HER2/neu Evaluation of Breast Cancer in 2019

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ERBB2 (HER2) Background

- 185-kDa membrane protein expressed in normal secretory epithelial cells (breast, pancreas, intestine and salivary gland)

- HER1, HER2, HER3 and HER4 (epidermal growth factors ERBB1, ERBB2, ERBB3 and ERBB4, respectively) are transmembrane tyrosine kinases
  - Regulate cell growth and proliferation, adhesion, migration
  - Extracellular binding domain, a transmembrane lipophilic segment, and an intracellular tyrosine kinase domain (except HER3)
  - Ligand binding induces activation of the tyrosine kinase domains by both homodimerization and heterodimerization
ERBB2 (HER2) Background

- Extracellular domain of HER2 adopts a fixed confirmation resembling a ligand-activated state
  - Dimerization in the absence of a ligand
  - Receptor overexpression or mutation can also induce dimerization
  - HER2 signaling promotes cell proliferation through the RAS-MAPK pathway and inhibits cell death through the PIK3-AKT-mTOR pathway
HER2 is Both a Prognostic and Predictive Marker

• Prognostic marker
  • Aggressive tumors with high-risk of metastasis
  • Decreased survival compared to HER2 negative/non-amplified tumors
    • Associated with worse clinical outcome in the absence of therapy
• Predictive marker
  • Predicts likelihood of responding to HER2 targeted therapies
• 1980s: Slamon et al identified that HER2 gene amplification was associated with worse clinical outcomes

• Late 1980s they described that HER2 protein overexpression might be a potential predictive tool for clinical use

• Phase II clinical trial (Baselga et al, 1996) showing Herceptin monotherapy was effective in pts who failed prior chemotherapy
  • 46 pts with extensive metastatic BC that overexpressed HER2
  • Overall response rate (11.6%)
• Herceptin becomes FDA approved for the treatment of metastatic breast cancer in 1998
2001: Slamon et al published first-generation trial of trastuzumab added to chemotherapy in metastatic BC

- Longer time to disease progression (median, 7.4 vs 4.6 months; P<0.001),
- Longer duration of response (median, 9.1 vs 6.1 months; P<0.001)
- Lower rate of death at 1 year (22 percent vs 33 percent, P=0.008)
- Longer survival (median survival, 25.1 vs 20.3 months; P=0.046)
Initial Clinical Trials

• Trastuzumab binds the juxtamembrane portion of the extracellular domain of HER2 receptor
  • Prevents activation of intracellular tryosine kinase, prevents dimerization and increases endocytic destruction of the receptor

• HER2 positive in initial trials was defined as:
  • HER2 expression determined by IHC (central lab)
  • 3+ more than moderate staining in >10% of tumor cells
Additional Clinical Trials

• 2005: First generation of trastuzumab adjuvant trials show improvement in disease-free and overall survival

• Second-generation studies in metastatic disease led to the approval of several new HER2-targeted therapies
Additional Clinical Trials

- Supported second-generation adjuvant and neoadjuvant trials testing single and dual HER2-targeted agents
  - Lapatinib: Small molecule tyrosine kinase inhibitor
  - Pertuzumab: HER2/HER3 antibodies
  - Adotrastuzumab emtansine: Antibody chemotherapy conjugate
  - Neratinib: Tyrosine kinase inhibitor
What is the definition of HER2 positive?

- Moving target
  - Depends on the year in which you are asking
  - Depends on which methodology is being used to assay for HER2
### Which assay to use?

