HER2 Testing and Correlation With Efficacy of Trastuzumab Therapy

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As a result of the availability and clinical efficacy of trastuzumab (Herceptin), clinicians are now faced with a dilemma regarding the accurate identification of patients with HER2 overexpression. In the October 2002 issue of ONCOLOGY, Monica Fornier and colleagues critically reviewed the methodologies currently available for HER2 testing in their article, "HER2 Testing and Correlation With Efficacy of Trastuzumab Therapy."[1]

The most widely used technique for detection of HER2 overexpression at the protein level is immunohistochemistry (IHC). However, the problems with this technique are, as correctly stated by the authors, that multiple primary antibodies—each of which binds to different epitopes and has differing sensitivities and specificities—are currently in clinical use. Moreover, different cutoff values are used to distinguish overexpressing from non-overexpressing tumors.[2] Detection of HER2/neu alteration is therefore not well standardized, and although IHC detection methods are widely commercially available, some of these assays may be suboptimal for this purpose.

Other IHC Limitations
Another concern is that the degree of IHC staining intensity for HER2 is subjective and qualitative. As a consequence, it may be difficult to know whether a sample scored as HER2-positive in one laboratory will be confirmed as HER2-positive by another laboratory. Furthermore, formalin fixation of tumor samples and storage in paraffin can result in epitope degradation so that sensitivity is lost over time in archival clinical material.[3-5]

To overcome this problem, some assays use a technique called antigen retrieval, which consists of boiling the tumor sample using heat or microwave radiation, in order to rehabilitate epitopes that have been lost during tissue processing and storage. Many believe that the loss of antigenicity in paraffin-embedded specimens can be corrected by application of heat-induced antigen retrieval to the paraffin-embedded tissue sections. However, this approach has not been validated, before introduction of these reagents and methods, by systematic evaluation of clinical breast cancer specimens with known amplification/overexpression levels.

Detection Rate Variability
As summarized in the review by Fornier et al, reported detection rates for IHC differ among clinical laboratories. This discrepancy has generated considerable confusion among clinicians, pathologists, and patients regarding the appropriate methods for HER2 testing. To address this issue, we recently used each of the four US Food and Drug Administration (FDA)-approved assays for detecting HER2 alterations, to determine which is the most accurate. We tested these assays on breast cancer specimens in which we had previously determined amplification and overexpression levels using solid matrix blotting techniques as a "gold standard."

In this comparative study, the sensitivity of the fluorescence in situ hybridization (FISH) assays for HER2 gene amplification was high—95.4% for the Vysis PathVision assay (Vysis, Downers Grove, Ill) and 95.4% for the Ventana INFORM assay (Ventana, Tucson). These sensitivity levels were followed by those for IHC assays performed with the Ventana CB11 monoclonal antibody (72.1%) and the DAKO HercepTest (69.8%, DAKO, Carpinteria, Calif).[5] The HercepTest was performed according to the approved protocol, as described by the manufacturer, using heat treatment for antigen retrieval. The protocol provided by Ventana Medical Systems and a Ventana automated slide stainer were used for the CB11 IHC assay. Both FISH assays were performed according to their respective FDA-approved protocols.

The specificity of HER2 testing methods can also vary considerably among laboratory studies, as
pointed out in the article by Fornier et al. The largest number of false-positive HER2 test results have been observed among IHC cases scored as 2+. Thus, it has been widely recommended that IHC 2+ scores be validated using FISH. However, recent data from the National Surgical Adjuvant Breast and Bowel Project (NSABP) and the Mayo Clinic have also questioned the accuracy of test results among cases scored as 3+ by IHC, especially when these assays were performed in laboratories other than reference laboratories.

**Trastuzumab Trials**

Two of the four ongoing large-scale trials evaluating adjuvant trastuzumab (NSABP B-31 and Breast Intergroup N9831) accrued HER2-positive primary breast cancer patients into the studies based on HER2 assay results submitted by local laboratories. Central review of the HER2 results by the NSABP Pathology Laboratory and the Mayo Clinic suggest higher than expected false-positive rates of 18% and 24%, respectively, using the FDA-approved HercepTest for detection of HER2 overexpression.[6,7]

The discrepancies of test results between regional laboratories and the central testing facility were especially pronounced among small-volume laboratories that performed less than 100 tests per month. Importantly, the concordance between central testing for FISH and IHC was good, suggesting that the number of false-positive results can be reduced when IHC is performed in a large-volume reference laboratory. These results have led to the modification of testing requirements for the NSABP B-31 and the Breast Intergroup N8931 clinical trials to ensure enrollment of patients with the highest probability of benefiting from anti-HER2 monoclonal antibody therapy, and to protect those patients that do not overexpress HER2 from the potential risks of trastuzumab therapy.

Similarly, a review of the first 680 cases analyzed in our centralized Breast Cancer International Research Group (BCIRG) laboratory for evaluation of HER2 gene amplification, as part of the entry criteria for the BCIRG006 study (a three-armed, prospective, randomized trial evaluating trastuzumab in combination with docetaxel [Taxotere]/carboplatin [Paraplatin] in HER2-positive primary breast cancer patients selected by FISH), concurred with the outside or local IHC staining results in only 83% of breast cancers (unpublished data; most of these discrepancies were false-positive results in the outside laboratories). However, a high level of concordance (96%) was observed in the results obtained from local laboratories performing FISH assays (unpublished data).

**Likelihood of Response**

An important finding that has emerged from the completed trastuzumab clinical trials pertains to the likelihood of response to the antibody according to HER2 expression. Trastuzumab has the greatest activity in patients whose tumors contain the highest levels of HER2 expression or gene amplification. In a large monotherapy trial, objective responses only occurred in patients with 3+ (vs 2+) HER2 overexpression, as determined by IHC. Consistent with this observation, the response rate was 34% in those with HER2 gene amplification detected by FISH, as compared to 7% in those with FISH-negative tumors.[8] Similar results were obtained in a pivotal randomized trial.[9] Thus, optimal use of the antibody would target patients with 3+ HER2 overexpression or FISH-positive gene amplification. In fact, to date, gene amplification as detected by FISH appears to be the single best predictor of response to trastuzumab.[10]

**Conclusions**

FISH assays have proven to be highly sensitive and specific for detecting HER2 gene amplification, and our findings demonstrate that FDA-approved IHC assays were less sensitive in detecting HER2 overexpression.[4,5] One problem of “real-world” IHC is the high number of false-positive results, which might be reduced if testing were limited to high-volume reference laboratories. However, this will not solve the well-described problem of lower sensitivity of IHC compared to FISH in paraffin-embedded tissue sections.[2,4,5]

**References:**


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