Current Status of HER2 Testing
Caught Between a Rock and a Hard Place
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The analysis of breast cancer specimens for alterations in the HER2/neu (c-erb-B2) gene or its protein product has become common practice in surgical pathology laboratories. Although some clinicians use the information derived from these assays for assessing patient prognosis and evaluating the likelihood of response to various chemotherapeutic agents and to tamoxifen, the major clinical role of assessment of HER2 status is to help determine patient suitability for treatment with trastuzumab (Herceptin), a monoclonal antibody targeted to the HER2 protein.1,2

Although there are a variety of methods available to assess HER2 status in clinical breast cancer specimens, assessment of protein overexpression using immunohistochemical analysis and evaluation of gene amplification using fluorescence in situ hybridization (FISH) are the methods most commonly used in clinical practice today.3,4 There are currently 4 commercially available, US Food and Drug Administration–approved assays for determining HER2 status in breast cancer samples, although these have not all been approved for the same purpose Table 1. Among these 4 assays, 2 are immunohistochemical assays (HercepTest, DAKO, Carpinteria, CA; and Pathway, Ventana Medical Systems, Tucson, AZ) and 2 are FISH assays (PathVysion, Vysis, Downers Grove, IL; and Inform, Ventana). All of these assays can be performed using automated instrumentation. In addition, there are numerous commercially available anti-HER2 primary antibodies and immunohistochemical detection systems for HER2 testing for which Food and Drug Administration approval has not been sought or received.

While the clinical value of assessing HER2 status in human breast cancers to help determine suitability for trastuzumab therapy is indisputable, the manner in which the test should be performed and the results evaluated and reported is a matter of heated debate and controversy involving several groups with somewhat different (and sometimes conflicting) agendas including pathologists, medical oncologists, basic scientists, commercial vendors, the lay press, and patients and their families. The College of American Pathologists recently issued recommendations regarding HER2 testing for patients with breast cancer.5 However, these recommendations largely reflect the current lack of consensus with regard to how best to evaluate HER2 status.

### Table 1
US Food and Drug Administration–Approved Assays for HER2

<table>
<thead>
<tr>
<th>Test</th>
<th>Type</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>HercepTest (DAKO, Carpinteria, CA)</td>
<td>Immunohistochemical</td>
<td>Suitability for trastuzumab (Herceptin)</td>
</tr>
<tr>
<td>Pathway (Ventana Medical Systems, Tucson, AZ)</td>
<td>Immunohistochemical</td>
<td>Suitability for trastuzumab</td>
</tr>
<tr>
<td>PathVysion (Vysis, Downers Grove, IL)</td>
<td>Fluorescence in situ hybridization</td>
<td>Assessing prognosis; predicting response to chemotherapy</td>
</tr>
<tr>
<td>Inform (Oncor/Ventana)</td>
<td>Fluorescence in situ hybridization</td>
<td>Assessing prognosis</td>
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Immunohistochemical analysis offers advantages over FISH in that it is relatively inexpensive and fast, uses a light microscope, and can be performed in most pathology laboratories. In addition, the results have been linked to prognosis and response to treatment.

Furthermore, it seems more logical to determine HER2 protein expression (by immunohistochemical analysis) than the level of gene amplification (by FISH) when treatments such as trastuzumab are specifically targeted toward the HER2 protein on the cell surface. Immunohistochemical analysis should, therefore, be the more biologically relevant assay, particularly in cases in which there is discordance between gene copy number and level of protein expression. However, the numerous commercially available anti-HER2 antibodies vary widely in sensitivity and specificity, staining results are subject to variations in tissue fixation and processing, there is no standard scoring system, there is no uniformly accepted threshold for positivity, and the results are not quantitative. FISH offers several advantages over immunohistochemical analysis in that the options for reagents are limited (there are only 2 commercially available reagent kits available at this time), the threshold for positivity has been standardized, the results are quantitative, and there are internal positive controls. Moreover, DNA, the target for FISH, seems less affected by variations in tissue fixation and processing than does protein, the target for immunohistochemical analysis. However, the capability for performing FISH is limited to a much smaller number of pathology laboratories than is immunohistochemical analysis, and FISH is more expensive, is technically more cumbersome, requires longer procedure and interpretation times than does immunohistochemical analysis, and requires a fluorescence microscope for interpretation. In addition, although published data have supported a link between FISH results and prognosis, data linking FISH assay results and response to therapy (including response to trastuzumab) are more limited.

