What has changed (again) in HER2 testing of breast cancers

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Disclosures

• None
ISSUES

• Changing guidelines / positivity rates
• Discordance between labs
• IHC vs FISH
1998

First genetically engineered drug treatment for advanced breast cancer
HER2 Targeted Therapies

Metastatic
HER2 Targeted Therapies

Metastatic

Adjuvant

Neoadjuvant
What we have learned in 20 years

• HER2 targeted therapy significantly improves outcome in metastatic, adjuvant and neoadjuvant settings

• However, this improvement is limited to HER2 positive cancers

• Definition of HER2 positivity has been a moving target, frustrating clinicians and pathologists alike

• Initial reported rates of 25%-30% is NOT correct. It is about 15%.
Do HER2 negative tumors benefit from targeted therapies?

Some patients tested positive at local hospitals and entered trial but were found to be HER2 negative on central testing
Do HER2 negative tumors benefit from targeted therapies?

| Table 1. Relative Risks of Disease Progression and Death among Patients in the ACTH Group as Compared with the ACT Group.* |
|---|---|---|---|---|---|
| **End Point and Central HER2 Assay†** | **ACT** | **ACTH** | **Relative Risk (95% CI)** | **P Value** | **P Value for the Interaction** |
| **no. of events/total no. of events** | | | | | |
| **Disease progression** | | | | | |
| HER2-positive | 163/875 | 85/804 | 0.47 (0.37–0.62) | <0.001 | 0.47 |
| HER2-negative | 20/92 | 7/82 | 0.34 (0.14–0.80) | 0.014 | |
| **Death** | | | | | |
| HER2-positive | 55/875 | 38/804 | 0.66 (0.43–0.99) | 0.047 | 0.08 |
| HER2-negative | 10/92 | 1/82 | 0.08 (0.01–0.64) | 0.017 | |

Paik et al, NEJM 2008
NSABP-47
Do women with HER2-low cancer improve DFS with targeted therapy?

[Diagram showing stratification and randomization process with details on treatment options for Arm 1 and Arm 2.]
## NSABP-47
**HER2 IHC 1+ or 2+**

<table>
<thead>
<tr>
<th></th>
<th>Chemotherapy</th>
<th>Chemotherapy + Herceptin</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive Disease-free Survival</td>
<td>89.2%</td>
<td>89.6%</td>
<td>0.90</td>
</tr>
<tr>
<td>Recurrence-free Survival</td>
<td>92.2%</td>
<td>92.0%</td>
<td>0.97</td>
</tr>
<tr>
<td>Distant Recurrence-free Survival</td>
<td>92.7%</td>
<td>92.7%</td>
<td>0.55</td>
</tr>
<tr>
<td>Overall Survival</td>
<td>94.8%</td>
<td>94.8%</td>
<td>0.14</td>
</tr>
</tbody>
</table>
NSABP-47

Do women with HER2-low cancer improve DFS with targeted therapy?

NO
HER2 Testing Issues

*Community vs Central Lab*

18-26% of community based positive assays could not be confirmed in central lab

<table>
<thead>
<tr>
<th>Central HercepTest™ score†</th>
<th>0</th>
<th>1+</th>
<th>2+</th>
<th>3+</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Local HER2 testing</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IHC‡</td>
<td>8</td>
<td>9</td>
<td>12</td>
<td>81</td>
<td>110</td>
</tr>
<tr>
<td>FISH</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>9</td>
<td>10</td>
<td>12</td>
<td>88</td>
<td>119</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Central FISH result§</th>
<th>Not amplified</th>
<th>Amplified</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>Local HER2 testing</td>
<td></td>
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<td>73</td>
<td>110</td>
</tr>
<tr>
<td>FISH</td>
<td>3</td>
<td>6</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>79</td>
<td>119</td>
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Paik et al JNCI 2002
Roche et al JNCI 2002
IHC vs FISH

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

Perez et al JCO 2006
IHC vs FISH

• Discordance rate between local and central HER2 test results:
  • IHC: 18.4%
  • FISH: 11.9%

<table>
<thead>
<tr>
<th>Test at Local Laboratory</th>
<th>Specimens Confirmed by Central Testing* (No.)</th>
<th>Agreement With Central Laboratory</th>
</tr>
</thead>
<tbody>
<tr>
<td>HercepTest</td>
<td>1,063</td>
<td>% 81.6</td>
</tr>
<tr>
<td>Non-HercepTest</td>
<td>636</td>
<td>75.0</td>
</tr>
<tr>
<td>FISH</td>
<td>813</td>
<td>88.1</td>
</tr>
</tbody>
</table>

NOTE. HercepTest, DAKO, Carpinteria, CA. Abbreviation: FISH, fluorescence in situ hybridization.
*Testing using the same method at both laboratories was not possible for 23 specimens.
Is FISH more reproducible than IHC?

