Pathology (June 2019) **51(4)**, pp. 345–348

EDITORIAL

ASCO/CAP 2018 breast cancer HER2 testing guidelines: summary of pertinent recommendations for practice in Australia

Gelareh Farshid[1], Michael Bilous[2], Adrienne Morey[3], Stephen Fox[4], Sunil Lakhani[5], Sherene Loi[6], Richard Bell[7], Andrew Spillane[8]

[1]SA Pathology, Royal Adelaide Hospital, Adelaide, SA, Australia; [2]Western Sydney University, and Australian Clinical Labs, Sydney, NSW, Australia; [3]ACT Pathology, Canberra Hospital, and ANU Medical School, Canberra, ACT, Australia; [4]Department of Pathology, Peter MacCallum Cancer Centre, and the University of Melbourne, Melbourne, Vic, Australia; [5]The University of Queensland Centre for Clinical Research, and Pathology Queensland Research, Brisbane, Qld, Australia; [6]Medical Oncology, Peter MacCallum Cancer Centre, Melbourne, Vic, Australia; [7]Medical Oncology, Deakin University, Vic, Australia; [8]Surgical Oncology, The University of Sydney, and Mater Hospital North Sydney, Sydney, NSW, Australia

Summary

The latest update to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) HER2 testing in breast cancer guidelines was published in 2018. A multidisciplinary expert committee, convened under the auspices of the Royal College of Pathologists of Australasia (RCPA) Structured Pathology Reporting framework, evaluated the implications of these guidelines for clinical practice in Australia. Following feedback from professional bodies, including the RCPA and CanSAC, peer review was invited. The final document prepared by the authors, endorsed by the Expert Committee RCPA Structured Pathology Reporting of Breast Cancer and by CanSAC, is published herein.

Key words: HER2 immunohistochemistry; *in situ* hybridisation; breast cancer.

Received 24 November, revised 24 January, accepted 3 February 2019
Available online 7 May 2019

SUMMARY OF KEY POINTS

- Testing of core biopsies for HER2 evaluation [including *in situ* hybridisation (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[1] there are no circumstances in which cancers with 0 or 1+ immunohistochemistry (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. STANDARDISATION OF PRE-ANALYTICAL FACTORS

a. Time to fixation (cold ischaemic time): $\leq$1 hour.
b. Duration of fixation: 6–72 hours.

These requirements apply to core biopsies and resections. There are no changes to the American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) 2013 HER2 testing guidelines[2] and their 2010 guidelines for the immunohistochemical testing of oestrogen and progesterone receptors[3] 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.

Print ISSN 0031-3025/Online ISSN 1465-3931
DOI: https://doi.org/10.1016/j.pathol.2019.02.004

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 over 95% of test results. For the 5% of cancers in groups 2–4, strategies for further investigation are proposed, emphasising concurrent IHC, dual probe ISH and second opinions. Final results are categorised per Table 1 and Fig. 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.

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.

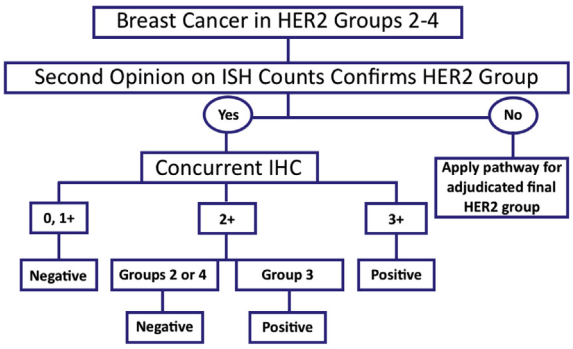

Fig. 1 Flow chart for determining the HER2 status of invasive breast cancer in ASCO/CAP HER2 groups 2–4.

b. ISH testing of cases with 3+ IHC

The Australian Pharmaceuticals Benefits Scheme provides subsidised 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 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 heterogenous 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

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

standard practice for resolving HER2 equivocal cases. Correlation with clinical factors, such as grade and histological 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 $\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.'

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 $\leq$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.'

FOOTNOTES AND PRACTICE POINTS

Questions concerning the practical clinical implementation of these recommendations:

1. To what extent does this document apply to practice to the wider Australasian region?

These recommendations pertain to the implementation of the 2018 ASCO/CAP HER2 testing guidelines in Australia. RCPA fellows practicing in other countries are best placed to address the implications for their own jurisdiction, cognisant of the regulatory and funding arrangements for access to HER2 treatments in their own region.

2. Given that core needle biopsies are now recommended as the preferred sample for biomarker evaluation is it now envisaged that BreastScreen will cover the cost of the HER2 testing that will be required on the core biopsies?

While as a scientific committee we have endorsed the ASCO/CAP position with regards to biomarker testing on core biopsies, at present the MBS funding rules have not been altered by the relevant regulatory agencies to reflect this clinical recommendation. This matter has been raised with the relevant departments and discussions are ongoing.