<table>
<thead>
<tr>
<th>Diagnostic Name</th>
<th>Diagnostic Manufacturer</th>
<th>Trade Name (Generic)</th>
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<tbody>
<tr>
<td>INFORM HER-2/neu</td>
<td>Ventana Medical Systems, Inc.</td>
<td>Breast cancer Herceptin (trastuzumab)</td>
</tr>
<tr>
<td>PathVysion HER-2 DNA Probe Kit</td>
<td>Abbott molecular Inc.</td>
<td>Breast cancer Herceptin (trastuzumab)</td>
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<td>Ventana Medical Systems, Inc.</td>
<td>Breast cancer Herceptin (trastuzumab)</td>
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<td>Dako Denmark A/S</td>
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<td>Hercep Test</td>
<td>Dako Denmark A/S</td>
<td>Breast cancer Herceptin (trastuzumab) Perjeta (pertuzumab) Kadcyla (ado-trastuzumab emtansine)</td>
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HER2 Testing Issues

• HercepTest antibody clone and those clones used in the initial clinical trials were not the same
  • HercepTest was not evaluated in a clinical trial before its FDA approval
  • 79% concordance with clinical trials assay scores
• For roughly the first decade of HER2 testing there were no guidelines on:
  • Standardization of pre-analytic factors (fixative type, length of fixation or cold ischemic time)
  • Interpretation and reporting
HER2 Testing Issues

- NSABP B-31 trial evaluated the addition of Herceptin to the adjuvant chemotherapeutic regimen of doxorubicin and cyclophosphamide followed by paclitaxel in the treatment of stage II BC
- HER2 overexpression or gene amplification
- Eligibility was based on HER2 results submitted by the accruing institutions
- Central review of 1st 104 cases entered based on 3+ IHC results found that 18% of cases from community/originating labs could not be verified as positive on central testing with HercepTest or FISH

Paik et al. NCI 2002.
• Prospective randomized, phase III trial evaluating women with primary, operable, histologically confirmed, node-positive BC that were 3+ overexpressing or HER2 amplified as determined by local laboratory randomized to doxorubicin plus cyclophosphamide followed by paclitaxel with or without trastuzumab

• Confirmatory central testing of HER2 status was performed using HercepTest IHC and Vysis PathVysion FISH
• 1st 119 patients enrolled were centrally tested
  • Six of nine (67%) of the specimens submitted by local laboratories as FISH positive were confirmed by central assay
  • Only 74% concordance between central and local HercepTest results
• Essentially first proficiency testing for HER2
• Two cases shared between 35 different labs
  • 100% concordance by FISH (one amp and one non amp)
Press et al reviewed 117 breast carcinomas with known HER2 amplification and assessed these cases with four different IHC assays and two different FISH assays.

- The two different FISH assays had 97.4% and 95.5% accuracy for identifying amplification.
- Four different IHC assays had accuracy ranging from 88.9% to 96.6% in identifying overexpression.

**HER2 Testing by Local, Central, and Reference Laboratories in Specimens From the North Central Cancer Treatment Group N9831 Intergroup Adjuvant Trial**

- Perez et al provided an updated analysis from the N9831 trial addressing the discordance between local and central testing.
  - If same methodology is employed, the discordance rate for IHC is 18.4% while the discordance rate for FISH is 11.9%.

IHC vs FISH

- Breast Cancer International Research Group (BCIRG)
- ~2600 women, prospective, Herceptin based clinical trials
- Local labs vs Central Labs:
  - 79% agreement between local IHC and central FISH
  - 77.5% agreement between local IHC and central IHC
  - 92% agreement between local FISH and central FISH
ASCO/CAP Guideline Recommendations for HER2 Testing in Breast Cancer

• To improve accuracy of HER2 testing by developing an algorithm that specifies specimen handling, assay exclusion, and reporting criteria while defining positive, equivocal, and negative values

• Methodologies to detect HER2
  • IHC
  • Fluorescence in situ hybridization and bright-field in situ hybridization
Variables in HER2 Testing

• Pre analytical
  • Fixation, time, length, and type

• Analytical
  • Equipment calibration, assay validation, training and competency of staff, antigen retrieval, reagents

• Post analytical
  • Interpretation criteria, quality assurance procedures (i.e. laboratory accreditation and proficiency testing)
• FDA Criteria
• 2007 ASCO/CAP Guidelines
• 2013 ASCO/CAP Guidelines
• 2018 ASCO/CAP Focused Update
0 Negative
• No staining is observed, or membrane staining is observed in <10% of the tumor cells

