



Adrian Deckbar. *Mirror Image*. Pastel, 20" × 27". Courtesy of the Hanson Gallery, New Orleans, Louisiana.

Accurate assessment of the tumor HER2 status is necessary for optimal breast cancer management.

Laboratory Testing for HER2/neu in Breast Carcinoma: An Evolving Strategy to Predict Response to Targeted Therapy

Nils M. Diaz, MD

Background: *Laboratory testing of HER2/neu in breast carcinoma has become vital to patient care following the approval of trastuzumab as the first therapy to target the HER2/neu oncoprotein. Initial clinical trials used immunohistochemistry (IHC) to test for HER2/neu overexpression in order to select patients for therapy. Fluorescence in situ hybridization (FISH), which tests for gene amplification, is more specific and sensitive than IHC when either assay is compared with HER2/neu overexpression as determined by Northern or Western blot analysis. Many weak overexpressors on IHC testing are not gene amplified on FISH analysis. Such weak overexpressors may be considered false-positives and raise the question of how best to test for HER2/neu.*

Methods: *The literature was surveyed regarding testing for HER2/neu overexpression in breast carcinomas and alternative testing strategies.*

Results: *False-positive results are a significant problem when IHC is exclusively used to test for HER2/neu overexpression. The false-positives are overwhelmingly confined to the group of 2+ positives and do not respond to targeted therapy. In contrast, concordance between IHC and FISH is high when immunostaining is interpreted as either negative or strongly positive (3+). Whereas some recent studies have suggested that FISH may better predict response to anti-HER2/neu therapy than IHC, others have indicated that IHC is as effective a predictor as FISH. IHC is less technically demanding and costly than FISH.*

Conclusions: *IHC analysis of HER2/neu in breast carcinoma is a useful predictor of response to therapy with trastuzumab when strongly positive. Negative immunostaining is highly concordant with a lack of gene amplification by FISH. Most weakly positive overexpressors are false-positives on testing with FISH. Thus, screening of breast carcinomas with IHC and confirmation of weakly positive IHC results by FISH is an effective evolving strategy for testing HER2/neu as a predictor of response to targeted therapy.*

From the Interdisciplinary Oncology Program at the H. Lee Moffitt Cancer Center & Research Institute at the University of South Florida, Tampa, Florida.

Submitted July 1, 2001; accepted August 9, 2001.

Address reprint requests to Nils M. Diaz, MD, Interdisciplinary Oncology Program, H. Lee Moffitt Cancer Center & Research Institute, 12902 Magnolia Dr, Tampa, FL 33612. E-mail: diaznm@moffitt.usf.edu

Introduction

Since amplification of the HER2/neu (human epidermal growth factor receptor 2, also referred to as *c-erbB-2*) gene in invasive breast carcinomas was initially associated with a poor prognosis,¹ interest in the biology of the protein was directed to its potential as a target for therapy. Trastuzumab (Herceptin), a humanized monoclonal anti-HER2 antibody^{2,3} is the first therapy approved by the Food and Drug Administration (FDA) that is directed against the HER2/neu oncoprotein. Its efficacy in treating patients with metastatic breast carcinoma is predicted by either HER2/neu protein overexpression or gene amplification.⁴ This article reviews how the strategic testing for HER2/neu is evolving together with targeted therapeutic options.

Expression and Overexpression of HER2/neu

The HER2/neu oncogene encodes a transmembrane protein with tyrosine kinase activity. The 185-kd protein is expressed in nonneoplastic breast epithelial cells and other normal cells. When the protein is overexpressed in carcinoma cells, the tyrosine kinase is constitutively activated, leading to increased mitogenic signal transduction.⁵ Between 20%-30% of breast carcinomas overexpress HER2/neu. The protein is overexpressed as a result of HER2/neu gene amplification, ie, the normal complement of one gene per each chromosome 17 is increased.

Testing for HER2/neu

Because anti-HER2/neu therapy benefits only patients with invasive breast carcinomas overexpressing HER2/neu, testing is used to identify those patients most likely to respond to anti-HER2/neu therapies. The potential side effects of Herceptin⁶ and the cost of therapy increase the importance of identifying HER2/neu overexpression. Given the latter considerations and the fact that the majority of patients with carcinoma overexpressing HER2/neu do not benefit from trastuzumab, testing may also be conceptualized as a mode of selecting patients who lack HER2/neu overexpression and thus should not be treated with Herceptin.

HER2/neu status in breast carcinomas can be determined by testing for (1) gene amplification by Southern blot, polymerase chain reaction, or fluorescence in situ hybridization (FISH), (2) mRNA using Northern blot, or (3) protein overexpression via enzyme linked immunosorbent assay, Western blot on cytosols, or immunohistochemistry (IHC).