• Breast Cancer International Research Group (BCIRG)
• ~2600 women, prospective, Herceptin based clinical trials
• Outside/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

• CAP
  • 100% agreement between FISH labs
  • 72.3% agreement between IHC labs
What is HER2 Positive?
Initial Clinical Trials

HER2 positive defined as weak to moderate (2+) or strong (3+) circumferential membrane staining in >10% of the tumor cells

HER2 positive metastatic breast cancer:
- Herceptin monotherapy effective in patients who failed treatment with prior chemotherapy
- Herceptin + chemotherapy is more effective than chemotherapy alone
Despite targeted therapy companion diagnostic test we have had two decades of problems
HER2 Testing Issues

• Antibody used in HercepTest and in the antibodies used in clinical trials (4D5 and CB11) are not the same.
• HercepTest was not evaluated in a clinical trail before its FDA approval
• It shows 79% concordance with clinical trials assay
• There was no standardization of pre-analytic factors (ischemic time, fixation time)
• Variations in testing, interpretation and reporting
Early days of testing

• FDA Criteria
  • 2007 ASCO/CAP Guidelines
  • 2013 ASCO/CAP Guidelines
  • 2018 Modifications to 2013 Guidelines
• Lack of standardization
  • Preanalytical: ischemic time, fixation time
  • Analytic
  • Post-analytic
• High number of false positives
• FDA Criteria

• 2007 ASCO/CAP Guidelines
• 2013 ASCO/CAP Guidelines
• 2018 Modifications to 2013 Guidelines
## ASCO/CAP Guidelines

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<td>Reduce false positive results</td>
<td>Ratio &gt;2.2 (dual probe) ≥6 HER2 (single probe)</td>
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<td>Addresses issues with less common dual FISH pattern</td>
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What has NOT changed?

Specimen handling is **critical!**

- Breast tissue undergoes ischemic changes from the minutes it is removed from the patient
- Enzymatic activity is not stopped until fixation begins
- Breast tissue should be cut and placed in 10% NBF within less than 1 hour of removed from the patient
Tumor stained as ‘2+’ for HER2 at 0.5 h of delayed fixation (a), but demonstrated reduction in staining at 3 h (b) and was completely negative at 24 h (c) and 48 h (d).

Time to Fixation: HER2 Testing IHC and FISH

30 minutes

a. 30 min IHC; b. 30 min FISH; c. 4 h immunohistochemistry; d. 4 h FISH

HER2/CEP17 = 0.98

HER2/CEP17 = 0.29

Time in Fixation

- 6-72 hours
- Cores and excisions need similar time in fixation
2018 ASCO / CAP Update

Human Epidermal Growth Factor Receptor 2 Testing in Breast Cancer

American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update

Antonio C. Wolff, M. Elizabeth Hale Hammond, Kimberly H. Allison, Brittany E. Harvey, Pamela B. Mangu, John M.S. Bartlett, Michael Bilous, Ian O. Ellis, Patrick Fitzgibbons, Wedad Hanna, Robert B. Jenkins, Michael F. Press, Patricia A. Spears, Gail H. Vance, Giuseppe Viale, Lisa M. McShane, Mitchell Dowsett
Clinical Question 1:
What is the most appropriate definition for IHC 2+ (IHC equivocal)?

2013 HER2 Testing Update as invasive breast cancer showing “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.”

Revised / 2018 definition of IHC 2+(equivocal) is invasive breast cancer with “weak to moderate complete membrane staining observed in > 10% of tumor cells”
Uncommon patterns that are not covered by these definitions but should be considered 2+ / equivocal:

- Moderate to intense but incomplete (basolateral or lateral) staining but can be found to be HER2 amplified
  - Micropapillary carcinoma

- Intense ≤10% circumferential membrane staining
Micropapillary carcinoma with incomplete basolateral staining where HER2 FISH was amplified
≤10% intense circumferential staining but still may be considered IHC 2+ equivocal
• Clinical Question 2
  • Must HER2 testing be repeated on a surgical specimen if initially negative test on core biopsy?

