ASCO/CAP HER2 guidelines update

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Outline

• The evolution of HER2 scoring recommendations by ASCO/CAP
• Changes in the 2018 focused update: critical discussion step by step
• The future of HER2 evaluation in breast cancer
Les fils rouges of ASCO/CAP recommendations

TO AVOID FALSE POSITIVE

- Review of pre-analytical and analytical steps
- Rising the thresholds for positive results (30% cut-off, \(\text{HER2}/\text{CEP17} > 2.2\))

TO AVOID FALSE NEGATIVE

- Sensitivity rather than specificity => thresholds lowered
- ISH algorithm => \(\text{HER2}/\text{CEP17}\) ratio and \(\text{HER2}\) copy number

TO ANSWER SPECIFIC CLINICAL QUESTIONS

- Integration of 2013 guidelines (i.e. “focused update”)
  - Score 2+ definition

5 Clinical questions addressed
Clinical question 1

What is the most appropriate definition for IHC 2+ (IHC equivocal)?
Score 2+ definition in 2013

=> “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.”
2018 ASCO/CAP recommendations

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 in > 10% of tumor cells*

IHC 3+ positive

Weak to moderate complete membrane staining observed in > 10% of tumor cells

IHC 2+ equivocal

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

IHC 1+ negative

No staining is observed or Membrane staining that is incomplete and is faint/barely perceptible and in ≤ 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)
Unusual Score 2+ staining patterns

Baso-lateral membrane staining
Unusual Score 2+ staining patterns

Complete intense membrane staining in $\leq 10\%$ of tumor cells
What to do?

• Test all available blocks (including the LND mets if any)
  
  => if still $\leq 10\%$: report the percentage of HER2 positive cells

Useful information for any retesting in case of relapse or metastatic disease
Impact of intra-tumor heterogeneity: the neoadjuvant setting
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pCR rates
Impact of HER2 heterogeneity on anti-HER2 treatment

Phase II Study Evaluating HER2 Heterogeneity as a Predictor of Response to Neoadjuvant T-DM1 and Pertuzumab (Dana-Farber Harvard Cancer Center 14-409)

Otto Metzger, Giuseppe Viale, Lorenzo Trippa, Tianyu Li, Denise Yardley, Ingrid Mayer, Vandana Abramson, Carlos Arteaga, Laura Spring, Adrienne Waks, Michalina Janiszewska, Eileen Wrabel, Michelle DeMeo, Shayna Stein, Franziska Michor, Aditya Bardia, Tari King, Kornelia Polyak, Eric Winer and Ian Krop
Study Hypothesis and Rationale

- HER2 heterogeneity is associated with inferior pathologic complete response (pCR) rate to neoadjuvant targeted anti-HER2 therapy

Investigating the impact of HER2 heterogeneity on response to therapy is an important step while we try to de-escalate chemotherapy and rely on HER2-targeted Rx
Study Design

- Centrally-confirmed HER2+ BC
- Stage II and III (N = 164)

Single Arm

T-DM1 + Pertuzumab q3w x 6

Image-guided research biopsies

Surgery
HER2 Heterogeneity: Method of Evaluation

HER2 Heterogeneity defined as either

1) HER2 positivity by FISH in > 5% and < 50% of tumor cells (i.e., CAP guideline)
2) An area of tumor that tested HER2 negative.

Assessment performed by central laboratory (European Institute of Oncology, Milan) and blinded to treatment outcome

Vance GH et al. Arch Pathol Lab Med 2009
Results: Prevalence of Heterogeneity

- 16/157 (10%) of evaluable cases were classified as HER2 heterogenous
  - 13 (81%) hormone receptor positive and 3 (19%) hormone receptor negative

Example: Core biopsy site 1 amplified and site 2 non-amplified

FISH ratio = 3.85
FISH ratio = 1.1
Effect of Heterogeneity on pCR

Pathologic Complete Response
Residual Cancer Burden Class 0

The study met its primary endpoint by demonstrating a significant association between HER2 heterogeneity and pCR adjusted by ER status (p < 0.001)

