Fluorescence In Situ Hybridization Is the Preferred Approach over Immunohistochemistry for Determining HER2 Status

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The detection of human growth factor receptor 2 (HER2) surface protein expression by immunohistochemistry (IHC) was used as the "clinical trial assay" that led to US Food and Drug Administration clearance of trastuzumab for the treatment of HER2-overexpressing metastatic breast cancer (1). IHC staining remains the most frequent initial test for determining HER2 status and is performed in approximately 80% of the cases of newly diagnosed breast cancers in the US. IHC assessment of HER2 status is semiquantitative rather than qualitative, because HER2 is expressed at low concentrations (e.g., <20 000 receptor molecules per cell) in all breast epithelial cells. Despite the publication of a number of studies that show that the results of a well-performed IHC assay provide a good to excellent correlation between gene copy status and protein expression (2–4), the ability to accurately measure the expression status of the HER2 protein by IHC can be markedly affected by technical issues, such as accentuation by warm and cold tumor ischemia, the duration of tissue fixation in formaldehyde, the tissue-processing technique, and the embedding temperature of the heated paraffin wax (5). Although IHC testing has some advantages, including its wide availability, relatively low cost, easy preservation of stained slides, and the use of a familiar routine microscope, IHC also has substantial drawbacks. These deficiencies include the aforementioned preanalytical issues, as well as the type and intensity of the antigen-retrieval procedure used, the type of antibody (polyclonal vs monoclonal), the lack of a signal from a positive internal control, the variation in system control samples, and, most importantly, the difficulties in applying a semiquantitative and subjective slide-scoring system. The problems with IHC standardization of slide scoring have been emphasized in studies of the patient response to trastuzumab (6). In some cases, but not all, IHC slide scoring can be improved by avoiding overinterpretation of sample edges, retraction artifacts, under- or overfixation artifacts, cases with substantial staining of benign ductal and lobular cells, staining of tumor cell cytoplasm, and membranous tumor cell staining that lacks a complete circumferential staining pattern (2). Therefore, it is not surprising that the results from the United Kingdom National External Quality Assessment Scheme for Immunocytochemistry (UK NEQAS-ICC) found that the lack of reproducibility of HER2 scoring between laboratories was not caused by tumor heterogeneity or differences in fixation or processing but rather by how the scoring system was applied (7). The use of a system for quantitative image analysis can reduce slide-scoring variation among pathologists, especially in 2+ cases (8).

Like IHC, fluorescence in situ hybridization (FISH)-based detection of the ERBB2 [v-erb-b2 erythroblastic leukemia viral oncogene homolog 2, neuro/glioblastoma derived oncogene homolog (avian); also known as HER2] gene copy number is a morphology-driven slide-based assay that features DNA hybridization with fluorescently labeled probes (9). Both the hybridization steps and slide scoring can be automated. Compared with IHC, FISH has major advantages: a more hardy target (DNA) that is more resistant to alterations caused by preanalytical issues (including ischemia, fixation, and tissue processing), a more objective scoring system, and the presence of a built-in internal control consisting of the 2 ERBB2 gene signals that are present both in benign cells and in malignant cells that do not feature ERBB2 gene amplification. In addition, FISH testing has the ability to detect aneuploidy that may reflect both an adverse overall prognosis and the likelihood of downstream HER2 protein overexpression. Although FISH testing is more costly, may require a longer time for slide scoring if not auto-

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5 Nonstandard abbreviations: HER2, human growth factor receptor 2; IHC, immunohistochemistry; UK NEQAS-ICC, United Kingdom National External Quality Assessment Scheme for Immunocytochemistry; FISH, fluorescence in situ hybridization.
ated, uses more-expensive equipment, must be imaged for result preservation, and does not provide a good background morphology, numerous studies have found it to be more accurate and predictive of trastuzumab benefit (2, 10–15). Given the inability to recognize the detailed background morphology during signal counting, a potential cause of false-positive FISH test results is the scoring of ERBB2-amplified areas of ductal carcinoma in situ in a tumor that has invasive carcinoma areas that lack ERBB2 amplification. Because the technique features a built-in internal-control system, false-negative FISH results are rare but may occur when the slide scorer fails to identify the amplified regions in a tumor with heterogeneity of ERBB2 gene amplification. Considering that ERBB2 gene amplification can be heterogeneous in a subset of HER2-positive invasive breast cancers, diligence and care on the part of the slide scorer are required when the patient’s sample is scanned at low magnification (2).

In summary, although the FISH method is more expensive and time-consuming than IHC, numerous studies have concluded that this cost is well justified by the increased accuracy and more precise use of anti-HER2 targeted therapies (14). A number of systematic reviews have considered FISH to be more objective and reproducible (2). In a recent survey, the College of American Pathologists reported that FISH was more precise and accurate than IHC. In one study, the concordance rates between IHC and FISH were highest for tumors scored by IHC as 0 and 1+ and lowest for 2+ and 3+ cases. Despite the fact that the majority of instances of primary HER2 testing in the US currently commence with a screen by IHC (with results of 0 and 1+ considered “negative,” 2+ considered “equivocal” and referred for FISH testing, and 3+ considered positive), the objectivity and accuracy of FISH continues to stimulate an increase in primary FISH testing for HER2 status in clinical breast cancer samples. Finally, although the FISH-based approach may be preferable to IHC for evaluating HER2 status in breast cancer, the advantages of FISH may not be as great when applied to gastric and gastrointestinal junction adenocarcinomas, the most recently approved indication for anti-HER2 targeted therapy with trastuzumab (15). In the ToGA trial (15)—in which both IHC and FISH were performed in all cases and patients were allowed to enter the trial if the central laboratory scored either test as positive—it was noted that the marked heterogeneity of HER2 amplification/overexpression in upper gastrointestinal cancers, combined with the minute amounts of tumor tissue available to assess for cases with endoscopically obtained biopsies only, might give IHC advantages over FISH in this setting.

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