In most studies, the concordance rates between immunohistochemical and FISH results are in the 80% to 95% range, with the highest levels of concordance among cases that are either completely negative or strongly positive by immunohistochemical analysis. Qualitatively more consistent agreement between immunohistochemical analysis and FISH results are seen for cases in which the immunohistochemical result is less than strongly positive.

So, given that immunohistochemical analysis and FISH each have their advantages and disadvantages, what is the practicing pathologist to do in order to best serve the clinical needs of patients with breast cancer and the physicians caring for them? Unfortunately, there is no definitive answer to this question, and, in a way, pathologists are caught between the proverbial rock and hard place. How, then do we move forward? As we see it, there are a number of options, with each having its pros and cons:

**Abandon immunohistochemical analysis and use FISH as the exclusive method for evaluating HER2 status in breast cancers.** A number of investigators have argued that because of the inherent limitations of immunohistochemical analysis for evaluating HER2 protein overexpression on fixed, paraffin-embedded tissue sections, FISH should be considered the method of choice for determining HER2 status in clinical breast cancer samples and could be used as a surrogate for HER2 protein expression. While this argument certainly has merit, there currently are numerous practical barriers to the routine use of FISH for determining HER2 status in breast cancers. In particular, as noted, the procedure is more complicated, time consuming, and expensive than immunohistochemical analysis, requires a fluorescence microscope, and requires considerably more pathologist interpretation time than does immunohistochemical analysis. It is possible that the development of automated methods for performing the assay and screening the slides may make FISH more practical for the routine determination of HER2 status.

**Use immunohistochemical analysis as a screening step followed by FISH in selected cases.** Many authors recently have advocated the use of a combined approach for assessing HER2 status. In this scenario, immunohistochemical analysis is used as the initial screening step for all cases, and, following evaluation of the immunohistochemical stains, a subset of cases is subjected to FISH, which is considered the “gold standard.” The most commonly proposed algorithm is to screen cases with immunohistochemical analysis and then perform FISH on cases in which the immunohistochemical stains show only weak or moderate membrane staining (“2+” in the HercepTest scoring system), since many such cases seem to represent immunohistochemical false-positive results. While this represents a reasonable suggestion, a number of studies have clearly shown that some immunohistochemically negative cases show gene amplification and some strongly positive cases (“3+” in the HercepTest scoring system) lack gene amplification. Moreover, preliminary data suggest that among cases that are strongly positive (“3+”) by immunohistochemical analysis, only the cases that also show gene amplification by FISH respond to trastuzumab treatment. Thus, limiting FISH testing to cases scored as “2+” by immunohistochemical analysis will not eliminate all false-positive and false-negative immunohistochemical results, and precisely which subset of cases should be subjected to FISH following immunohistochemical analysis remains a matter of debate. Furthermore, it is not at all clear which immunohistochemical assay should be used as the screening test in such a scenario.
Develop methods to standardize immunohistochemical analysis and/or render it more objective and quantitative. One of the major problems with immunohistochemical analysis is the lack of standardization with regard to primary antibodies, detection systems, epitope/antigen retrieval, interpretation, and reporting of results. The DAKO HercepTest represented an important step toward standardizing immunohistochemical staining for HER2 by introducing prediluted reagents in kit form and a scoring system. However, some studies have reported that this test has lower than desirable specificity, whereas others have found that it has lower than desirable sensitivity. Nevertheless, standardization of immunohistochemical methods, with appropriate quality control, remains an important goal.

Another major criticism of immunohistochemical analysis has been that interpretation of the results is subjective and prone to interobserver variability, particularly at intermediate levels of protein expression (ie, “2+” by the HercepTest scoring system). Recently, a number of investigators have attempted to overcome this limitation of immunohistochemical analysis by using computer-assisted image analysis to quantitate HER2 immunohistochemical results. For example, in the October 2001 issue of the Journal, Wang et al retrospectively studied 189 breast cancer cases using immunohistochemical analysis and FISH and quantitated the immunohistochemical analysis staining using the Automated Cellular Imaging System (ACIS; ChromaVision Medical Systems, San Juan Capistrano, CA). The results of this study indicated that immunohistochemical analysis quantitated with ACIS showed a higher level of concordance with FISH than immunohistochemical stains interpreted using visual inspection (91% vs 86%) and that ACIS provided higher sensitivity (but slightly lower specificity) than visually interpreted immunohistochemical stains. Similar results using other image analysis software have recently been reported by others. However, Wang et al also appropriately noted a number of limitations to the ACIS method, which also apply to other computer-assisted image analysis methods, including the cost of purchasing and maintaining the hardware and computer software required for the quantitation, unresolved technical issues, and lack of data about clinical validation of quantitative immunohistochemical analysis for HER2 protein determination. Perhaps most important, unless the immunohistochemical analysis procedure being subjected to image analysis has been optimized by calibrating it against an appropriate standard, quantification may simply provide an objective way to report an incorrect result rather than a more reliable or accurate result. Thus, while quantitation of immunohistochemical results using computer-assisted image analysis could provide certain important advantages over qualitatively or semiquantitatively interpreted immunohistochemical stains, there are a number of barriers and limitations to the widespread implementation of this type of technology.

