

Guideline

Subject: **ASCO CAP 2018 HER2 Testing for Breast Cancer Guidelines - Recommendations for Practice in Australasia**

Approval Date: December 2018

Review Date: December 2022

Review By: HER2 testing for Breast Cancer Working Party; Cancer Services Advisory Committee

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Associated Documents

ASCO CAP 2018 GUIDELINES and SUPPLEMENTS -
<http://ascopubs.org/doi/full/10.1200/JCO.2018.77.8738>

Summary of Key Points

- Testing of core biopsies for HER2 evaluation (including ISH, when necessary) is recommended. If core biopsies cannot be tested, evaluation of resection specimens is acceptable, if compliance with recommended cold ischaemic time and fixation time is ensured. Repeat testing of resection specimens should only be required in some circumstances, e.g. negative core biopsy result in a heterogeneous *tumour*, unknown result or non-diagnostic core. Communication between pathologists and other clinicians will help avoid unnecessary duplication.
- In the 2018 guidelines there are no circumstances in which cancers with 0 or 1+ IHC are considered HER2 Positive. Routine ISH testing of cases with a 0 or 1+ IHC result is not recommended. A case should not be classified as gene-amplified, if the IHC result is 0 or 1+. Implicit in this change is a need for quality assurance of HER2 IHC.
- In Australia, PBS-subsided access to anti-HER2 therapies requires demonstration of HER2 gene amplification by ISH, including cases with 3+ IHC.
- For cases with 2+ or 3+ IHC, the determination of the final HER2 status requires concurrent evaluation of both gene amplification and protein overexpression to determine classification into the appropriate HER2 subgroup.
- Specific criteria for the classification of five subgroups of dual probe HER2 ISH findings are provided.

1. Standardization of Pre-Analytical Factors

- a. Time to fixation (cold ischaemic time): ≤ 1 hour
- b. Duration of fixation: 6-72 hours

These requirements apply to core biopsies and resections. There are no changes to ASCO CAP's 2013 HER2 testing guidelines and their 2010 guidelines for the immunohistochemical testing of oestrogen and progesterone receptors that recommended all biomarker testing be performed on core biopsies of the primary tumour.

2. Clarification of the IHC 2+ Category

The IHC 2+ category reverts to the commonly accepted definition of weak to moderate complete membrane staining in $>10\%$ of tumour cells.

3. Discretionary Repeat HER2 Testing

Further HER2 testing on the resection specimen in cases with a negative initial HER2 test on the needle core biopsy is now at the discretion of the pathologist, rather than mandatory.

4. The Five ASCO-CAP Groupings of Dual ISH HER2 Test Results

Five clinical scenarios encountered in HER2 evaluation of breast cancers are enumerated. Groups 1 and 5 comprise 95% of test results. For the 5% of cancers in groups 2-4, strategies for further investigation are proposed, emphasizing concurrent IHC, dual probe ISH and second opinions. Final results are categorized per Table 1 and Figure 1.

Concurrent evaluation of IHC

This involves concurrent IHC testing by the laboratory performing the ISH evaluation, using sections from the same tissue block used for ISH.

The aim is to: i) score the IHC to resolve the HER2 status, and ii) guide the selection of areas to evaluate by ISH.

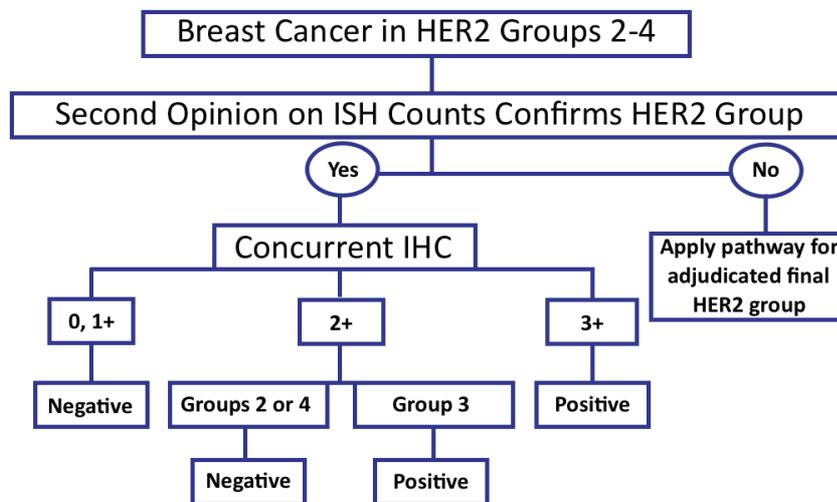
Second opinion

- Is required when the IHC result is 2+ and the case is in groups 2-4.
- Involves recount of ISH in at least 20 cells, in an area with 2+ IHC staining, by an additional observer, blinded to the first ISH results.
- If the second observer's counts assign the results into another ISH category, the final results are adjudicated, per internal procedures.
- For cases with 2+ IHC, if the initial observer's ISH counts are confirmed, the final HER2 status is negative (with specific comments), in HER2 groups 2 and 4, but positive in group 3.
- Specific comments are recommended to be included in reports of cases in HER2 groups 2-4 that are ultimately categorised as negative. See Appendix A.