The membrane localization of the protein forms the basis of IHC, the most commonly used method of testing for HER2/neu overexpression. The first IHC assay to be approved by the FDA as a response indication for Herceptin therapy, the HercepTest (Dako Corp, Carpinteria, Calif), uses a polyclonal antibody.⁷ The antibody approval was based on good concordance of its ability to detect HER2/neu overexpression with the monoclonal clinical trials assay antibodies used for determining eligibility in the initial trastuzumab clinical trials. Subsequently Pathway (Ventana Medical Systems, Tucson, Ariz), a monoclonal preparation (CB11), has also been approved by the FDA for the same response indication.

FISH, the other test currently utilized in clinical practice to select patients for Herceptin use, and IHC have distinct advantages and disadvantages. IHC is performed in more clinical laboratories, and it is less expensive and less labor intensive than FISH. FISH requires fluorescence microscopes rather than the light microscope used for routine microscopic evaluation by pathologists (chromogenic reagents under study may obviate this issue regarding in situ hybridization). Both methods test routinely processed surgical specimens (formalin fixed, paraffin-embedded tissue) and allow for analysis within individual cells. Because FISH tests for the gene rather than the HER2/neu protein tested for by IHC, FISH does not have the potential problem of antigen loss associated with formalin fixation. Reliable IHC, based on quality assurance and concordance studies with standard positive and negative results that correlate with either clinical outcome or with another IHC assay with documented correlation with outcome data, are required for this quantitative assay. IHC testing for HER2/neu should not be done when assay requirements, such as fixative type, are not met. Parenthetically, negative IHC results on archival material should be interpreted with caution because the negative result may be associated with prolonged fixation or storage considerations.

False-Positive Results Using IHC

Jacobs et al⁸ drew attention to the presence of false-positive HercepTest results by retesting breast carcinomas, which were interpreted as negative by FISH and by other IHC methods using the same antibody. This finding highlights and suggests several important concepts relating to IHC testing for HER2/neu expression. First, such testing is intended as a quantitative test. Second, the test result will vary according to the method utilized, including the primary antibody used, antigen detection and retrieval techniques, the scoring system, the expertise of the pathologist analyzing the test, and the use of

Concordance Between Different IHC Antibody Reagents and FISH Testing for HER2/neu Status in Breast Carcinoma

IHC Result \ IHC Reagent	% Gene Amplified (FISH-positive)			
	CTA ⁴	HercepTest ⁹	CB11 ¹⁰	HercepTest ¹¹
0-1+	4%	2%	7%	0%
2+	24%	36%	24%	17%
3+	89%	100%	85%	89%

other technologies (eg, image analysis). For example, Jacobs and associates⁸ reduced the number of false-positive results using the HercepTest kit by modifying the scoring system to take into account the level of staining of nonneoplastic epithelium.

Several reports have now verified that false-positive results are an issue in testing for HER2/status by IHC.^{4,9-14} The “gold standard” used to identify these false-positive results has been testing for HER2/neu gene amplification by FISH. This is due to the greater specificity and sensitivity of FISH when either test is compared with HER2/neu overexpression as determined by Northern and Western blot analyses.^{15,16} However, errors may also occur with FISH analysis (approximately 5%). The percentage of IHC false-positive results varies by different IHC antibodies and methods. Representative studies that compare overexpression by IHC testing and gene amplification by FISH in the assessment of HER2/neu status in breast carcinomas are presented in the above Table.

Studies have shown that the problem of false-positive results involves IHC techniques most significantly when the result is 2+.^{4,9-14} As few as 17% of 2+ HercepTest carcinomas demonstrate gene amplification by FISH.¹³ The overwhelming majority of 3+ positives are amplified. However, in a study by Mass et al,⁴ 11% of 3+ positive tumors did not demonstrate gene amplification by FISH, indicating that false-positive results are a potential problem in this group as well. False-negative results, ie, IHC-negative/FISH-positive carcinomas, are rare in the 0-1+ group.

HER2/neu Status as a Predictor of Response to Targeted Therapy

In any given case, the key determinant of the utility of a test for HER2/neu is whether it is predictive of a patient’s response to targeted therapy, which presently consists of trastuzumab. Review of the results using the clinical trials assay (CTA), an

immunostaining procedure that employs monoclonal antibodies directed against HER2/neu, suggests that most patients with a beneficial clinical response to Herceptin had tumors with the highest levels of HER2/neu overexpression: 3+ in a 0-3+ scoring system.⁴ Unless 2+ positive overexpression of HER2/neu was confirmed by FISH assay for gene amplification, the probability of a therapeutic response was 0% in second- and third-line monotherapy. Some recent studies also suggest that FISH may be a better predictor than IHC of response to Herceptin.^{17,18} Such results have contributed to proposals that FISH should serve as the front-line test for selecting patients for Herceptin therapy. In contrast, Seidman et al¹⁹ found that IHC was as effective as FISH as a predictor of response to Herceptin. The predictive value of IHC and FISH may prove to vary according to how anti-HER/new therapy is used in monotherapy and in combination with nontargeted chemotherapeutic agents.

