HER2-LOW BREAST CANCERS

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BREAST CANCER CONTEXT

Treatment decisions in clinical practice are based on conventional histopathological factors.

BC is divided into 4 surrogate subgroups: luminal A-like, luminal B-like (HER2-negative), HER2-positive and triple-negative BC (TNBC).

This classification has both prognostic and predictive value.

**HER2 gene amplification** = Strong oncogenic driver ➔ HER2 receptor overexpression ➔ ~15% of BC

HER2 amplification = **actionable pathway targetable** for >20 years.

OUTLINES

Latest HER2 testing guidelines

Defining and targeting HER2 low
CONSTANT AND CONTINUOUS EXPRESSION OF HER2 IN BREAST CANCER

Images courtesy of Frédérique Penault-Llorca, MD, PhD
LATEST CLINICAL GUIDELINES FOR MEASURING HER2
FDA, Food and Drug Administration; IHC, immunohistochemistry; ISH, in situ hybridization.
THE HISTORY OF HER2 TESTING: THE CUT-OFF CONTROVERSY

Trastuzumab approval: HER2, IHC 3+ (10%) or FISH+ = HER2 positive

Change in IHC definition 3+ (>30%)

One step back! 3+ (>10%)

2000

2006/2007

2013

2018

ISH+ ≥4 copies or ≥ratio 2

>6 copies or ratio >2.2

...equivocal category

4–6 copies ratio <2

ASCO/CAP 2018 new guidelines

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; FISH, fluorescent in situ hybridization;
HER2 GUIDELINES: WHEN TO TEST

**ASCO/CAP:** Primary, recurrent, and metastatic cancers, if tissue sample is available

**EU:** All invasive primary breast carcinoma and in recurrent and metastatic tumours where biopsy tissue is available

Every primary invasive breast cancer and on metastatic site, if specimen available
HER2 GUIDELINES: ACCEPTABLE METHODS

**UK/EU:** validated standardised IHC

ISH includes FISH technique; bright-field ISH, which can be used to assess HER2 status with a regular light microscope; can be used as an alternative to FISH

Currently, other available alternative HER2 testing techniques (polymerase chain reaction, enzyme-linked immunosorbent assay, Southern blotting, mRNA assays, and DNA microarray) should be used for research only.
HER2 GUIDELINES: WHICH SPECIMEN?

EU: core biopsy or surgical specimen

Excellent concordance between core biopsy and surgical specimens has been shown using a combination of IHC and ISH

ASCO/CAP: core biopsy or incisional or excisional biopsy

- 2013: if negative on core, must repeat on excision
- 2018: if negative on core, may repeat on excision

ASCO, American Society of Clinical Oncology; CAP, College of American Pathologists; IHC, immunohistochemistry; ISH, in situ hybridization.

IMMUNOHISTOCHEMISTRY RULES

HER2 testing (invasive component) by validated IHC assay

Batch controls and on-slide controls show appropriate hybridization

No staining is observed
Or Membrane staining that is incomplete and is faint/barely perceptible and in ≤10% of tumour cells

IHC 0 negative

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

IHC 1+ negative

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

IHC 2+ equivocal
Must order reflex test (same specimen using ISH) or order a new test (new specimen if available, using IHC or ISH)

Circumferential membrane staining that is complete, intense, and in >10% of tumour cells*

IHC 3+ positive

IHC, immunohistochemistry.
HER2 SCORING AND MAGNIFICATION

IHC, immunohistochemistry.
Courtesy of Josef Rüschoff: microscope’s rule for HER2 scoring.
ALGORITHM FOR EVALUATION OF HER2 AMPLIFICATION ASSAY

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

**HER2/CEP17 ratio <2.0**
- **Group 2**: Average HER2 copy number <4.0 signals/cell
- **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

**Additional work-up required**
Assess IHC using sections from the same tissue sample used for ISH

IHC 0 or 1+ = HER2 negative with comment
IHC 2+ = varies from group 2 vs 3 vs 4
IHC 3+ = HER2 positive

CEP17, chromosome enumeration probe 17; IHC, immunohistochemistry; ISH, hybridization in situ
*Recommended instead of single-probe ISH assays.
ALGORITHM FOR EVALUATION OF HER2 AMPLIFICATION ASSAY

**ALGORITHM FOR EVALUATION OF HER2 AMPLIFICATION ASSAY**

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

Batch controls and on-slide controls show appropriate hybridization

**Group 1**
Average HER2 copy number ≥ 4.0 signals/cell

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

*Recommended instead of single-probe ISH assays.