Table 1: Test result scenarios and recommended final HER2 status

Group	Biology	HER2/CEP 17 Ratio	Mean HER2 copy number	2018 ASCO CAP Recommendation
1	Classic HER2 amplified cancer	≥2.0	≥4.0	Positive
2	Monosomy 17	≥2.0	<4.0	Negative, unless concurrent IHC 3+
3	Co-amplification, previously polysomy 17	<2.0	≥6.0	Negative, unless concurrent IHC 2+ or 3+
4	Borderline / equivocal	<2.0	≥4.0 and <6.0	Negative, unless concurrent IHC 3+
5	Classic HER2 non-amplified cancer	<2.0	<4.0	Negative

Figure 1: Flow chart for determining the HER2 status of invasive breast cancer in ASCO CAP HER2 groups 2-4.



Notes for IHC and ISH Testing

- a. Implication for cases with 0 and 1+ IHC
 In Australia many laboratories have been performing dual testing of IHC and ISH for all breast cancers. This is not a regulatory requirement. Under the 2018 ASCO CAP HER2 testing guidelines, there are no scenarios whereby cases with 0 or 1+ IHC will be classified as HER2 Positive. This eliminates the rationale for ISH testing of cases with 0 or 1+ IHC. This critical role of IHC in determining eligibility for subsidised therapy highlights the particular importance of quality assurance of IHC testing.
- b. ISH testing of cases with 3+ IHC
 The Australian Pharmaceuticals Benefits Scheme provides subsidized access to approved anti HER2 therapies based on the demonstration of HER2 gene amplification. This includes cancers with 3+ protein expression by IHC. Therefore, ISH testing of IHC 3+ cancers will continue to be required.
- c. Dual versus single probe ISH assays
 The guidelines recommend the preferential use of dual probe rather than single probe ISH assays. Single probe ISH assays are still considered acceptable for routine testing.

5. Heterogeneity

Heterogeneity is defined as the presence of any aggregate population of amplified cells comprising >10% of the tumour cells on the slide (not scattered single cells in a mosaic pattern). This is rare and usually identified on whole slide sections of the resected specimen. The amplified and non-amplified areas of the case must be evaluated separately and average HER2 copy number and HER2/CEP 17 ratios be provided for each tumour sub-population. The percentage of the total tumour population with amplification should also be reported. Cases containing amplified and non-amplified areas should be reported as Positive for HER2. A morphologically heterogeneous tumour on excision can be considered for retesting.

6. Use of Alternate Chromosome 17 Probes or Other Genetic Methods

In view of absence of outcome data, alternate chromosome 17 probes or other genetic methods should not be used as standard practice for resolving HER2 equivocal cases.

Correlation with clinical factors, such as grade and histologic subtype, repeat testing of other tumour tissue samples, expert consultation which may include alternative probes and other genetic methods may be considered in particularly challenging cases.

There was insufficient evidence to warrant inclusion of mRNA assays (e.g. using rtPCR) to determine HER2 status in unselected patients.

Appendix A: Recommended Comments for Inclusion in Reports of Groups 2-4 with Negative Final HER2 Results

ASCO CAP Expert Panel Recommended Comment for Group 2 with a Negative ISH Result

“Evidence is limited on the efficacy of HER2-targeted therapy in the small subset of cases with a HER2/CEP17 ratio of ≥ 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.”

ASCO CAP Expert Panel Recommended Comment for Group 3 with a Negative ISH Result

“There are insufficient data on the efficacy of HER2-targeted therapy in cases with a HER2 ratio of < 2.0 in the absence of protein overexpression because such patients were not eligible for the first generation of adjuvant trastuzumab clinical trials. When concurrent IHC results are negative (0 or 1+), it is recommended that the specimen be considered HER2 negative.”

ASCO CAP Expert Panel Recommended Comment for Group 4 with a Negative ISH Result

“It is uncertain whether patients with an average of > 4.0 and ≤ 6.0 HER2 signals per cell and a 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.”