• HER2 testing *may* be repeated on the surgical specimen if initially negative on core biopsy
## ASCO/CAP Guidelines

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2018 ASCO / CAP Update

FISH related questions

Clinical Question 3

Should invasive cancers with an HER2/chromosome enumeration probe 17 (CEP17) ratio of ≥2.0 but an average HER2 copy number of <4.0 signals per cell be considered ISH positive?

Clinical Question 4

Should invasive cancers with an average HER2 copy number of ≥6.0 signals per cell but a HER2/CEP17 ratio of <2.0 be considered ISH positive?

Clinical Question 5

What is the appropriate diagnostic workup for invasive cancers with an average HER2 copy number of ≥4.0 but <6.0 signals per cell and an HER2/CEP17 ratio of <2.0, and initially deemed to have an equivocal HER2 ISH test result?
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

Michael E. Press, Guido Sauter, Marc Buyse, Hélène Fourmanoir, Emmanuel Quinaux, Denice D. Tsao-Wei, Wolfgang Eiermann, Nicholas Robert, Tadeusz Pienkowski, John Crown, Miguel Martin, Vicente Valero, John R. Mackey, Valerie Bee, Yanling Ma, Ivonne Villalobos, Anaamika Campeau, Martina Mirlacher, Mary-Ann Lindsay, and Dennis J. Slamon
<table>
<thead>
<tr>
<th>ASCO-CAP FISH Group</th>
<th>Description of HER2 FISH Category</th>
<th>No. of Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ratio ≥ 2.0, HER2 average ≥ 4.0</td>
<td>4,269 (40.8)</td>
</tr>
<tr>
<td>2</td>
<td>Ratio ≥ 2.0, HER2 average &lt; 4.0</td>
<td>71 (0.7)</td>
</tr>
<tr>
<td>3</td>
<td>Ratio &lt; 2.0, HER2 average ≥ 6.0</td>
<td>55 (0.5)</td>
</tr>
<tr>
<td>4</td>
<td>Ratio &lt; 2.0, HER2 average ≥ 4.0, &lt; 6.0</td>
<td>432 (4.1)</td>
</tr>
<tr>
<td>5</td>
<td>Ratio &lt; 2.0, HER2 average &lt; 4.0</td>
<td>5,641 (53.9)</td>
</tr>
<tr>
<td>Total*</td>
<td></td>
<td>10,468* (100.0)</td>
</tr>
</tbody>
</table>
Patients screened successfully in central lab (N = 10,468)

HER2 not amplified (n = 6,199; 59.2%)
  - BCIRG-005 participants (n = 3,298)
    - Arm 1: AC-T (n = 1,649)
    - Arm 2: TAC (n = 1,649)
  - Arm 1: AC-T (n = 1,073)
  - Arm 2: ACTH (n = 1,074)
  - Arm 3: TCH (n = 1,075)

HER2 amplified (n = 4,269; 40.8%)
  - BCIRG-006 participants (n = 3,222)
  - BCIRG-007 participants (n = 263)
HER2 testing (invasive component) by validated dual-probe ISH assay

Batch controls and on-slide controls show appropriate hybridization *

**HER2/CEP17** ratio ≥ 2.0 *

**HER2/CEP17** ratio < 2.0

**Average HER2 copy number ≥ 4.0 signals/cell**

ISH positive

Group 1

**Average HER2 copy number < 4.0 signals/cell**

ISH positive

Group 2

Average **HER2 copy number ≥ 6.0 signals/cell**

ISH positive

Group 3

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

ISH equivocal

Group 4

Average **HER2 copy number < 4.0 signals/cell**

ISH negative

Group 5
Group 1
HER2/CEP17 ≥ 2.0
Average HER2 signal / cell ≥ 4.0 (FISH Positive)

Table 4. Comparison of HER2 Ratio and Average HER2 Gene Copy Number and ASCO-CAP Groupings With Clinical Outcomes in BCIRG-006