CEP17, chromosome enumeration probe 17; IHC, immunohistochemistry; ISH, hybridization in situ.
ALGORITHM FOR EVALUATION OF HER2 AMPLIFICATION ASSAY

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

Batch controls and on-slide controls show appropriate hybridisation

**HER2/CEP17 ratio ≥2.0**
- Group 2
  - Average HER2 copy number <4.0 signals/cell

**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

Additional work-up required
Assess IHC using sections from the same tissue sample used for ISH

IHC 0 or 1+ = HER2 negative with comment
IHC 3+ = HER2 positive
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*Recommended instead of single-probe ISH assays.

CEP17, chromosome enumeration probe 17; IHC, immunohistochemistry; ISH, hybridation in situ.
ALGORITHM FOR EVALUATION OF HER2 AMPLIFICATION ASSAY: SUMMARY

Steps in review process for these uncommon groups are the same

Applies to all 3 uncommon groups:

- **Group 2** HER2/CEP17 ratio >2.0, HER2 copies <4 (monosomy)
- **Group 3** HER2/CEP17 ratio <2.0, HER2 copies >6 (co-amplified-polysomy)
- **Group 4** HER2/CEP17 ratio <2.0, HER2 copies >4 but <6 (ex equivocal cases)

HER2 IHC done on the same sample and ideally adjacent slide is reviewed along with ISH result

If **HER2 IHC is 3+**, diagnosis is HER2-positive

If **HER2 IHC is 0, 1+**, diagnosis is HER2-negative

If **HER2 IHC is 2+**, ISH slide must undergo additional review:
More cells must be counted in HER2 IHC 2+ area by second observer **blinded** to first result

CEP17, chromosome enumeration probe 17; IHC, immunohistochemistry; ISH, hybridation in situ
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 F. 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
GROUP 2 HER2/CEP17 RATIO >2.0, HER2 COPIES <4
(MONOSOMY)

M Press analysis:
- 0.7% of >10,000 patients; rare
- In BCIRG-006 no apparent benefit of trastuzumab in terms of DFS or OS

If IHC is 2+ ➔ consider as HER2 negative
GROUP 4 HER2/CEP17 RATIO <2.0, HER2 COPIES >4 BUT <6 (EX EQUIVOCAL CASES)

80–90% are ER+ - luminal B like

Press analysis:
- 4.1% of >10,000 patients
- Patients in this category had similar DFS and OS without trastuzumab when compared with patients with ratio <2 and copy number <4

If IHC is 2+ ➔ consider as HER2 negative
GROUP 3 HER2/CEP17 RATIO <2.0, HER2 COPIES >6 (CO-AMPLIFIED-POLYSOMY)

M Press analysis:
- 0.5% of >10,000 patients; rare
- Unclear whether trastuzumab benefits, but standard remains to treat with trastuzumab

Consider as HER2 positive after multidisciplinary tumour board discussion

UNRESOLVED ISSUES FOR HETEROGENEITY

Image courtesy of F. Penault-Llorca

HER2 heterogeneous primary BC

HER2 negative metastatic BC
**DIFFICULT CASES: HETEROGENEITY**

**1st situation:** zonal or regional heterogeneity

Contingent of non amplified 0/1+/HER2 and contingent of 3+/HER2 amplified in the same tumour

- Review IHC slide
- Exclude a technical artefact (fixation)
- Test another block or a node+
- **Give the % of contingent + and of contingent –**
- Score as HER2 positive if amplified >10%
- Discuss in MTB

IHC, immunohistochemistry; MTB, molecular tumour board.

2\textsuperscript{ND} SITUATION: COMPLEX AND SCATTERED HETEROGENEITY

2+ score with isolated amplified tumour cells

- Unknown significance
- Check node metastasis before conclusion

Images courtesy of Frederique Penault-Llorca, CJP, Clermont-Ferrand and Dr Alexander Valent, IGR, Villejuif, France

HETEROGENEITY AND LONG-TERM OUTCOMES

280 consecutive invasive breast cancer cases (breast-conserving surgery or modified radical mastectomy)

Clinical outcome investigation

HETEROGENEITY AND ANTI-HER2 THERAPIES

Trastuzumab-based neoadjuvant chemotherapy efficacy model

HETEROGENEITY AND ANTI-HER2 THERAPIES

Single-arm, neoadjuvant Phase 2 study of T-DM1 plus pertuzumab 6 cycles enrolling centrally confirmed HER2+ heterogeneous breast cancer

HER2 heterogeneity defined as either:
1. HER2 positivity by FISH in >5% and <50% of tumour cells (i.e., CAP guidelines)
2. An area of tumour that tested HER2 negative

HER2 heterogeneity ➔ strong predictor of non pCR to a dual-HER2 targeted therapy regimen
HER2 heterogeneity should be considered in selection of patients for HER2-targeted regimens without chemotherapy in the curative setting

