HER2 (Core and Non-core)

A subset of breast carcinomas (approximately 15 to 20%) overexpress human epidermal growth factor receptor 2 (HER2; HUGO nomenclature *ERBB2*). Protein overexpression is usually due to gene amplification. Assays for gene copy number, mRNA quantity, and protein generally give similar results; gene amplification correlates with protein overexpression in about 95% of cases. In a small subset of carcinomas (probably <5%), protein overexpression may occur by different mechanisms.

Overexpression is both a prognostic and predictive factor.

HER2 status is primarily evaluated to determine patient eligibility for anti-HER2 therapy. It may also identify patients who have a greater benefit from anthracycline-based adjuvant therapy.

HER2 status can be determined in formalin-fixed paraffin-embedded tissue by assessing protein overexpression on the membrane of tumour cells using immunohistochemistry (IHC) or by assessing the number of HER2 gene copies using in situ hybridization (ISH). When both IHC and ISH are performed on the same tumour, the results should be correlated. The most likely reason for a discrepancy is a false result of one of the assays, but in a small number of cases there may be protein overexpression without amplification, amplification without protein overexpression (especially in low-level amplification), or marked intratumoural heterogeneity.

There are many preanalytic, analytic and postanalytic variables that can affect test results, and the assays must be validated to ensure their accuracy. External quality assurance proficiency testing is essential to ensure accurate performance of testing. External quality assurance (EQA) HER2 surveys are available from established EQA scheme providers.

It is recommended that testing and scoring be carried out according to recommendations made by professional bodies including American Society of Clinical Oncology (ASCO), College of American Pathologists (CAP), The Royal College of Pathologists UK (RCPath), and The Royal College of Pathologists of Australasia (RCPA).[1-3]

The majority of laboratories worldwide use first line IHC testing with reflex ISH gene assessment for borderline 2+ cases only.

Differences in recommendations for positive versus negative classification of some ISH results have emerged recently relating to Chromosome 17/HER2 gene ratio and HER2 gene copy number findings (see below). Table 6 includes recommendations on reporting results of HER2 testing by ISH from ASCO/CAP,[1] however as these are not universally adopted, it is recommended that laboratories follow the recommendations pertinent to their geographic location.

Table 6: Reporting results of HER2 testing by in situ hybridization (dual-probe assay) based on ASCO/CAP[1] 2018 focused update guidelines.

Test result	Scoring criteria
Negative	HER2/CEP17 ratio <2.0 AND average HER2 copy number <4.0 signals/cell (Group 5)
Negative* (see comment)	HER2/CEP17 ratio ≥2.0 AND average HER2 copy number <4.0 signals/cell (Group 2) and concurrent IHC 0-1+ or 2+ HER2/CEP17 ratio <2.0 AND average HER2 copy number ≥ 6.0 signals/cell (Group 3) and concurrent IHC 0-1+ HER2/CEP17 ratio <2.0 AND average HER2 copy number ≥4.0 and <6.0 signals/cell (Group 4) and concurrent IHC 0-1+ or 2+
Positive*	HER2/CEP17 ratio ≥2.0 AND average HER2 copy number <4.0 signals/cell (Group 2) and concurrent IHC 3+ HER2/CEP17 ratio <2.0 AND average HER2 copy number ≥ 6.0 signals/cell (Group 3) and concurrent IHC 2+ or 3+ HER2/CEP17 ratio <2.0 AND average HER2 copy number ≥4.0 and <6.0 signals/cell (Group 4) and concurrent IHC 3+
Positive	HER2/CEP17 ratio ≥2.0 AND average HER2 copy number ≥ 4.0 signals/cell (Group 1)

*For Groups 2-4 final ISH results were based on concurrent review of IHC, with recounting of the ISH test by a second reviewer if IHC is 2+.

- Comment for Group 2 Negative result: Evidence is limited on the efficacy of HER2-targeted therapy in the small subset of cases with HER2/CEP17 ratio ≥2.0 and an average HER2 copy number <4.0/cell. In the first generation of adjuvant trastuzumab trials, patients in this subgroup who were randomized to the trastuzumab arm did not appear 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 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 lack of protein overexpression.
- Comment for Group 3 Negative result: There are insufficient data on the efficacy of HER2-targeted therapy in cases with HER2/CEP17 ratio <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 (0-1+), it is recommended that the specimen be considered HER2 negative.
- Comment for Group 4 Negative result: It is uncertain whether patients with ≥4.0 and <6.0 average HER2 signals/cell and HER2/CEP17 ratio <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.

References

1 Wolff AC, Hammond MEH, Allison KH, Harvey BE, McShane LM and Dowsett M (2018). HER2 Testing in Breast Cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update Summary. *J Oncol Pract* 14(7):437-441.

2 Royal College of Pathologists of Australasia (2018). *ASCO CAP 2018 HER2 Testing for Breast Cancer Guidelines - Recommendations for Practice in Australasia*. Available from: https://www.rcpa.edu.au/getattachment/fecd094c-aaf4-416b-9ed5-4a61f5ac1a93/ASCO-CAP-2018-HER2-Testing-for-Breast-Cancer-Guide.aspx (Accessed 26th March 2020).

3 Royal College of Pathologists and National Coordinating Committee for Breast Pathology (2016). *Pathology reporting of breast disease in surgical excision specimens incorporating the dataset for histological reporting of breast cancer*. Available from: https://www.rcpath.org/profession/guidelines/cancer-datasets-and-tissue-pathways.html (Accessed 26th March 2020).