- Heterogeneous: N = 16
- Non-Heterogenous: N = 141

55%
Conclusions

- Our study met its primary endpoint by demonstrating a significant association between HER2 heterogeneity and pCR
  - This effect was independent of ER status and HER2 protein expression by IHC
- The use of a clinical definition of HER2 heterogeneity defined by FISH should facilitate efforts to validate in other studies
- T-DM1 plus Pertuzumab is a well tolerated regimen with 95% of pts completing six cycles of tx
  - In the non-heterogeneous group pCR rate is 55%
- HER2 heterogeneous cancers may represent a distinct subset of HER2+ breast cancer
  - Lower rates of pCR
  - Lower levels of HER2 protein expression
  - Possibly require different treatment approaches
Clinical question 2

Must HER2 testing be repeated on a surgical specimen if initially negative on core biopsy?
HER2 repeat following a negative result on core biopsy

If the initial HER2 test result in a core needle biopsy specimen of a primary breast cancer is negative, a new HER2 test may be ordered on the excision specimen if one of the following is observed:

- Tumor is grade 3
- Amount of invasive tumor in the core biopsy specimen is small
- Resection specimen contains high-grade carcinoma that is morphologically distinct from that in the core
- Core biopsy result is equivocal for HER2 after testing by both ISH and IHC
- There is doubt about the handling of the core biopsy specimen (long ischemic time, short time in fixative, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error

- Integration of core biopsy and surgical specimen info (importance of accurate clinical info)
- Preanalytical phase control
Clinical questions 3, 4, 5

Unusual ISH patterns
2018 ASCO/CAP recommendations

**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 FISH test required

**HER2/CEP17 ratio < 2.0**

- **Group 3**
  - Average HER2 copy number ≥ 6.0 signals/cell
  - Additional FISH test required

- **Group 4**
  - Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell
  - Additional FISH test required

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

**FISH Groups according to Press M et al, J Clin Oncol 2016**
Unraveling the chromosome 17 patterns of FISH in interphase nuclei: an in-depth analysis of the HER2 amplicon and chromosome 17 centromere by karyotyping, FISH and M-FISH in breast cancer cells

Milena Rondón-Lagos1,2, Ludovica Verdun Di Cantogno3, Nelson Rang1,4, Teresa Mele5, Sandra R Ramírez-Clavijo6, Giorgio Scaglotti7, Caterina Marchió1,2,1* and Anna Sapino1,2,1*
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=> Complex patterns of Chr 17 genetic rearrangement in breast carcinomas
Does chromosome 17 centromere copy number predict polysomy in breast cancer? A fluorescence in situ hybridization and microarray-based CGH analysis

Caterina Marchiò,1,2 Maryou B Lambros,1 Patrizia Gugliotta,2 Ludovica Verdun Di Cantogno,2 Cristina Botta,2 Barbara Pasini,3 David SP Tan,1 Alan Mackay,1 Kerry Fenwick,1 Naninder Tamber,1 Gianni Bussolati,2 Alan Ashworth,1 Jorge S Reis-Filho1 * and Anna Sapino2 *
2018 ASCO/CAP recommendations

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HER2/CEP17 ratio ≥ 2.0

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  - ISH positive

- Group 2: Average HER2 copy number < 4.0 signals/cell
  - Additional FISH score

HER2/CEP17 ratio < 2.0

- Group 3: Average HER2 copy number ≥ 6.0 signals/cell

- Group 4: Average HER2 copy number ≥ 4.0 and < 6.0 signals/cell

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

FISH Groups according to Press M et al, J Clin Oncol 2016
2018 ASCO/CAP recommendations

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

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- **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

*FISH Groups according to Press M et al, J Clin Oncol 2016*
Additional work-up means ...