WE HAVE OPTIMISED HER2 TESTING TO GUIDE TREATMENT DECISIONS FOR USE OF CLASSICAL ANTI-HER2 THERAPIES
Summary of HER2 FISH results in 1044 HER2 IHC equivocal cases according to the ASCO®/CAP 2007, 2013, and 2018 Guidelines

<table>
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<tr>
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<tbody>
<tr>
<td></td>
<td>No.</td>
<td>%</td>
<td>No.</td>
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<tr>
<td>Positive</td>
<td>112</td>
<td>10.7</td>
<td>182</td>
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<tr>
<td>Equivocal</td>
<td>118</td>
<td>11.3</td>
<td>38</td>
</tr>
<tr>
<td>Negative</td>
<td>814</td>
<td>78.0</td>
<td>824</td>
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“The 2018 update heralds a potential change in therapeutic options for a significant number of patients, with 2.9% of FISH-positive tumours according to 2007 and 2013 guidelines now categorised as HER2 negative”
MESSAGES FOR HER2 ASCO/CAP NEW GUIDELINES

Simplification of HER2 2+
No longer systematic re-testing

- **Difficult ISH categories:**
  - Interpretation with IHC++++
  - Independent (second reader for ISH) for 2+
  - Disappearance of equivocal ISH category
    - Category 2 (monosomy): rather negative
    - Category 3 (co-ampl): rather positive
    - Category 4 (ex-equivocal): rather negative

- Avoid single probe ISH
ALGORITHM FOR EVALUATION OF HER2 AMPLIFICATION ASSAY

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Batch controls and on-slide controls show appropriate hybridization

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**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

Additional work-up required
Assess IHC using sections from the same tissue sample used for ISH

IHC 0 or 1+ = HER2 negative with comment
IHC 3+ = HER2 positive
ICH 2+ = varies from group 2 vs 3 vs 4

*Recommended instead of single-probe ISH assays.*

NEW DEFINITIONS OF HER2 STATUS

HER2-low patients who may derive benefit from emerging therapies
CONSTANT AND CONTINUOUS EXPRESSION OF HER2 IN BREAST CANCER

Images courtesy of Frédérique Penault-Llorca, MD, PhD
HER2 POSITIVE OR “HIGH”

Images courtesy of Frédérique Penault-Llorca, MD, PhD

Oncogene addiction
HER2-LOW BREAST CANCERS CONTEXT

Recently, a subset of HER2-expressing BC not addicted to HER2 could also derive benefit from targeting this receptor with new drugs.

~55% of all BC are HER2-low cancers (IHC 2+ non amplified and IHC 1+)
HER2 “LOW”

HER2 testing (invasive component) by validated IHC assay

Batch controls and on-slide controls show appropriate hybridization

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

IHC 1+

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

IHC 2+ Non amplified

HER2 IHC EXPRESSION IN HER2-NEGATIVE (NON AMPLIFIED OR NON 3+) BREAST CANCER

Cases on file, from Centre Jean Perrin, with an over-representation of referred 2+ cases (FISH reference centre)

49% of HER2 “low” expression

- TNBC
  - HER2 0: 51%
  - HER2 1+: 15%
  - HER2 2+ NA: 34%
  - HER2 2+ NA: 34%
  - HER2 2+ NA: 34%

607 cases

75% of HER2 “low” expression

- RH+
  - HER2 0: 25%
  - HER2 1+: 27%
  - HER2 2+ NA: 48%

5563 cases

Frédérique Penault-Llorca, Md, PhD, Personal Communication
HER2 “LOW”

Score 2+ non amplified

Score 1+ non amplified

Images courtesy of Frédérique Penault-Llorca, MD, PhD
IMPORTANCE OF DISTINGUISHING SCORE 1+ FROM 0

In the literature, the definition of HER2 score 0 varies

As no therapies nor FISH are required for them, 1+ and 0 scores are frequently pooled under the denomination of HER2 negative or zero

Only 15/102 breast tumours locally scored IHC 0 were centrally confirmed; the remaining 87 cases scored 1+ or 2+, ➔ 85% of these patients would have been excluded from HER2-low trials

In fact, in the literature, HER2 score 0 varies from 14% to 80%

➔ with the perspective of new therapies for HER2 low BC ➔ the proportion of HER2 score 0 will decrease

STAINING OF NORMAL BREAST TISSUE

Depends on the clone of HER2 antibody, and on the technical conditions, as HER2 receptors are also expressed in normal cells.

