Symposium article

Current use of HER2 tests

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Summary
Reliable detection of HER2 overexpression is important for the success of trastuzumab (Herceptin®) therapy. Several methods are available for measuring HER2 expression at the DNA, RNA or protein level. The method most frequently employed is immunohistochemical (IHC) detection of the HER2 receptor in paraffin sections. Advantages include the precise localization of the HER2 protein, the availability of paraffin material and the ease of the procedure. However, IHC can be influenced by the sensitivity/specificity of the antibody, tissue treatment and, in particular, subjective assessment. These disadvantages do not exist in the detection of gene amplification by fluorescence in situ hybridization (FISH) or polymerase chain reaction. However, FISH requires expensive equipment that is not widely available in pathology laboratories. Another approach quantitates shed HER2 antigen in the serum by an enzyme-linked immunosorbent assay. The key advantage of this method is the ease of sampling blood, however, serum HER2 concentrations do not accurately reflect the tumor status. Furthermore, this method does not register single-cell expression, which is important for therapeutic decision making. In addition to improving the accuracy and comparability of HER2 assays, these optimized protocols may further enhance the efficacy of trastuzumab therapy by selecting those patients most likely to respond.

Key words: ELISA, FISH, HER2, Herceptin, IHC, PCR, trastuzumab

Introduction
The human epidermal growth factor receptor-2 (HER2) protein is overexpressed in 20%-30% of breast cancers and is associated with a poor clinical outcome [1, 2]. HER2 belongs to the family of growth factor receptors and is a component of a signal-transduction pathway involved in cellular growth [3]. The HER2 protein plays a key role in malignant transformation and its extracellular location provides a therapeutic target through which the transduction of proliferation signals can be limited [4-6].

The humanized anti-HER2 monoclonal antibody trastuzumab (Herceptin®) has been developed as a therapeutic agent and early clinical trials demonstrated that it is effective and well tolerated in the treatment of relapsed HER2-positive breast cancer patients [7]. A pivotal randomized, multicenter, phase III study also demonstrated that trastuzumab in combination with chemotherapy was a more effective first-line treatment for HER2-positive metastatic breast cancer than chemotherapy alone [8]. The anti-tumor effect of trastuzumab in combination with chemotherapy is related to the amount of HER2 expressed by the tumor cells; HER2 status therefore serves as a useful parameter for the identification of tumors that are most likely to respond to this therapy (Figure 1) [9].

The accurate determination of HER2 expression by a tumor is a valuable prognostic indicator and an important predictive factor for the response to trastuzumab therapy. Until recently, the routine clinical use of HER2 as a tumor marker has been limited by a lack of standardization in HER2 assays. However, significant progress has been made towards the development of widely available, reliable assays for the routine measurement of tumor HER2 status.

Methods for the detection of HER2 status
A wide range of assay methods have been used to assess HER2 status. Overexpression of the HER2 protein is largely the result of HER2-gene amplification, which correlates well with the levels of HER2 mRNA and protein expressed by the tumor cell [10]. There are, therefore, a number of target molecules available for assay including DNA, mRNA, cell surface protein and the HER2 extracellular domain (ECD) that is shed into the blood (Figure 2). Most of the tests measure either protein or DNA, and the most useful of these reveal the HER2 status of individual tumor cells.

Some of the HER2 assays are not suitable for routine pathology laboratory use. For example, the blotting techniques (Western, Southern and Northern) can be used to detect the various HER2 target molecules, although they are technically demanding and impractical for routine screening of tumors. They also require extraction of the tissue and quantitation is influenced by
Figure 1. Treatment effect vs. level of HER2 expression. Response rates are presented for patients who were IHC 2+ and IHC 3+ in the pivotal trials of trastuzumab (Herceptin®).

Table 1. HercepTest scoring system.