1+ Negative
• A faint/barely perceptible membrane staining is detected in >10% of tumor cells
• The cells exhibit incomplete membrane staining

2+ Weakly Positive*
• A weak to moderate complete membrane staining is observed in >10% of tumor cells

3+ Positive
• A strong complete membrane staining is observed in >10% of tumor cells


- Standardization of immunohistochemistry and FISH assays
- Specified tissue handling and formalin fixation times
- Mandated external proficiency testing

<table>
<thead>
<tr>
<th>Method</th>
<th>Negative</th>
<th>Equivocal</th>
<th>Positive</th>
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<tr>
<td>IHC</td>
<td>No staining or weak, complete membrane staining &lt;10%</td>
<td>Weak, non-uniform staining ≥10% or Uniform intense membrane staining ≤30%</td>
<td>Uniform intense membrane staining &gt;30%</td>
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<td>FISH</td>
<td>Single probe: &lt;4/cell Dual probe: <strong>Ratio &lt; 1.8</strong></td>
<td>Single probe: 4-5.9/cell Dual probe: <strong>Ratio 1.8-2.2</strong></td>
<td>Single probe: ≥6.0/cell Dual probe: <strong>Ratio &gt;2.2</strong></td>
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Data Leading to Changes in the 2007 Guidelines

- Perez EA et al. Predictability of adjuvant trastuzumab benefit in N9831 patients using the ASCO/CAP HER2-positivity criteria
- The ASCO/CAP 2007 guidelines were a modified version of the US Food and Drug Administration (FDA) criteria that were used in adjuvant trastuzumab trials
  - Breast tumor samples from 2904 patients enrolled in the randomized phase 3 N9831 trial that investigated trastuzumab as an adjuvant therapy for patients who had HER2-positive resected early breast cancer
    - 107 (3.7%) of 2904 patients identified as HER2 positive by IHC and enrolled in N9831 would not have been eligible for enrollment
      - (3+) in 10-30%
      - If these patients were retested using FISH, the number of ineligible patients would have been reduced to 29 (1.0%)

JNCI 2012 Jan 18;104(2):159-62.
Updated ASCO/CAP Guidelines in 2013

- Tissue handling procedures (pre-analytical variables)
  - Cold ischemia time (minimize <1 hour)
  - Fixative: 10% neutral buffered formalin
    - Fixatives other than NBF have shown inconsistent results
  - Minimum duration of fixation: 6 hours
  - Maximum duration of fixation: 72 hours
    - Over or under fixation can lead to erroneous results
  - Fixation time points need to be documented in accession or report

## Updated Guidelines (2013)

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HER2 testing (invasive component) by validated IHC assay

Batch controls and on-slide controls show appropriate staining

- Circumferential membrane staining that is complete, intense, and within > 10% of tumor cells*
  
  **IHC 3+ positive**

- Circumferential membrane staining that is incomplete and/or weak/moderate and within > 10% of tumor cells*
  or
  Complete and circumferential membrane staining that is intense and within ≤ 10% of tumor cells*

  **IHC 2+ equivocal**

- Incomplete membrane staining that is faint/barely perceptible and within > 10% of tumor cells*

  **IHC 1+ negative**

- No staining is observed* or Membrane staining that is incomplete and is faint/barely perceptible and within ≤ 10% of tumor cells

  **IHC 0 negative**

*Must order reflex test (same specimen using ISH) or order a new test (new specimen if available, using IHC or ISH)
**HER2 FISH**

**HER2 gene** is located at **17q12.1** on the long arm of chromosome 17.

Chromosome 17

- Centromere

**HER2 gene**
HER2 Non-amplified

HER2 Amplified
**HER2 testing (invasive component) by validated dual-probe ISH assay**

