HER2 Testing and Correlation With Efficacy of Trastuzumab Therapy

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By Monica Fornier, MD [2], Mauro Risio, MD [3], Catherine Van Poznak, MD [4], and Andrew D. Seidman, MD [5]

The need for accurate detection of HER2 status is becoming more apparent, as therapeutic decisions are influenced by this information in both the adjuvant and advanced-stage setting. Since the US Food and Drug

Molecular markers are studied for their potential to act as prognostic or predictive factors. A prognostic factor influences the clinical outcome independently of treatment, whereas a predictive factor correlates with prognosis because it is linked to the response to a particular therapy. HER2 may function as both a prognostic and a predictive factor; in addition, HER2 can be the target of therapy.

The HER2/neu oncogene, also referred to as c-erbB-2/neu, encodes a protein with a molecular weight of 185,000 daltons (p185). The gene product is a transmembrane tyrosine kinase receptor belonging to a family of epidermal growth factor receptors (EGFRs) that are structurally related to the human EGFR.[1,2] The other members of this family are HER1 (also known as EGFR), HER3, and HER4. This receptor family is known as the type 1-receptor tyrosine kinases.

Ligands for this family of receptors include the epidermal growth factor (EGF) and neuregulins, also known as neu differentiation factors or heregulins. At least six different ligands, the EGF-like ligands, activate the EGF receptor and cause formation of homodimers—an event believed to activate the intrinsic tyrosine kinase, resulting in transautophosphorylation of tyrosine residues. EGF-like ligands can also induce heterodimerization between other members of the HER family, forming heterodimers such as HER1/HER2, HER1/HER3, and HER1/HER4 (Figures 1 and 2).[3] The HER2 receptor is partially homologous to the EGFR. However, to date, unlike for EGFR, HER3, and HER4, no ligand for HER2 has been identified.[4]

The second class of ligands for the HER receptors, collectively termed neuregulins, bind directly to HER3 and HER4, but not to HER2 or the EGFR. It is hypothesized that the main role of HER2 may be to dimerize with the other members of the HER family of receptors. Interestingly, HER2 is the preferred heterodimerization partner within the family and is frequently transactivated by EGF-like ligands or neuregulins resulting from the formation of heterodimerization with other members of the HER family. This heterodimerization allows the participation of HER2 in signal transduction, even in the absence of a cognate ligand.

HER2

The HER2 gene was first identified as a transforming oncogene in the DNA of chemically induced neuroblastomas in the rat.[5] HER2 is overexpressed or amplified in approximately 20% to 25% of human breast cancers.[6] The HER2 gene product is composed of a cytoplasmic domain with tyrosine kinase activity, a transmembrane domain, and an extracellular domain that may be shed from the surface of breast cancer cells. HER2 may be involved in the pathogenesis and clinical aggressiveness of HER2-overexpressing tumors. Indeed, evidence supports a direct role for HER2 overexpression in the pathogenesis and poor clinical outcome of human tumors.[7] When the mutated gene is transfected into mouse fibroblast cells (NIH-3Y3), it causes transformation, and the resulting cells are tumorigenic in nude mice.[8] Transgenic mice that overexpress the neu gene (the rodent homolog of the human HER2 gene) develop breast cancer.[9] Specific antibodies to the extracellular domain of the human HER2/neu gene product inhibit the growth of experimental tumors that overexpress the gene.[10] HER2 has been shown to be overexpressed in several human carcinomas including, but not limited to, breast, ovarian, gastric, colon, and non-small-cell lung cancer.[11] Cellular proliferation is regulated by extracellular factors that trigger signal transduction cascades from surface receptors.
through cytoplasmic effectors and ultimately control progression through the cell cycle. To date, the exact mechanisms by which oncogenic HER2 affects cell proliferation and the cell-cycle regulatory components involved have not been identified.

**HER2 Overexpression as a Predictive Factor**

Amplification of the HER2 proto-oncogene and overexpression of its protein product in patients with breast cancer have been linked to a poor prognosis; i.e., a more aggressive clinical course and shortened survival.[12,13] Although inconclusive, data have also suggested that HER2 overexpression may be useful as a predictive factor, raising the possibility of pretreatment selection of patients who might benefit from particular therapeutic strategies. Recent data indicate that patients with lymph node-positive breast cancer whose tumors overexpress HER2 may obtain additional benefit from adjuvant anthracycline-containing chemotherapy (as opposed to non-anthracycline-containing regimens).[14,15]

The role of HER2 status in predicting response to adjuvant systemic therapy and the method of defining HER2 status require further exploration before definitive recommendations can be made on how best to utilize this biomarker.[16] Further investigations are also necessary to define the responsiveness of HER2-positive tumors to hormonal therapy with selective estrogen-receptor modulators (SERMs) and aromatase inhibitors.