An Evolving Strategy for Determining HER2/neu Status

A consensus is evolving that a useful strategy for determining whether anti-HER2/neu therapy should be offered to a patient in appropriate clinical circumstances include both IHC and FISH analysis. Such a strategy will require modification according to which laboratory testing methods best predict response to available and future anti-HER2/neu-directed therapies. Specifically, IHC should presently be used as a screen for overexpression of the oncoprotein based on the following factors: (1) IHC is less expensive, more routine in clinical laboratories, and less labor intensive than FISH, (2) IHC has a high concordance with FISH when the result is negative (0 or 1+) or strongly positive (3+), and (3) studies have, as yet, not shown that nonoverexpressors — 0 or 1+ by IHC but FISH-positive — benefit from targeted therapy. If the IHC result is weakly positive or indeterminate (2+), testing the carcinoma by FISH for gene amplification is recommended to help in selecting patients for the anti-HER2/neu therapy trastuzumab.

References

1. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER2/neu oncogene. *Science*. 1987;235:177-182.
2. Slamon DJ, Leyland-Jones B, Shak S, et al. Addition of Herceptin (humanized anti-HER-2 antibody) to first line chemotherapy for HER-2 overexpressing metastatic breast cancer markedly increases anticancer activity: a randomized, multinational controlled phase III trial. *Proc Annu Meet Am Soc Clin Oncol*. 1998;17:A377. Abstract.
3. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. *J Clin*

Oncol. 1999;17:2639-2648.

4. Mass RD, Sanders C, Kasian C, et al. The concordance between the clinical trials assay (CTA) and fluorescence in situ hybridization in the Herceptin pivotal trials. *Proc Annu Meet Soc Am Soc Clin Oncol.* 2000;19:A291. Abstract.

5. Lemoine NR, Staddon S, Dickson C, et al. Absence of activating transmembrane mutations in the c-erbB-2 proto-oncogene in human breast cancer. *Oncogene.* 1990;5: 237-239.

6. Ewer MS, Gibbs HR, Swafford J, et al. Cardiotoxicity in patients receiving trastuzumab (Herceptin): primary toxicity, synergistic or sequential stress, or surveillance artifact? *Semin Oncol.* 1999;26(suppl 12):96-101.

7. Graziano C. HER-2 breast assay, linked to Herceptin, wins FDA's okay. *CAP Today.* 1998;12:1,14-16.

8. Jacobs TW, Gown AM, Yaziji H, et al. Specificity of HercepTest in determining HER-2/neu status of breast cancers using the United States Food and Drug Administration-approved scoring system. *J Clin Oncol.* 1999;17:1983-1987.

9. Ridolfi RL, Jamehdor MR, Arber JM. HER-2/neu testing in breast carcinoma: a combined immunohistochemical and fluorescence in situ hybridization approach. *Mod Pathol.* 2000;13:866-873.

10. Tubbs RR, Pettay JD, Roche PC, et al. Discrepancies in clinical laboratory testing of eligibility for trastuzumab therapy: apparent immunohistochemical false-positives do not get the message. *J Clin Oncol.* 2001; 19:2714-2721.

11. Hoang MP, Sahin AA, Ordonez NG, et al. HER-2/neu gene amplification compared with HER-2/neu protein overexpression and interobserver reproducibility in invasive breast carcinoma. *Am J Clin Pathol.* 2000; 113:852-859.

12. Wang S, Saboorian MH, Frenkel E, et al. Laboratory assessment of the status of Her-2/neu protein and oncogene in breast cancer specimens: comparison of immunohistochemistry assay with fluorescence in situ hybridisation assays. *J Clin Pathol.* 2000;53:374-381.

13. Lebeau A, Deimling D, Kaltz C, et al. Her-2/neu analysis in archival tissue samples of human breast cancer: comparison of immunohistochemistry and fluorescence in situ hybridization. *J Clin Oncol.* 2001;19: 354-363.

14. 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; 18:3651-3664.

15. Pauletti G, Godolphin W, Press MF, et al. Detection and quantitation of HER-2/neu gene amplification in human breast cancer archival material using fluorescence in situ hybridization. *Oncogene.* 1996;13:63-72.

16. Press MF, Bernstein L, Thomas PA, et al. HER-2/neu gene amplification characterized by fluorescence in situ hybridization: poor prognosis in node-negative breast carcinomas. *J Clin Oncol.* 1997;15:2894-2904.

17. Vogel CL, Cobleigh MA, Tripathy D, et al. Superior outcomes with Herceptin (trastuzumab) (H) in fluorescence in situ hybridization (FISH)-selected patients. *Proc Annu Meet Soc Am Soc Clin Oncol.* 2001; 20:A86. Abstract.

18. Mass RD, Press M, Anderson S, et al. Improved survival benefit from Herceptin (trastuzumab) in patients selected by fluorescence in situ hybridization (FISH). *Proc Annu Meet Soc Am Soc Clin Oncol.* 2001;20:A85. Abstract.

19. Seidman AD, Fornier MN, Esteva FJ, et al. Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification. *J Clin Oncol.* 2001;19:2587-2595.