Batch controls and on-slide controls show appropriate hybridization

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**HER2/CEP17 ratio ≥ 2.0***

- **Average HER2 copy number ≥ 4.0 signals/cell***
  - **ISH positive**

- **Average HER2 copy number < 4.0 signals/cell***
  - **ISH positive†**

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**HER2/CEP17 ratio < 2.0**

- **Average HER2 copy number ≥ 6.0 signals/cell***
  - **ISH positive**

- **Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell***
  - **ISH equivocal**

- **Average HER2 copy number < 4.0 signals/cell***
  - **ISH negative**

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Must order a reflex test (same specimen using IHC), test with alternative ISH chromosome 17 probe, or order a new test (new specimen if available, ISH or IHC)
How did the 2013 ASCO/CAP Guidelines change HER2 FISH results?
Trends following the implementation of the 2013 Guidelines

- **HER2** FISH tests performed on primary or metastatic breast cancers between January 2011 and August 2013 originally scored with 2007 ASCO/CAP guidelines were identified

- FISH was manually scored by at least two people (including one pathologist and one technician) and originally reported per 2007 ASCO/CAP guidelines

- HER2 and CEN17 copy numbers per cell were recorded in addition to the **HER2/CEN17** ratio

Presented at SABCS 2014
Applying the 2013 ASCO/CAP guidelines, HER2 status was re-assigned when applicable:

- HER2/CEN17 ratio of $\geq 2.0$ or a HER2 average copy number of $\geq 6$ per cell (with HER2/CEP17 ratio of $<2.0$) as amplified
- Average HER2 copy number $\geq 4$ but $<6$ per cell as equivocal
- HER2/CEP17 ratio of $<2.0$ as non-amplified
3,647 samples were tested by HER2 FISH in our laboratory

With the 2007 ASCO/CAP guidelines:
- 391 (11.7%) were amplified (dual probe ratio >2.2)
- 112 (3.3%) were equivocal (dual probe ratio of 1.8 to 2.2)
- 2,845 (85%) were non-amplified (dual probe ratio <1.8)
- 299 (8.2%) did not have an interpretable FISH result
Trends following the implementation of the 2013 Guidelines

• Of the FISH equivocal cases, 40 (35.7%) had a \( \text{HER2/CEN17} \) ratio between 2.0-2.2 (reclassified as amplified with 2013 guidelines)

• Of the remaining 72 equivocal cases which had a \( \text{HER2/CEN17} \) ratio <2.0
  • 34 (47.2%) had \( \text{HER2} \) copy number ≥4 but <6 per cell number
  • 38 (53%) were re-classified as non-amplified based on a \( \text{HER2/CEP17} \) ratio of <2.0

• Of the 2,845 non-amplified cases, 2,810 (98.5%) remained non-amplified following new guidelines
  • 35 (1.2%) had a \( \text{HER2} \) copy number ≥4 but <6 per cell

Presented at SABCS 2014
Trends following the implementation of the 2013 Guidelines

- Inclusion of cases with HER2/CEN17 ratios between 2.0-2.2 and cases with average HER2 copy number of ≥6 (in dual probe assays) in the amplified category, as expected, resulted in an increase in amplified cases.

- Other institutions have similarly re-evaluated HER2 FISH results with the updated guidelines and showed a similar increase in amplification rates.

Presented at SABCS 2014
HER2 testing (invasive component) by validated dual-probe ISH assay

Batch controls and on-slide controls show appropriate hybridization

HER2/CEP17 ratio $\geq 2.0^*$

- Average HER2 copy number $\geq 4.0$ signals/cell*
  - ISH positive

- Average HER2 copy number $< 4.0$ signals/cell*
  - ISH positive*

HER2/CEP17 ratio $< 2.0$

- Average HER2 copy number $\geq 4.0$ signals/cell*
  - ISH positive

- Average HER2 copy number $\geq 4.0$ and $< 6.0$ signals/cell*
  - ISH positive

- Average HER2 copy number $< 4.0$ signals/cell
  - ISH negative

Must order a reflex test (same specimen using IHC), test with alternative ISH chromosome 17 probe, or order a new test (new specimen if available, ISH or IHC)
Intrachromosomal ("stacked" signals)