Preclinical studies have suggested that estrogen-dependent cultured human breast cancer cell lines are rendered hormone-independent after transfection with multiple copies of the HER2/neu gene.[17] Some studies have suggested that patients whose tumors overexpress HER2/neu are less likely to respond to tamoxifen and may have a worse outcome, compared to patients with normal HER2/neu expression.[18,19] However, other studies have reported no worse outcome with the use of adjuvant tamoxifen in patients with HER2-overexpressing tumors.[20,21]

The American Society of Clinical Oncology (ASCO) recently updated its recommendations for the use of tumor markers.[16] In this document, ASCO recommends the evaluation of HER2/neu status in all primary breast cancers, at either the time of diagnosis or the time of recurrence. However, the data are currently insufficient to recommend the routine use of HER2 overexpression to identify patients with a higher risk of relapse. Complicating the evaluation of the published data on HER2 as a prognostic factor is the lack of uniform use of assays in assessing HER2 by immunohistochemistry or fluorescent in situ hybridization (FISH).

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**HER2 Testing: Which Is the Optimal Method?**

A wide range of assay methods have been used to assess the HER2 status of fresh and archival surgical specimens from breast carcinoma patients. Methods to assess protein overexpression include immunohistochemistry, enzyme immunoassay, and Western blot analysis; methods to evaluate gene amplification include FISH, Southern blot analysis, and polymerase chain reaction (PCR); and methods to assess messenger (m)RNA overexpression include Northern blot analysis and in situ hybridization (Table 1).[22,23] In addition, circulating levels of the shed extracellular domain of the HER2 receptor protein can be detected by serum enzyme-linked immunosorbent assay (ELISA).

All of these assays have been used in research laboratories, but some have not been routinely used in hospitals because they require specialized equipment and/or the use of radioisotopes. On the other hand, a wide range of factors affect both HER2 testing and determination of HER2 status, including standardization of methods, standardization of antibodies (monoclonal/polyclonal) used for immunohistochemistry, agreement on the adoption of common grading criteria, scoring systems, and correlation of various assays.

**Immunohistochemistry**

Immunohistochemistry and FISH are the techniques routinely recommended for determining HER2 status, and both have been approved by the US Food and Drug Administration (FDA) for use in the selection of patients for therapy with the monoclonal antibody trastuzumab (Herceptin). The other available techniques should be used for research purposes only. However, many factors may compromise successful immunohistochemical testing of HER2 status.

**Form of Fixation**

The fixative should preserve antigenic integrity and limit extraction, diffusion, or displacement of antigen during subsequent processing. Formalin-fixed, paraffin wax-embedded tumor tissue samples are appropriate for immunohistochemical assessment of HER2, whereas other methods of tissue fixation can adversely affect reactivity.[24] The rate of false-positive cases rises to
50% with the use of an unsuitable fixative. Evidence suggests that HER2 protein reactivity may deteriorate in fixed paraffin wax sections after prolonged storage; the immunohistochemistry test should, therefore, be performed on block sections that have been stored for no more than a few months.

**Antigen Retrieval** Excessive antigen retrieval can artifactually increase immunoreactivity of cancer cells and bring about spurious membrane reactivity in normal breast epithelial cells, as well as carcinoma cells.

**Antibody** Several antibodies are commercially available, including CB11 (mouse antihuman monoclonal antibody, Ventana, Tucson), TAB 250 (mouse antihuman monoclonal antibody, Zymed, San Francisco), and HercepTest (rabbit antihuman HER2/neu polyclonal antibody, DAKO, Carpinteria, Calif). No single antibody has been consistently demonstrated to be superior in terms of sensitivity and specificity (Figure 3).

**Scoring System** HercepTest is the currently recommended scoring method. A semiquantitative system based on the intensity of cell-membrane immunostaining and the percentage of positive cells, it has a score range from 0 to 3+. Samples scoring 3+ are regarded as unequivocally positive and 0/1+ as negative. Borderline 1+/2+ and 2+ require confirmation with an alternative method, preferably FISH.