<table>
<thead>
<tr>
<th>Tumor cells with membrane staining</th>
<th>0%</th>
<th>&lt;10%</th>
<th>Complete</th>
<th>&gt;10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kind of membrane staining</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Staining intensity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Herceptin®</td>
<td>0+</td>
<td>0+</td>
<td>0+</td>
<td>0+</td>
</tr>
<tr>
<td>Paclitaxol</td>
<td>0+</td>
<td>0+</td>
<td>0+</td>
<td>0+</td>
</tr>
<tr>
<td>Herceptin® + paclitaxol</td>
<td>0+</td>
<td>0+</td>
<td>0+</td>
<td>0+</td>
</tr>
<tr>
<td>Herceptin® + AC</td>
<td>0+</td>
<td>0+</td>
<td>0+</td>
<td>0+</td>
</tr>
</tbody>
</table>

the proportion of tumor cells in the biopsy. The most useful techniques for the measurement of tumor HER2 status are outlined below.

Assays for HER2-protein expression

Immunohistochemical (IHC) detection of HER2 receptor on the cell surface is the method most frequently employed for determination of HER2 status. IHC staining is particularly useful because it reveals HER2-protein expression in individual tumor cells. However, many variables can reduce the reliability of this technique, including the method of tissue preservation, the age of the embedded tissue, and the specificity and sensitivity of the antibody or the detection system used. In addition, the accuracy and reproducibility of all IHC techniques are limited by the subjective interpretation of staining results.

Optimal IHC staining is obtained with frozen sections, which preserve tissue architecture and allow the precise localization of the HER2 protein on the cell membrane. Although subject to antigen deterioration due to harsh fixation, paraffin sections are also widely used. They are technically easier to handle and material preserved in paraffin is more readily obtained.

The availability of commercial IHC kits for HER2 detection has led to greater standardization of the assay. The FDA has approved the use of the commercial HercepTest (Dako) for the routine selection of metastatic breast cancer patients who are appropriate candidates for trastuzumab therapy. The test can be conducted on paraffin sections or fresh tissue samples, and both the processing and the assessment of the sections are standardized. The HercepTest includes a scoring system designed to increase objectivity and reduce variability in the interpretation of IHC (Table 1). A series of decisions are made about the tumor staining and a final score is generated for HER2 expression. The first step in the scoring system is to ascertain whether >10% of the tumor cells are stained. The next step is to determine if the membrane staining is partial or complete, and in the final step the intensity of the staining is graded. The use of commercial IHC kits should provide for more reproducibility and less interlaboratory variation in the determination of HER2 status. However, questions have been raised about the sensitivity of the HercepTest, with some investigators finding high HER2 positivity rates using this test [11, 12]. These findings have been contradicted by other researchers, who have found levels of HER2 positivity using the HercepTest to be as expected [13].

Enzyme-linked immunosorption assay (ELISA) can be used to detect the HER2 protein in tumor homogenates. ELISA is a relatively simple technique that is quantitative and well suited to automation. The specificity and sensitivity of the technique is affected by the antibody and the detection system used. In addition, the proportion of tumor cells in the homogenized sample can affect the reliability of HER2 quantitation.

HER2 ECD, which is shed into the blood during tumor cell turnover, can be readily quantitated in serum by ELISA. The advantage of this method is that blood samples are easy to collect and changes in circulating HER2 antigen in response to physiologic stimuli or...
therapeutic agents can be monitored easily. Unfortunately, circulating HER2 ECD does not necessarily correlate with tumor load [14, 15]. The assay, however, may be useful for the monitoring of tumor progression.

Detection of HER2-gene amplification

The HER2 status of a tumor can also be determined by testing for amplification of the HER2 gene. DNA is more stable than the HER2 protein and detection is possible in old and harshly fixed paraffin material. Two techniques, polymerase chain reaction (PCR) and fluorescence in situ hybridization (FISH), can be used for the routine analysis of HER2-gene amplification in tumors.