Develop methods to assess HER2 gene copy numbers that can be evaluated using routine light microscopy. A method that would combine the advantages of both immunohistochemical analysis and FISH might well be the most reliable and practical means to routinely assess HER2 status (ie, a DNA-based method that could be readily interpreted using routine light microscopy). One such method is chromogenic in situ hybridization (CISH). This approach permits the identification and quantitation of HER2 gene copies using light microscopy since it uses a chromogenic rather than a fluorescent detection system. One study has indicated a high level of concordance between CISH and FISH in enumerating HER2 gene copy numbers. However, additional studies, including those assessing the clinical value of CISH, are still needed. Until such data are available, CISH should be considered an investigational technique.

Develop tests that better predict the response of patients to HER2-targeted therapy. Even if there were a method that had 100% sensitivity, specificity, and accuracy in the determination of HER2 status of breast cancers, available data suggest that this would still not permit the identification of all patients who might or might not respond to HER2-targeted antibody treatment with trastuzumab. Therefore, there is great interest in developing a test that better predicts response to such treatment. In particular, it is possible that evaluation of the activated (phosphorylated) form of HER2, assessment of the level of expression of other members of the epidermal growth factor receptor family in conjunction with assessment of HER2, or assessment of downstream effector molecules may provide more accurate information than simply measuring HER2 status. However, additional studies are needed to assess the clinical value of these alternative testing approaches.

Abandon tissue-based HER2 testing altogether. Some investigators have studied the use of assays for circulating HER2, in particular the measurement of the serum level of the HER2 extracellular domain (ECD) in determining HER2 status. Although the early results of such studies showed promise, several potential problems exist in using a serum assay for HER2 ECD. First, the assay has low sensitivity compared with immunohistochemical analysis or FISH on corresponding tissue samples. Detection of ECD is dependent on several factors, such as the level of antigen production and release by the patient’s tumor and the half-life of the protein in the serum. In addition, use of this assay is subject to problems similar to those currently experienced with immunohistochemical analysis and FISH, such as standardization of method and determination of appropriate assay cutoff levels.
Await results of studies directly comparing different assays for HER2 with regard to their relationship to clinical outcome. Perhaps more important than anything else at this time, the various available assays for determining HER2 status need to be directly and rigorously compared within the setting of prospective clinical trials to determine their relative merit and also to determine the appropriate threshold for HER2 positivity. It should be noted, however, that the threshold for HER2 positivity may vary, depending on the clinical question being asked. For example, the level of HER2 protein expression required to declare a case “HER2-positive” may be different when evaluating response to treatment with trastuzumab than when determining sensitivity to anthracyclines. Similarly, determining gene copy number (eg, by FISH) may be more appropriate in some clinical settings than others. Some recently published23 and ongoing studies are attempting to address these issues.

In the meantime, in the absence of definitive answers, it seems most prudent for pathologists to closely follow the developments in this field and to work with their clinical colleagues to formulate an approach to HER2 testing that is most suitable at their institution. Ideally, before it is introduced into clinical laboratory practice, any immunohistochemical assay should be validated against another assay such as FISH or a polymerase chain reaction–based assay.14 Based on extensive discussions with our clinical colleagues, the approach we have settled on at our institution is to initially test all cases by immunohistochemical analysis using a method we have validated against FISH.3,24 For cases that are strongly positive or clearly negative by immunohistochemical analysis, no further testing is performed. For cases that are equivocal or weakly positive by immunohistochemical analysis (corresponding to “14” or “2+” by HercepTest scoring) we perform FISH. We view this as an interim solution until the issue of how best to assess the HER2 status of breast cancers becomes more clearly defined.

References