<table>
<thead>
<tr>
<th>HER2 FISH (HER2/CEP17) Ratio</th>
<th>HER2 Copies per Cell</th>
<th>No. of Subjects</th>
<th>DFS Control Events/No. of Subjects</th>
<th>DFS Trastuzumab Events/No. of Subjects</th>
<th>DFS P for Log-Rank Test</th>
<th>OS Control</th>
<th>OS Trastuzumab</th>
<th>OS, HR (95% CI)*</th>
<th>OS P for Log-Rank Test</th>
<th>ASCO-CAP FISH Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 2.0</td>
<td>&lt; 4.0</td>
<td>46</td>
<td>4/18</td>
<td>6/28</td>
<td>1.10 (0.31 to 3.89)</td>
<td>2/18</td>
<td>4/28</td>
<td>3.15 (0.35 to 28.63)</td>
<td>28/39</td>
<td>Group 2</td>
</tr>
<tr>
<td>≥ 4</td>
<td>3,109</td>
<td>251/1,031</td>
<td>391/2,078</td>
<td>&lt; .0001</td>
<td>38/1,031</td>
<td>202/2,078</td>
<td>0.69 (0.56 to 0.85)</td>
<td>.0006</td>
<td>Group 1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>3,156</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. The HRs are for trastuzumab treatment arms compared with control chemotherapy-only arm. There were too few patients (n = 6) accrued to BCIRG-006 with a HER2 FISH ratio < 2.0 and ≥ 4.0 average HER2 gene copy number/tumor cell for analysis of the HR.

Abbreviations: BCIRG, Breast Cancer International Research Group; CAP, College of American Pathologists; DFS, disease-free survival; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; OS, overall survival.

*Trastuzumab-containing treatment arms compared with control (chemotherapy alone) treatment arm.
Group 2
HER2/CEP17≥2.0

Average HER2 signal / cell < 4.0 (FISH Positive)
Group 3

HER2/CEP17 < 2.0

Average HER2 signal / cell ≥ 6.0 (FISH Positive)

---

Table 3. Comparison of HER2 Ratio and Average HER2 Gene Copy Number and ASCO-CAP Groupings With Clinical Outcomes in BCIRG-005

<table>
<thead>
<tr>
<th>HER2 FISH (HER2/CEP17) Ratio</th>
<th>HER2 Copies per Cell</th>
<th>No. of Subjects</th>
<th>DFS, No. of Events</th>
<th>OS, No. of Events</th>
<th>DFS HR (95% CI) and P for Log-Rank Test*</th>
<th>OS HR (95% CI) and P for Log-Rank Test*</th>
<th>ASCO-CAP FISH Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2.0</td>
<td>&lt; 4.0</td>
<td>3,079</td>
<td>971</td>
<td>608</td>
<td>1.0 (reference)</td>
<td>1.0 (reference)</td>
<td>Group 5</td>
</tr>
<tr>
<td>4.01-6.0</td>
<td>176</td>
<td>51</td>
<td>30</td>
<td></td>
<td>0.923 (0.697 to 1.224)</td>
<td>0.878 (0.609 to 1.267)</td>
<td>Group 4</td>
</tr>
<tr>
<td>≥ 6</td>
<td>11</td>
<td>6</td>
<td>4</td>
<td></td>
<td>2.502 (1.121 to 5.583)</td>
<td>2.351 (0.879 to 6.284)</td>
<td>Group 3</td>
</tr>
</tbody>
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NOTE. The hazard ratios are for each ASCO group compared with ASCO Group 5 taken as the reference. There were too few patients accrued to BCIRG-005 with a HER2 FISH ratio ≥ 2.0 for analysis of DFS or OS.

Abbreviations: BCIRG, Breast Cancer International Research Group; CAP, College of American Pathologists; DFS, disease-free survival; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; OS, overall survival.

*Group 5 (reference) compared with each other group in BCIRG-005 (HER2 not amplified breast cancers).
Group 4

HER2/CEP17 < 2.0

Average HER2 signal / cell ≥ 4.0 and < 6.0 (FISH Equivocal)
**Group 5**

HER2/CEP17 < 2.0

Average HER2 signal / cell < 4.0 *(FISH Negative)*

---

**Table 3.** Comparison of HER2 Ratio and Average HER2 Gene Copy Number and ASCO-CAP Groupings With Clinical Outcomes in BCIRG-005

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<th>No. of Subjects</th>
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Abbreviations: BCIRG, Breast Cancer International Research Group; CAP, College of American Pathologists; DFS, disease-free survival; HER2, human epidermal growth factor receptor 2; HR, hazard ratio; OS, overall survival.

