E14. Optimal HER2 testing

Giuseppe Viale*

Department of Pathology, European Institute of Oncology and University of Milan, School of Medicine, Milan, Italy

Keywords: Breast cancer; HER2; Gene amplification; Over-expression; Immunohistochemistry; FISH

HER2 gene over-expression and/or amplification in breast carcinoma has been repeatedly documented as a very important prognostic and predictive parameter,[1,2] with special emphasis on its role for the accurate selection of candidate patients to trastuzumab and to other HER2-targeting therapies.[3–7] Accordingly, all primary breast carcinomas should be tested for HER2 status assessment at the time of the diagnosis or at the time of the recurrence, if not previously tested.

Several assays are currently available for the assessment of HER2 status, either on formalin-fixed and paraffin-embedded tissue sections, or on extracted DNA and mRNA. Immunohistochemistry (IHC) and *in situ* hybridisation assays with fluorochrome-labelled probes (FISH) or chromogenic reactions (CISH and SISH) are most widely used in the clinical practice,[8,9] and their results are highly correlated, with >90% concordance among the results of different assays. IHC is more commonly adopted as the screening test, because it is easier to perform, less expensive and less time-consuming, while *in situ* hybridisation assays (either FISH, CISH or SISH) are mainly used to clarify equivocal IHC results, or to confirm the reliability of the IHC results.

Despite the number of available assays and the publication of several guidelines and recommendations for an optimal HER2 testing by national and international agencies,[10] it must be admitted that the current intra- and inter-laboratory reproducibility of HER2 status assessment still is very unsatisfactory, with a false-positive rate of 20% to 30% for immunohistochemistry and of almost 12% for FISH assays.[11,12] Data on the actual false-negative rate of the assays are not easily obtainable, but it may well be that it is of the same magnitude as for the false-positive counterpart.

The reasons for these discordant results may be manifold, and include pre-analytical, analytical, and interpretative issues. In the pre-analytical phase, the most critical step is the type and length of fixation, while the choice of the reagents and of the tissue pre-treatments may be important variables of the analytical phase. The incorrect interpretation of the assay results, however, is by far the most common cause of discrepancy in the assessment of HER2 status. A weak to moderate staining of the tumour cells, or an incomplete decoration of the cell membranes or an purely cytoplasmic immunoreactivity are often misinterpreted as a truly positive IHC result, whereas false-positive *in situ* hybridisation assays are more often due to the detection of gene amplification in the intraductal component of the tumour, that may be associated with a non amplified invasive component, or to a non-specific binding of the probes to the cell nuclei.

To avoid an incorrect assessment of the HER2 status, special attention should be paid first to the proper fixation of the tissue specimens. Surgical specimens (mastectomy, quadrantectomy or lumpectomy specimens) must be promptly sliced (3–5 mm thick slices) and placed in neutral buffered formalin for 6 to 48 h. A shorter fixation will increase the risk of false-positive IHC result, whereas a prolonged fixation may lead to false negative IHC and FISH assays. It is necessary to use validated assays for both IHC and *in situ* hybridisation experiments. Commercially-available kits for these assays have been extensively tested, validated, and eventually approved by the United States Food and Drug Administration. These kits must be used strictly following the recommendations of the manufacturers, without modifications. The use of home-brewed assays requires their preliminary validation against a validated assay.

Whenever possible, the tissue sections submitted to the assay should include both a representative component of the invasive tumour (avoiding necrotic and highly inflamed areas) and at least a minor component of non neoplastic breast tissue as a built-in internal control. In evaluating the results, the examiner should first assess the reliability of the assay by checking the normal tissue for the expected reactivity. Non neoplastic breast epithelia should exhibit a very faint membrane immunoreactivity and a normal copy number of the HER2 gene. The assay results are not reliable in case of moderate to strong immunoreactivity of the normal breast tissue, or if *in situ* hybridisation signals are fewer or more than expected.

The HER2 status of any intraductal or *in situ* component of the tumour may be different from that of the invasive component, thus the results of the assays must be based on the evaluation of the invasive component only.

High-grade invasive ductal carcinomas with a negative hormone receptor status are more likely to carry *HER2* gene over-expression and amplification, whereas well differentiated carcinomas, both of ductal and lobular

histotype, very rarely – if ever – show an abnormal HER2 status. This should always be kept in mind when evaluating the results of any HER2 assays to avoid many misdiagnoses.

HER2 status of breast carcinoma is a very important prognostic and predictive parameter, so that all efforts should be made to improve the consistency and the reproducibility of the testing results. It is mandatory that the final report be validated by a pathologist with a proper knowledge of breast pathology and with sound expertise in the technical and interpretative issues of the testing procedures. The adoption and continuous implementation of internal and external quality assurance and quality control programmes are very helpful in achieving and maintaining an adequate reliability of the testing results.

Conflict of interest statement

None declared.

References

[1] Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science* 1987;235:177–82.

[2] Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. *Science* 1989;244:707–12.

[3] Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N Engl J Med* 2001;344:783–92.

[4] Marty M, Cognetti F, Maraninchi D, et al. Randomized phase II trial of the efficacy and safety of trastuzumab combined with docetaxel in patients with human epidermal growth factor receptor 2-positive metastatic breast cancer administered as first-line treatment: the M77001 study group. *J Clin Oncol* 2005;23:4265–74.

[5] Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. *N Engl J Med* 2005;353:1659–72.

[6] Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. *N Engl J Med* 2005;353:1673–84.

[7] Joensuu H, Kellokumpu-Lehtinen PL, Bono P, et al. Adjuvant docetaxel or vinorelbine with or without trastuzumab for breast cancer. *N Engl J Med* 2006;354:809–20.

[8] Press MF, Slamon DJ, Flom KJ, Park J, Zhou JY, Bernstein L. Evaluation of HER-2/neu gene amplification and overexpression: comparison of frequently used assay methods in a molecularly characterized cohort of breast cancer specimens. *J Clin Oncol* 2002;20:3095–105.

[9] Pauletti G, Dandekar S, Rong H, et al. Assessment of methods for tissue-based detection of the HER-2/neu alteration in human breast cancer: a direct comparison of fluorescence in situ hybridization and immunohistochemistry. *J Clin Oncol* 2000;18:3651–64.

[10] Wolff AC, Hammond ME, Schwartz JN, Hayes DF. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. *Arch Pathol Lab Med* 2007;131:18–43.

[11] Gancberg D, Jarvinen T, Di LA, et al. Evaluation of HER-2/NEU protein expression in breast cancer by immunohistochemistry: an interlaboratory study assessing the reproducibility of HER-2/NEU testing. *Breast Cancer Res Treat* 2002;74:113–20.

[12] Perez EA, Suman VJ, Davidson NE, et al. HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 Intergroup adjuvant trial. *J Clin Oncol* 2006;24:3032–38.