PCR is a very sensitive technique that can be performed on a small volume of tumor tissue and it is easy to automate and standardize. However, the value of PCR is limited by the fact that it is difficult to quantitate and involves DNA extraction providing a mean value of the HER2 expression in the tumor sample.

FISH is a widely used and valuable assay. The advantages of this technique are the stability of chromosomal DNA and the ability to assess gene amplification within the individual tumor cells of a tissue section. FISH is based on the in situ visualization of the HER2 gene using a specific fluorescence-labeled oligonucleotide probe (Figure 3). Currently two commercial, FDA-approved test kits are available. PathVysion™ (Vysis™) detects the HER2 gene with a directly-labeled probe. One advantage of this kit is that it includes an internal control probe for the assessment of polysomy. The control probe simultaneously detects the centromere of chromosome 17, the locus of the HER2 gene. The Inform™ (Ventana™) kit uses a biotin-labeled oligonucleotide probe that is detected using an avidin-biotin-FITC system for signal amplification. The FISH technique is very reliable, providing 96.5% sensitivity and 100% specificity for the detection of HER2-gene amplification [16]. The major limitation of FISH is that it requires a high-grade fluorescence photomicroscope with digital image recording. This specialist equipment is expensive and not widely available in diagnostic pathology laboratories.

Currently only two methods, IHC and FISH, can be recommended for the routine determination of tumor HER2 status. The equipment and expertise required for IHC are widely available in routine pathology laboratories. However, the results may be variable depending upon the tissue and the antibody used and the experience of the pathologist in using the procedure. FISH, on the other hand, offers very high diagnostic reliability but is technically demanding and expensive. A combined protocol has been devised that exploits the advantages and minimizes the disadvantages associated with both techniques. In combination, IHC and FISH offer enhanced reliability for the determination of HER2 status and the selection of patients appropriate for trastuzumab therapy.

Combination of IHC and FISH increases the accuracy of HER2 assessment

In our laboratory, IHC has been combined with FISH in a two-step procedure for the determination of HER2 status (Figure 3). Firstly, the HercepTest is used for the IHC determination of HER2 score. Trastuzumab treatment is primarily indicated with tumors having a HER2 score of 3+; tumors that are scored 2+ by IHC are then subjected to FISH analysis for HER2-gene amplification. With these tumors, an amplification of > 10 gene copies is regarded as a positive indication for trastuzumab therapy. Survival data correlates more closely with HER2-gene amplification [1, 2] than with HER2-protein expression and only patients with a 2+ IHC score with HER2-gene amplification (>10 copies) were selected for trastuzumab therapy.

These selection criteria are supported by the finding that HER2 gene amplification is present in all of the tumors with a 3+ IHC score (Figure 4). In contrast, only 25% of the tumors with a 2+ IHC score have gene amplification. This is unexpected as HER2-gene amplification has been demonstrated to correlate well with HER2-protein expression in previous studies (reviewed
The HER2 status of a breast tumor provides valuable prognostic and predictive information. The information is vital for the selection of metastatic breast cancer patients who are appropriate for trastuzumab therapy. Of the many assays available for the assessment of tumor HER2 status, IHC and FISH are the most reliable and sensitive. It is of critical importance to standardize methods used for staining and to apply common interpretation criteria to enable direct comparison of results between laboratories. The availability of standardized commercial kits for both of these techniques has led to greater accuracy and comparability in the routine assessment of HER2 status.

The accuracy of HER2 testing may be increased by using a combination of assays. In combination, IHC and FISH offer enhanced accuracy in the measurement of HER2. Both PCR and ELISA are readily automated and well suited to routine screening laboratories. With further development, these may prove more suitable assays to combine with IHC or FISH. The more stringent testing of patients with serial assays should improve HER2 assessment and may result in an increase in improved selection of patients for trastuzumab therapy.

Note

The authors have not reported any financial relationships with companies whose products are mentioned in the text.

References