When dealing with the analysis of an image, both brightness and spatial resolution play crucial roles. Basically, the finer the resolution, the closer we approach the original appearance of the image. The quality of resolution, in turn, is significantly impaired by a so-called contouring phenomenon, which mainly affects the perimeter evaluation of images, such as HER2 immunostain on the cell membrane. Taken together, these optical phenomena are likely to influence interpretation of immunohistochemistry. Interobserver variation in the assessment of staining is considerable and can lead to misclassification of HER2 status. A simplified immunohistochemistry scoring method is advisable.

The accuracy, sensitivity, and reproducibility of HER2 immunohistochemical assay can be substantially improved with the use of an image analyzer system to quantify the immunohistochemical staining. The Automated Cellular Imaging System (ACIS, ChromaVision, San Juan Capistrano, Calif) automatically scans the immunohistochemically stained slide, and clearly distinguishes and quantifies cell membrane staining from cytoplasmic staining using so-called color-space transformation proprietary technology.

With the ACIS system, HercepTest is scored as a number between 0 and 4, with a score < 2 indicating a negative result and > 2, a positive result. FISH analysis is requested for scores ranging from 0.5 to 1.9. When compared with the FISH standard, the ACIS immunohistochemical assay notably reduces intraobserver and interobserver disagreement, thus improving the concordance rate and sensitivity of the manual immunohistochemical assay.

**False-Positive Results** Another pitfall of immunohistochemistry is the possibility of false-positive results. In over 90% of cases, HER2 protein overexpression is related to HER2/neu gene amplification, which results in increased mRNA transcription and, ultimately, increased synthesis of the glycoprotein receptor. When technical artifacts (eg, excessive antigen retrieval) can be ruled out, HER2 overexpression in the absence of genomic amplification could be the effect of a transcriptionally up-regulated overexpression. HER2 is a growth factor, and enhanced transcription in the absence of gene amplification is a well-recognized mechanism of cellular function through enhanced production of mRNA by phosphorylation of tyrosine kinase acting on growth factors and other regulators of cell growth and proliferation.

Transiently enhanced transcription unlinked with the corresponding oncogene amplification has been immunohistochemically seen in colonic regenerating epithelium and is a well-recognized mechanism in cellular homeostasis. This does not seem to be the case for HER2 in breast cancer, however, and most HercepTest-positive cases with a score of 2+ and a normal gene copy should be regarded as true false-positives, unresponsive to trastuzumab. In contrast, gene amplification without protein overexpression is an artifact and can only be due to a failure of the immunohistochemistry assay; ie, posttranslational modifications of the mRNA transcript may go undetected by inappropriate or inadequate antibody detection methods.

HER2 gene copy enumeration by FISH is based on a count of immunofluorescent signals contained within the nucleus of invasive carcinoma cells. The normal resting cell has two copies of the HER2 oncogene, although cells not amplified for HER2 can show 0 to 4 signals by FISH analysis because of variations in proliferative activity and, above all, plane of sections. FISH studies of archival
formalin-fixed, paraffin-embedded tissue can be performed directly on sectioned and mounted material or on extracted nuclei. Both methods are reliable, and each has advantages and disadvantages.

When examining tissue sections, it is often difficult to evaluate overlapping nuclei, due to the presence of several cell layers and cut nuclei (21% to 26%), leading to artificial signal loss.[33] These two problems can be solved by using nuclear-extraction techniques. In such a case, however, normal epithelial breast cells and ductal carcinoma in situ cells mix with invasive cancer cells, therefore biasing the correct assessment of HER2 status.

In order to correct the HER2 signal number for chromosome 17 aneusomy (occurring in about 50% of human breast cancers), FISH enumeration of chromosome 17 should always be associated. Results are expressed as the ratio of HER2 signal to chromosome 17 signal, and a tumor cell with a ratio > 2 is considered positive (or amplified). Cutoff values for HER2 gene amplification in the absence of a chromosome 17 control have not been established, although cells containing more than four signals per nucleus have been arbitrarily defined as amplified.[34]

Although we lack evidence that storage of blocks or slides causes deterioration of signal, the reliability of the FISH assay decreases after cut sections have been stored for 6 to 12 months. Tumor heterogeneity (1% to 2% of cases), which could potentially affect the correct evaluation of genomic amplification of HER2, is balanced by scoring more than 60 cells from three distinct tumor fields. The low interobserver variation (10% to 15%) seen in nonamplified cases significantly increases in amplified samples, but this is not critical when the ratio is greater than 4.[26]

Although FISH is not as widely available and is more costly than immunohistochemistry, economic modelling shows that it represents the most effective and cost-effective option in terms of total population cost and life-years gained.[35] From a macroeconomic perspective, the additional cost of better tests to optimally assess patients who may benefit from therapies such as trastuzumab may ultimately save health-care systems money.