Extrachromosomal

Co-amplification
Copy Number Gains in the Context of the Cancer Genome

- Entire genome may be present in 3 or more copies (on average), i.e. “polyploidy”, confounding the definition of “normal” or “control” for the genome
- Adult solid tumors are known to have complex genomes, characterized by gains, losses, allelic imbalances encompassing large portions of the genome
- Absolute copy number per cell can be estimated by some techniques, but not others
  - FISH, flow cytometry, cytogenetics: individual cell analysis
- Reference/ “control” region(s) may also be abnormal
Reflex HER2 FISH Assay

HER2/RAI1

HER2/RAI1 ratio < 2.0: Non-amplified
HER2/RAI1 ratio ≥ RAI1: Amplified*

*arbitrary cutoff per HER2 guidelines
FISH Scoring

Avg HER2/RAI = 1.0
Not amplified

Avg HER2/RAI1 = 2.5
Amplified
RAI1 to Resolve HER2 FISH Equivocal Cases

- 97 invasive breast carcinomas which were equivocal by FISH during the study period
- 33 (34%) had prior equivocal (2+) immunohistochemical staining requiring reflex FISH testing
- The remaining cases had FISH as the primary testing methodology

Presented at SABCS 2015
RAI1 to Resolve HER2 FISH Equivocal Cases

- RAI1 probe identified 39.2% (38/97) cases as amplified
  - HER2:RAI1 ratio ranging from 2.0 to 3.2 (average ratio: 2.37)
- RAI1 probe identified 57.7% (56/97) cases as non-amplified
- 3.1% (9/97) were still unclassifiable owing to a deletion of RAI1

Presented at SABCS 2015
Resolution of Equivocal HER2 FISH

- ASCO-CAP 2013 Guidelines recommended using an alternate control probe for a gene on chromosome 17
- No guidelines on which probe to use, how to interpret, how to report, no data to support using it
  - Just because it has a ratio >2, does it really mean this material is positive and will respond to therapy?
• 3,630 cases with 137 FISH equivocal (ratio <2 with average \(\text{HER2}\) copy number ≥4 and <6) breast cancers identified from 2000-2010 were assessed with an alternative probe (\(\text{SMS}\))
  • 35 were upgraded to amplified with the alternative probe
  • 5 year DFS and OS for this group were the same as the non-amplified group
310 cases classified as equivocal by the 2013 guidelines (ratio <2 with an average HER2 copy ≥4 and <6.0) were evaluated with the D17S122 alternative probe.

- 39/310 (12.6%) cases designated as equivocal by 2013 guidelines were reclassified as amplified.
- Tumors were Stage 1, intermediate to high histologic grade, hormone receptor positive and lymph node negative.

Assessment of ERBB2/HER2 Status in HER2-Equivocal Breast Cancers by FISH and 2013/2014 ASCO-CAP Guidelines

• Retrospectively assessed alternative control probes (TP53, C17S122, SMS, RARA, TOP2A) in 1980 patients
  • Data and materials were available through an international trial on adjuvant chemo in invasive, node positive breast cancer patients
  • Assess frequency of heterozygous deletions of alternative control probes in equivocal carcinomas, assess HER2 protein expression in the group and compare clinical outcomes

100 HER2 FISH equivocal breast cancers had frequent heterozygous deletions (most commonly in SMS, TP53 and D17S122)
  - p-arm more frequently than the q-arm
100 HER2 FISH non-amplified cancers also had heterozygous deletions (also more commonly on the p-arm)
Both groups had similar low levels of HER2 protein expression by IHC
• ISH equivocal patients who were called amplified based on an alternative probe had a similar DFS and OS to those who were ISH negative
Relative Copy Number of ERBB2 and Genomic Sites Used as Alternative Controls for Assessment of HER2 Status by FISH (METABRIC Cohort, SNP Chip Data for 1980 Patients)