*Group 5 (reference) compared with each other group in BCIRG-005 (HER2 not amplified breast cancers).
HER2 testing (invasive component) by validated dual-probe ISH assay

Batch controls and on-slide controls show appropriate hybridization

**HER2/CEP17 ratio ≥ 2.0**
- **Group 1**
  - Average HER2 copy number ≥ 4.0 signals/cell
  - ISH positive

**HER2/CEP17 ratio < 2.0**
- **Group 5**
  - Average HER2 copy number < 4.0 signals/cell
  - ISH negative

95% of cases
HER2 testing (invasive component) by validated dual-probe ISH assay

Batch controls and on-slide controls show appropriate hybridization

- HER2/CEP17 ratio ≥ 2.0
  - Group 1: Average HER2 copy number ≥ 4.0 signals/cell
    - ISH positive
  - Group 2: Average HER2 copy number < 4.0 signals/cell
    - Additional work-up required (see Fig 4)

- HER2/CEP17 ratio < 2.0
  - Group 3: Average HER2 copy number ≥ 6.0 signals/cell
    - Additional work-up required (see Fig 5)
  - Group 4: Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell
    - Additional work-up required (see Fig 6)
  - Group 5: Average HER2 copy number < 4.0 signals/cell
    - ISH negative

5% of the cases

Addressed in 2018 ASCO/CAP Update
2018 ASCO/CAP Update for Less Common FISH Patterns

• It is not based only on FISH but a combination of FISH and IHC testing.
• Requires review of IHC before designation of HER2 status (positive or negative)
HER2 testing (invasive component) by validated dual-probe ISH assay

Batch controls and on-slide controls show appropriate hybridization

**HER2/CEP17 ratio ≥ 2.0**
- **Group 1**
  - Average HER2 copy number ≥ 4.0 signals/cell
  - ISH positive
- **Group 2**
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**HER2/CEP17 ratio < 2.0**
- **Group 3**
  - Average HER2 copy number ≥ 6.0 signals/cell
  - Additional work-up required (see Fig 5)
- **Group 4**
  - Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell
  - Additional work-up required (see Fig 6)
- **Group 5**
  - Average HER2 copy number < 4.0 signals/cell
  - ISH negative
Clinical Question 3 (Group 2):

- FDA: trastuzumab regardless of HER2 copy number; 2013 ASCO/CAP considered these as positive
- Rare: 0.8% in HERA trial; 0.7% in BCIRG
- HERA trial: “Sample size insufficient to rule out benefit”
- Almost always HER2 negative by IHC
- Most are estrogen receptor (ER) positive
Clinical Question 3 (Group 2):

1. HER2/CEP17 ratio $\geq 2.0$
   Average HER2 signals/cell < 4.0
   - Assess IHC using sections from the same tissue sample used for ISH
     - IHC 0 or 1+:
       - HER2 negative with comment*
     - IHC 2+:
       - Observer blinded to previous results; recounts ISH, counting at least 20 cells
         - HER2 positive
     - HER2/CEP17 Ratio $\geq 2.0$
       Average HER2 signals/cell < 4.0
       - HER2 negative with comment*
     - Other ISH result
       - Result should be adjudicated per internal procedures to determine final category
Clinical Question 4 (Group 3):

- Heterogeneous group: HER2 + and HER2-ive by IHC
  HERA trial: 75% of 20 cases were IHC positive / 3+
  Trial with three centers: 31% of 63 cases were IHC positive / 3+
  USC: 8.3% of 48 cases were IHC positive / 3+
2018 ASCO / CAP Update

Clinical Question 4 (Group 3):

- **HER2/CEP17** ratio < 2.0
  - Average HER2 signals/cell ≥ 6.0
  - Assess IHC using sections from the same tissue sample used for ISH

  - IHC 0 or 1+
    - HER2 negative with comment
  - IHC 2+
    - Observer blinded to previous results recounts ISH, counting at least 20 cells