Images courtesy of Frédérique Penault-Llorca, MD, PhD
HER2-LOW DISEASE AND BENEFIT FROM TRASTUZUMAB
**NSABP B-31: QUESTIONED POTENTIAL BENEFIT OF TRASTUZUMAB IN HER2-NEGATIVE POPULATION**

**HER2 Status and Benefit from Adjuvant Trastuzumab in Breast Cancer**

<table>
<thead>
<tr>
<th>Endpoint and central HER2 assay†</th>
<th>ACT # events/total # events</th>
<th>ACTH # events/total # events</th>
<th>Relative Risk (95% CI)</th>
<th>p-value</th>
<th>p-value for interaction</th>
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<tbody>
<tr>
<td>Disease progression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2-positive</td>
<td>163/875</td>
<td>85/804</td>
<td>0.47 (0.37–0.62)</td>
<td>&lt;0.001</td>
<td>0.47</td>
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<tr>
<td>HER2-negative</td>
<td>20/92</td>
<td>7/82</td>
<td>0.34 (0.14–0.80)</td>
<td>0.014</td>
<td></td>
</tr>
<tr>
<td>Death</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HER2-positive</td>
<td>55/875</td>
<td>38/804</td>
<td>0.66 (0.43–0.99)</td>
<td>0.047</td>
<td>0.08</td>
</tr>
<tr>
<td>HER2-negative</td>
<td>10/92</td>
<td>1/82</td>
<td>0.08 (0.01–0.64)</td>
<td>0.017</td>
<td></td>
</tr>
</tbody>
</table>

*95% CI and p-values adjusted according to number of positive nodes and oestrogen-receptor status from univariate Cox proportional-hazards model for each subgroup in NSABP B-31 trial.

†Central HER2 assay results defined as negative if they were negative by both fluorescence in situ hybridization (PathVysion, Vysis) and immunohistochemical analysis (Herceptest, Dako) and defined as positive if either test was positive.

ACT denotes doxorubicin, cyclophosphamide, and paclitaxel; ACTH, ACT plus trastuzumab.

NSABP, National Surgical Adjuvant Breast and Bowel Project.


“Among the 1787 patients with follow-up data, 174 patients had breast cancers that were found to be central HER2-negative (9.7%), yet these patients also appeared to benefit from trastuzumab (RR for disease-free survival, 0.34; 95% CI, 0.14, 0.80; P=0.014).”

“…our findings suggest that the benefit of adjuvant trastuzumab may not be limited to patients with HER2 amplification.”

“Validation of the findings from central testing would justify a Phase 3 trial of adjuvant trastuzumab in women with breast cancers that do not meet established criteria for therapy.”
NSABP B-47: ADJUVANT TRASTUZUMAB FOR HER2-LOW BC

Absence of or low HER2 oncogenic addiction
Absence of efficacy of TZB for HER2-low

Hormonal therapy and radiation as indicated. Chemotherapy by MD choice:
*AC→WP: Doxorubicin 60 mg/m² and Cyclophosphamide 600 mg/m² q2 or 3 wks x 4 followed by qwk paclitaxel x 12
or TC: Docetaxel 75 mg/m² + Cyclophosphamide 600 mg/m² q3wk x 6.
About 55% of BCs express low levels of HER2 in the absence of gene amplification.

HER2-low are either ER+ or ER− (less frequent).

NSABP B-47 demonstrated that adjuvant trastuzumab is not effective in these tumours, likely due to their low or absent addiction to HER2 signalling. Similarly, other agents disrupting the HER2 pathway have shown modest activity in HER2-low tumours.
HER2-LOW DISEASE: CONCLUSION
In HER2 addicted breast cancer, (HER2 amplified/3+) targeting the HER2 pathway has proven efficacy.

Recently, a subset of HER2-expressing BC not addicted to HER2 could also derive benefit from targeting this receptor with new drugs.

~55% of all BC are HER2-low cancers (IHC 2+ non-amplified and IHC 1+).

In HER2-low disease, the benefit of therapy is likely achieved by delivering cytotoxic drugs to the target cells or by attracting immune cells ➔ HER2 being a targetable protein.

No anti-HER2 agent is yet approved for the treatment of HER2-low breast cancer, but promising agents are in late stage of development.
CONSTANT AND CONTINUOUS EXPRESSION OF HER2 IN BREAST CANCER = THE TARGET IS PRESENT

Images courtesy of Frédérique Penault-Llorca, MD, PhD

HER2 0

HER2 1+

HER2 2+

HER2 3+

TARGETABLE PROTEIN
HER2 is used as target to deliver cytotoxic drugs or to trigger IC

ACTIONABLE PATHWAY
Response to anti-HER2 therapies
EVOLUTION OF HER2 STATUS DEFINITION

The dichotomous view of HER2 status (either positive or negative) has come to an end.

Now it becomes clinically important to distinguish truly HER2-negative from HER2-low BC, as treatment options are becoming available.

In particular, it is relevant to accurately distinguish tumours with an IHC score 1+ from those scored 0.
HER2 EXPRESSION IS NOT BLACK OR WHITE

A continuous expression exists, and different cut-offs might be needed depending on the objective
THANK YOU!