Retrospective assessment of the 2013 ASCO/CAP guidelines on cases within Breast Cancer International Research Group (BCIRG -005, -006 and -007)

- Assigned 10,468 patients to the five ISH groups in the 2013 guidelines
  - 40.8% were ratio ≥2 with average HER2 >4
    - Correlated with 3+ IHC
    - Had improved DFS and OS when treated with trastuzumab
  - 0.7% were ratio ≥2 with average HER2 <4
    - Correlated with 0 or 1+ IHC
    - NO improved DFS or OS when treated with trastuzumab

HER2 Gene Amplification Testing by Fluorescent In Situ Hybridization (FISH):
Comparison of the ASCO-College of American Pathologists Guidelines With FISH Scores Used for
Enrollment in Breast Cancer International Research Group Clinical Trials

• 0.5% were <2 with average $HER2 \geq 6$
  • Worse DFS and OS than the non amp group; too limited for more analysis

• 4.1% were <2 with average $HER2 \geq 4$ and <6
  • Correlated with 0 or 1+ IHC; did not get trastuzumab and had outcomes similar to non amp group

• 53.9% were <2 with average $HER2 < 2$
  • Correlated with 0 or 1+ IHC

Clinical Question 1

• What is the most appropriate definition for IHC 2+ (IHC equivocal)?
  • Weak to moderate complete membrane staining observed in >10% of tumor cells

Clinical Question 2

• Must HER2 testing be repeated on a surgical specimen if initially negative test on core biopsy?
  • May
Requires concomitant IHC review with dual-probe ISH groups 2 to 4 to arrive at the HER2 status (positive or negative)

- Integrated final HER2 status
- Comments suggested by ASO/CAP
Group 2

- Typically ER+
- Usually 0 or 1+ on HER2 IHC
- Based on limited data, no benefit (DFS or OS) when treated with trastuzumab
2018 ASCO/CAP Update

Group 3

- More heterogeneous
- May be ER+ or ER-
- May be HER2+ or HER2- by IHC
- No good outcome data on these patients as they were not included in the initial trials
Group 4

- Typically ER+
- Usually 0 or 1+ on HER2 IHC
- Were not included in initial HER2 trials, but they have similar outcome without trastuzumab to HER2 negative patients
Compare *HER2* Results Using 2013 vs 2018 Guidelines

- Changes in the 2018 update focused on rare signal patterns expected to be seen in roughly 5% of breast cancers
  - Ratio <2 with *HER2* copy number >4 and <6
  - Ratio ≥2 with *HER2* copy number <4
  - Ratio <2 with *HER2* copy number >6
- Mayo and ARUP report that roughly 15% of the cases they evaluate fall into these categories
Morphology Matters

- Discordant IHC and FISH in micropapillary breast carcinoma
- HER2 overexpression in a phenotype that is usually negative
Invasive Micropapillary Carcinoma

- WHO 2012
- An invasive carcinoma composed of small, hollow or morula-like clusters of cells surrounded by clear stromal spaces
- Proclivity for lymphovascular space invasion and frequently present with axillary nodal disease at the time of diagnosis
2013 ASCO/CAP HER2 guidelines have a key recommendation for pathologists with respect to invasive micropapillary carcinoma (MPC)

- HER2 immunohistochemical staining that is intense but incomplete (basolateral or U shaped) and would be considered 1+ may be HER2 amplified by fluorescence in situ hybridization (FISH)

Pathologists should consider reporting these specimens as equivocal and perform an alternative testing methodology

- Recommendation is based largely on a single paper (Vingiana et al, 2013 Histopathology 63, 217-224)
  - A series of 23 MPC that were considered 1+ by HercepTest with HER2 amplification identified in 1 case (4%)
  - We sought to evaluate this possible discordance between IHC and FISH in a series of invasive carcinomas with micropapillary features that had both IHC and FISH testing methodologies performed
Micropapillary Features: IHC vs FISH