    - **HER2** positive

    - **HER2** positive

- **HER2/CEP17** ratio ≥ 2.0
  - Average HER2 signals/cell ≥ 6.0

  - HER2 positive

  - Other ISH result
    - Result should be adjudicated per internal procedures to determine final category
2013 ASCO/CAP
FISH Equivocal

• **Mayo Clinic**: 14% of all FISH cases were equivocal → 50% of which became positive with alternate probe (D17S122) increasing overall FISH positivity to **23.6%**

• **ARUP**: 15% of all FISH cases were equivocal → 30% of which became positive with alternate probe (RIA1) increasing overall FISH positivity to **21.6%**

• **Some labs used 4 or more FISH alternate probes, reported the positive one, increasing the overall FISH positivity rate even further**
Clinical Question 5 (Group 4):

NO ALTERNATE PROBE!
2018 ASCO / CAP Update

Clinical Question 5 (Group 4):

HER2/CEP17 ratio < 2.0
Average HER2 signals/cell ≥ 4.0 and < 6.0
Assess IHC using sections from the same tissue sample used for ISH

IHC 0 or 1+
HER2 negative with comment*

IHC 2+
Observer blinded to previous results recounts ISH, counting at least 20 cells

IHC 3+
HER2 positive

HER2/CEP17 ratio < 2.0
Average HER2 signals/cell ≥ 4.0 and < 6.0
HER2 negative with comment*

Other ISH result
Result should be adjudicated per internal procedures to determine final category
What to expect after 2018 ASCO/CAP Update?
HER2 testing (invasive component) by validated dual-probe ISH assay

Batch controls and on-slide controls show appropriate hybridization

HER2/CEP17 ratio ≥ 2.0

- **Group 1**: Average HER2 copy number ≥ 4.0 signals/cell
  - ISH positive

- **Group 2**: Average HER2 copy number < 4.0 signals/cell
  - Additional work-up required (see Fig 4)

HER2/CEP17 ratio < 2.0

- **Group 3**: Average HER2 copy number ≥ 6.0 signals/cell
  - Additional work-up required (see Fig 5)

- **Group 4**: Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell
  - Additional work-up required (see Fig 6)

- **Group 5**: Average HER2 copy number < 4.0 signals/cell
  - ISH negative

5% of the cases
<table>
<thead>
<tr>
<th>Table 3. Distribution by Dual Fluorescent In Situ Hybridization (FISH) and Immunohistochemistry (IHC) Testing Results in Reported Data Sets*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial Test Results</strong></td>
</tr>
<tr>
<td><strong>FISH distribution</strong></td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>Group 1 ratio ≥2.0; HER2 ≥4.0</td>
</tr>
<tr>
<td>Group 2 ratio ≥2.0; HER2 &lt;4.0</td>
</tr>
<tr>
<td>Group 3 ratio &lt;2.0; HER2 ≥6.0</td>
</tr>
<tr>
<td>Group 4 ratio &lt;2.0; HER2 ≥4.0 and &lt;6.0 (after alternative probe: pos, equivocal, neg)</td>
</tr>
<tr>
<td>Group 5 ratio &lt;2.0; HER2 &lt;4.0</td>
</tr>
<tr>
<td><strong>IHC distribution</strong></td>
</tr>
<tr>
<td>No.</td>
</tr>
<tr>
<td>0</td>
</tr>
<tr>
<td>1+ (including 0 or 1+)</td>
</tr>
<tr>
<td>2+ (including 1+/2+ or 2+/3+)(\d)</td>
</tr>
<tr>
<td>3+</td>
</tr>
</tbody>
</table>
### Table 3. Distribution by Dual Fluorescent In Situ Hybridization (FISH) and Immunohistochemistry (IHC) Testing Results in Reported Data Sets