3. Very often the core and the resection specimen will be reported by different laboratories. How will the pathologist reporting the resection specimen know whether HER2 testing should be carried out?

Improved communication between the requesting clinicians and the various laboratories is required to minimise unnecessary duplication of biomarker testing. Depending on local conditions, groups of professionals concerned may adopt local conventions to assist with this issue. It is foreseeable that laboratories evaluating the resection specimen may choose not to proceed with routine biomarker testing, unless specifically requested. The surgeons can play a critical role, recording and at least correlating the tumour grade and subtype, as reported by the different laboratories, so circumstances where repeat testing may be advisable are identified.

4. What is the revised HER2 testing workflow likely to be in the light of these changes?

Laboratories are likely to decide to first evaluate the breast biomarkers by immunohistochemistry and report the HER2 status as negative if the IHC is 0 or 1, without routine ISH testing.

For cases with 2+ or 3+ IHC results, reflex ISH testing can be initiated and a final HER2 status determined depending on the HER2 clinical subgroup.

This uncoupling of IHC and ISH testing will expedite the final reporting for the majority of cases that do not require ISH but add a slight delay for the remaining cases.

5. The HER2 equivocal category was specified previously and FISH testing was used for establishing a final result in these cases. How are such cases to be dealt with now?

The focus of the 2018 guidelines is to assist in the binary management decisions of whether to administer HER2 targeted therapy or not by offering strategies for resolving equivocal findings. In the uncommon 2–4 subtypes, in the first instance a second opinion from an independent observer is required to confirm the relevant clinical subgroup. If confirmed as belonging to subgroups 2–4, evaluation of the immunohistochemistry can be helpful in establishing the HER2 status of the case for the purposes of patient management. In such cases a finding of 3+ IHC leads to a positive HER2 status. In group 3 cases, either 2+ or 3+ IHC results in a positive result. IHC counts of 0 or 1+ will lead to a negative HER2 status, with the comments recommended by ASCO/CAP and reproduced in Appendix A.

Counts for ratio and copy number that border the specified numerical thresholds for these parameters are subject to biological and interobserver variation. Slight variations in counts

may result in categorisation of cases in different groupings. The ASCO/CAP 2018 guidelines refer to counts being repeated by one additional observer. Rather than mandating any specific protocol for resolving differences in opinion and counts, the discretion and local conventions for resolving such situations are invoked, referring to 'adjudication as per internal procedures'. In relation to subgroup 4 cases, the ASCO/CAP committee discourage multiple ISH testing of these cancers by ISH tests (SISH, FISH or different CEP 17 probes) arguing that for cancers that have HER2 scores near a threshold, random variation in scoring will result in some counts crossing the threshold, leading to a different clinical decision point just by chance alone, and without adding to the level of confidence in the clinical decision.

The guidelines refer to ISH. Although a specific scenario where FISH (rather than SISH) must be employed has not been identified, there are no statements in these guidelines that preclude the reporting pathologist from further assessment of the case, as they see fit.

6. What are the specific circumstances where new HER2 testing should be performed after an initial positive HER2 result?

These circumstances include histological grade 1 carcinoma of the following types:

- Infiltrating ductal or lobular carcinoma, ER and PR positive.
- Tubular (at least 90% pure).
- Mucinous (at least 90% pure).
- Cribriform (at least 90% pure).
- Adenoid cystic carcinoma (90% pure) and often triple negative.

7. Under which specific circumstances should a new HER2 test on the resection specimen be considered after a negative HER2 result on a core needle biopsy?

These include:

- Tumour is grade 3.
- Amount of invasive tumour 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 ischaemic time, short time in fixative, different fixative) or the test is suspected by the pathologist to be negative on the basis of testing error.

Acknowledgements: CanSAC and Prof S. O'Toole for their valuable feedback. Ms Meagan Judge for support with project management. The editors and anonymous reviewers of *Pathology* for their helpful suggestions that have assisted in the presentation of this work.

Conflicts of interest and sources of funding: Gelareh Farshid, Michael Bilous, Adrienne Morey and Stephen Fox were previously members of the Roche HER2 Testing Advisory Board in Australia. Sunil Lakhani has received educational funding from Roche/Ventana and is on the ASCO ER/PgR panel. Richard Bell served on Advisory Boards for Roche.

Address for correspondence: Prof Gelareh Farshid, SA Pathology, Royal Adelaide Hospital, Port Rd, Adelaide, SA, 5000, Australia. E-mail: gelareh.farshid2@sa.gov.au

References

1. Wolff AC, Hammond ME, Allison KH, *et al*. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline focused update. *J Clin Oncol* 2018; 36: 2105–22.
2. Wolff AC, Hammond ME, Hicks DG, *et al*. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists clinical practice guideline update. *J Clin Oncol* 2013; 31: 3997–4013.
3. Hammond MEH, Hayes DF, Dowsett M, *et al*. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Arch Pathol Lab Med* 2010; 134: 907–22.