• 31 cases with micropapillary features
  • 22 core needle biopsies and 9 resection specimens (1 representative slide)
  • Percentage of micropapillary features present ranged from 30-100%
  • Nottingham grade 2 or grade 3 (n=13 and 18, respectively)
Micropapillary Features: IHC vs FISH

- **DAKO HER2 IQFISH parmDx FISH testing**
  - 12/31 (39%) were HER2 FISH non amplified with corresponding HercepTest scores of 0 (n=1), 1+ (n=8) and 2+ (n=3)
  - 8/31 (26%) were HER2 amplified (n=2 by copy number >6 with copy numbers of 6.5 and 7.3; n=6 by ratio with HER2/CEN-17 ratios of 2.1-2.6) with corresponding HercepTest scores of 1+ (n=4) and 2+ (n=4)
  - 11/31 (35%) were HER2 equivocal with the DAKO HER2 IQFISH parmDx
    - Reflex probe testing identified an additional 4 non amplified cases with corresponding HercepTest results of 1+ (n=1) and 2+ (n=3)
    - 5 cases as amplified (HER2/RAI1 ratio ranging from 2.0-2.9) with corresponding HercepTest results of 1+ (n=2) and 2+ (n=3)
    - 2 cases could not be resolved with the reflex probe set owing to either a deletion or apparent amplification of the RAI1 probe (RAI1 copy number of 1.1 and 6.7, respectively
Micropapillary Features: IHC vs FISH

- Overall in this series, 16/29 (55%) were ultimately called HER2 non-amplified while 13/29 (45%) were designated as HER2 amplified
  - 6/13 (46%) had a corresponding HercepTest of 1+
  - Immunoreactivity included weak staining either along the apical border or along the periphery of the morula clusters without lateral staining in 3/6 (50%) of cases
  - Remainder with 1+ immunoreactivity had weak lateral staining and staining along with periphery of the morula clusters
Micropapillary Features: IHC vs FISH

- Cases scored as HercepTest 2+ were heterogeneous
  - Areas of tumor having no immunoreactivity while other areas had only weak linear staining with areas of focal circumferential staining in >10% of the tumor
- The cases that were not 100% micropapillary morphology had a component of invasive ductal carcinoma, NOS
  - HercepTest score within these different morphologies was similar
    - No components were of a higher IHC score
Micropapillary Features: IHC vs FISH

- The ASCO/CAP recommendation that this morphology may not stain in the typical pattern is highlighted
  - 6/13 (43%) of cases with micropapillary features and HER2 staining that would otherwise have been scored as 1+ were HER2 amplified by FISH, albeit low level amplification (2.0-2.9)
- Likely biased towards borderline cases
  - If this discrepancy is borne out in larger series, the potential for dual testing (IHC and FISH) for carcinomas with micropapillary features seems appropriate
Classical-Type Invasive Lobular Carcinoma With HER2 Overexpression
Clinical, Histologic, and Hormone Receptor Characteristics

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Tumor Type</th>
<th>Tubule Formation</th>
<th>Nuclear Grade</th>
<th>Mitosis</th>
<th>Ki-67 (%)</th>
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ER, estrogen receptor; IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; N, no; PR, progesterone receptor; Y, yes.

- Case 3, mixed classic (90%) and pleomorphic (10%) types of ILC; case 4, mixed ILC (95%) and IDC (5%); case 6, mixed ILC (70%, classical type) and IDC (30%); and case 9, mixed classic (75%) and pleomorphic (25%) types of ILC.
- The score of 3 for tubule formation indicates tubules in <10% of tumor. Nuclear grade refers to the classical-type ILC component. Mitosis score of 1 indicates 0-5 per 10 high-power fields; a score of 2 indicates 6-10 per high-power field.

THANK YOU!

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