<table>
<thead>
<tr>
<th>Initial Test Results</th>
<th>HERA Central Laboratory(^{15})</th>
<th>BCIRG Central Laboratory(^{10})</th>
<th>USC Breast Cancer Analysis Laboratory(^{12})</th>
<th>Mayo Clinic Cytogenetics Laboratory(^{11})</th>
<th>UK NEQAS 2009-2016(^{4})</th>
<th>Stanford/UCSF/UWMC(^{6})</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>FISH distribution</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>6018</td>
<td>10,468</td>
<td>7,526</td>
<td>2,851</td>
<td>11,116</td>
<td>8,068</td>
</tr>
<tr>
<td>Group 1 ratio ≥2.0; HER2 ≥4.0</td>
<td>55.0 (≥4.0, 48.7; ≥4.0, 5.6, 6.3)</td>
<td>40.8</td>
<td>17.7</td>
<td>11.8</td>
<td>14.2</td>
<td>13.8</td>
</tr>
<tr>
<td>Group 2 ratio ≥2.0; HER2 &lt;4.0</td>
<td>0.8</td>
<td>0.7</td>
<td>0.4</td>
<td>1.3</td>
<td>3.7</td>
<td>1.4</td>
</tr>
<tr>
<td>Group 3 ratio &lt;2.0; HER2 ≥6.0</td>
<td>0.4</td>
<td>0.5</td>
<td>0.6</td>
<td>3.0</td>
<td>1.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Group 4 ratio &lt;2.0; HER2 ≥4.0 and &lt;6.0 (after alternative probe: pos, equivocal, neg)</td>
<td>1.9</td>
<td>4.1</td>
<td>4.6</td>
<td>14.2 (7.5, 5.5, 1.3)</td>
<td>7.6</td>
<td>5.2</td>
</tr>
<tr>
<td>Group 5 ratio &lt;2.0; HER2 &lt;4.0</td>
<td>41.9</td>
<td>23.9</td>
<td>76.7</td>
<td>69.6</td>
<td>73.4</td>
<td>78.8</td>
</tr>
<tr>
<td><strong>IHC distribution</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>3,089</td>
<td>4,331</td>
<td>7,526</td>
<td>1,922</td>
<td>11,116</td>
<td>3,027</td>
</tr>
<tr>
<td>0</td>
<td>IHC 0-1+, 2.0</td>
<td>54.5</td>
<td>51.7</td>
<td>2.4</td>
<td>0.5</td>
<td>IHC 0-1+, 38.1</td>
</tr>
<tr>
<td>1+ (including 0 or 1+)</td>
<td>—</td>
<td>9.4</td>
<td>31.0</td>
<td>8.9</td>
<td>1.8</td>
<td>—</td>
</tr>
<tr>
<td>2+ (including 1+/2+ or 2+3+)†</td>
<td>61.8</td>
<td>13.7</td>
<td>9.0</td>
<td>87.14</td>
<td>96.54</td>
<td>2+, 46.6</td>
</tr>
<tr>
<td>3+</td>
<td>36.2</td>
<td>22.4</td>
<td>8.4</td>
<td>2.5</td>
<td>1.3</td>
<td>3+, 15.3</td>
</tr>
</tbody>
</table>
In most labs, these three groups will be ~5-10% of all FISH cases. However, the proportion will be much higher in reference lab setting.

Almost 1/4\(^{th}\) (127/521; 24.4%) of all HER2 FISH tests from primary or metastatic breast cancers at the University of Utah / ARUP Labs fell under the three groups (Groups 2, 3, and 4)

2018 ASCO/CAP recommendations may result in some drop in HER2 FISH positivity rate which may be limited to reference labs.
Reference Lab / ARUP HER2 FISH Results

2013 ASCO/CAP
(before alternate probe)

HER2 Negative 61%
HER2 Positive 18%
HER2 Equivocal 21%

2013 ASCO/CAP
(after alternate probe)

HER2 Equivocal 1%
HER2 Negative 74%
HER2 Positive 25%

2018 ASCO/CAP

HER2 Equivocal 1%
HER2 Positive 17%
HER2 Negative 83%
HER2/CEP17 Ratio < 2.0
HER2 signal /cell ≥ 4.0 and < 6.0
FISH Equivocal
HER2/CEP17 Ratio >2.0

FISH Positive
Common Problem in Interpretation of HER2 IHC

• Overcalling 2+ / Equivocal HER2 as positive (3+)

  • When there is heterogeneous IHC staining i.e. some areas look like 3+ and others 0-2+ → stop and think before calling it 3+

  • Most HER2 IHC positives (3+) are homogenously positive and you do not need a microscope to call it positive!
Lastly ...

If you are using ink for breast cores to prevent specimen mix-up, avoid using orange ink as it auto-fluoresces and interferes with FISH interpretation.