- Perform IHC/ review IHC (also on additional blocks)
  - Report acc. to IHC score
- If score 2+ confirmed:
  - Count additional cells (additional observer)

Discordant scoring: result adjudicated per internal procedures

Concordant scoring
Evidence is limited on the efficacy of HER2-targeted therapy in the small subset of cases with a HER2/CEP17 ratio of $\geq 2.0$ and an average HER2 copy number of $< 4.0$ per cell. In the first generation of adjuvant trastuzumab trials, patients in this subgroup who were randomly assigned to the trastuzumab arm did not seem to derive an improvement in disease-free or overall survival, but there were too few such cases to draw definitive conclusions. IHC expression for HER2 should be used to complement ISH and define HER2 status. If the IHC result is not 3+ positive, it is recommended that the specimen be considered HER2 negative because of the low HER2 copy number by ISH and the lack of protein overexpression.

(Type: evidence based; Evidence quality: intermediate; Strength of recommendation: strong). An algorithm for Clinical
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

IHC 3+
- 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
The Expert Panel recommends the following comment: It is uncertain whether patients with an average of \( \geq 4.0 \) and \( < 6.0 \) HER2 signals per cell and a \( \text{HER2/CEP17} \) ratio of \( < 2.0 \) benefit from HER2 targeted therapy in the absence of protein overexpression (IHC 3+). If the specimen test result is close to the ISH ratio threshold for positive, there is a high likelihood that repeat testing will result in different results by chance alone. Therefore, when IHC results are not 3+ positive, it is recommended that the sample be considered HER2 negative without additional testing on the same specimen. (Type: evidence based; Evidence quality: intermediate; Strength of recommendation: strong). An algorithm for Clinical
In HER2+ patients
Population of “HER2-low” breast cancer patients
Score 1+/2+ not amp (HER2/CEP17 <2 and HER2 copy number < 4)
The Dilemma of HER2 Double-equivocal Breast Carcinomas
Genomic Profiling and Implications for Treatment

Molecular subtyping by PAM50

Luminal B in the large majority (76%) HER2 mRNA levels much closer to HER2-neg HER2-enriched in 5% of cases
# HER2-directed ADC in clinical development

<table>
<thead>
<tr>
<th>Agent</th>
<th>Anti-HER2 MAb/payload (target)</th>
<th>Drug to antibody ratio</th>
<th>Linker drug</th>
<th>Phase of development</th>
<th>ORR in HER2-positive</th>
<th>ORR in HER2 low (IHC1+/2+/ISH-)</th>
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<tr>
<td>Trastuzumab-DM1 (T-DM1)(^7)</td>
<td>Trastuzumab/DM1 (anti-tubulin)</td>
<td>3.5</td>
<td>Noncleavable</td>
<td>US FDA Approved</td>
<td>43.6%</td>
<td>———</td>
</tr>
<tr>
<td>Trastuzumab duruxtecan (DS-8201a)(^39)</td>
<td>Trastuzumab/ exatecan derivative (topoisomerase I inhibitor)</td>
<td>8</td>
<td>Cleavable</td>
<td>II/III NCT03248492 NCT03529110 NCT03523585</td>
<td>54.5%</td>
<td>50%</td>
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<tr>
<td>SYD985(^40)</td>
<td>Duocarmycin derivative (alkylating agent)</td>
<td>2.8</td>
<td>Cleavable</td>
<td>III NCT03262935</td>
<td>33%</td>
<td>HR + 27% HR - 40%</td>
</tr>
<tr>
<td>XMT-1522(^41)</td>
<td>XMT-1519/ monomethyl auristatin (anti-tubulin)</td>
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<td>ARX788</td>
<td>Anti-HER2 MAb/ auristatin analog 269 (AS269) (anti-tubulin)</td>
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<td>DHES0815A</td>
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<td>2</td>
<td>Cleavable</td>
<td>I NCT03451162</td>
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</tr>
</tbody>
</table>

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Pernas S & Tolaney S, *Therapeutic Advances in Medical Oncology* 2019
What’s next?
HER2 negative

SCORE 0

SCORE 1+

SCORE 2+

SCORE 3+

“HER2 low”

ISH

NOT Amp

EQUIV

AMP

HER2 POSITIVE
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