of assays that are **not** recommended for use by ASCO Guidelines Committees because they don’t know how to apply clinically:

  - Sentinel lymph node testing with IHC or molecular markers to detect isolated tumor cells
  - Chemotherapy-sensitivity assays
  - IHC markers of proliferation: Ki67, cyclin E, etc.
  - MammaPrint assay (currently being prospectively evaluated in MINDACT trial)

References:
- Schrag et al. JCO 2004
- Lyman et al. JCO 2005
- Harris et al. JCO 2007
Overall Summary

- Pathologists will still play a key role in helping Oncologists individualize treatment recommendations for patients
  - Need to do things well that oncologists need (eg, Her2, ER testing)
  - Unclear who will perform some assays (genotyping, FISH)
  - Important to communicate with oncologists prior to implementing new technology to design appropriate trials to determine clinical utility
Thank you