CISH
Even if FISH is considered the most specific and sensitive method for detecting gene amplification in neoplastic tissues, it does not allow direct evaluation of the histopathologic features of examined samples. A new method of in situ hybridization—chromogenic in situ hybridization (CISH)—allows detection of gene amplification using conventional peroxidase reaction. Gene copies identified by CISH can be easily visualized under a light microscope in routinely counterstained tissue sections. CISH represents a useful alternative for determining HER2/neu gene amplification in paraffin-embedded tissue samples because it was found to correlate well with FISH in breast cancer (kappa coefficient ranging from 0.67 to 0.81). Discrepancies were confined to cases with low-level amplification by FISH, which turned out to be negative by CISH and immunohistochemistry. CISH, therefore, seems to be a reliable method of confirming HER2 immunohistochemical assays.[36,37]

Immunohistochemistry vs FISH
The degree of concordance between immunohistochemistry and FISH HER2 testing remains an ongoing debate. Several groups of investigators have reported comparisons of these two methods, with controversial results. This issue has become of paramount importance in the clinical setting, because the HER2 gene product represents a specific target for treatment with trastuzumab, and optimal use of this therapy requires accurate and reliable determination of HER2 status. Appropriate selection of patients for trastuzumab-containing therapies is also quite important when one considers the potential cardiotoxicity of trastuzumab, alone or in combination with doxorubicin or other drugs.[38]

Several studies have verified that false-positive results are an issue in immunohistochemical testing of HER2 status (Table 2).[25,32,39-41] The "gold standard" for identifying these false-positives has been testing for gene amplification by FISH (because of its greater specificity and sensitivity when both tests are compared with Northern and Western blot analyses of HER2 overexpression).[42] The percentage of false-positive results on immunohistochemistry varies by the antibodies and methods used, and studies have shown that the problem of false-positives involves immunohistochemical techniques most significantly when the result is 2+; as few as 17% of HercepTest 2+ carcinomas demonstrate gene amplification by FISH.[39]

Jacobs et al Study[25]Jacobs and colleagues tested 48 breast carcinoma specimens using the FDA-approved HercepTest.[25] These specimens had previously been found to be HER2-nonoverexpressing using two different immunohistochemical assays, and nonamplified by FISH analysis. When testing with the HercepTest kit, there was a high percentage (58.4%) of HER2 positivity, thus suggesting a high incidence of false-positives and a low specificity of the testing assay. However, with the use of a modified scoring system that took into account the level of
staining of nonneoplastic epithelium, specificity increased to 93.2%, suggesting that consideration of the level of staining of nonneoplastic epithelium might help to "normalize" the level of HER2 staining by serving as an internal control.

In a second report, the same authors found a high level of concordance (91%) between FISH and immunohistochemistry by HercepTest in the evaluation of HER2 status in 100 formalin-fixed, paraffin-embedded breast cancer specimens.[40] The authors specified that their findings were based on the analysis of consecutive cases that were fixed and processed in a relatively uniform manner in a single pathology department; this could partially explain the high level of correlation between FISH and immunohistochemistry, in contrast with other reports.

**Lebeau et al Study**[39] Lebeau et al compared immunohistochemistry and FISH testing methods for HER2 detection in 85 breast cancer specimens. HER2 overexpression was demonstrated in 42% of the tumors with the HercepTest polyclonal antibody. FISH performed on paraffin sections identified gene amplification in 28% of the tumors. Interestingly, strongly positive immunohistochemistry results (3+) were always associated with gene amplification, whereas 75% of weakly positive immunohistochemistry results (2+) lacked evidence of gene amplification.[39]

**Pauletti et al Study**[40] Pauletti et al reported their review of specimens from patients with stage I, II, and III breast carcinoma. A total of 900 cases were tested for HER2/neu gene amplification by FISH in paraffin-embedded, formalin-fixed archival material.[41] Of these, 856 were also tested for HER2 overexpression by non-antigen-retrieval immunohistochemistry with the polyclonal antibody R60, the sensitivity and specificity of which was preliminarily compared with HercepTest. When comparing the two immunohistochemical methodologies, HercepTest failed to improve sensitivity and introduced false-negatives. Therefore, patient survival was analyzed in relation to the presence of HER2 alteration as detected by FISH vs immunohistochemistry using the R60 antibody. Among the 900 patients, 21% tested positive by FISH, and 17% of 856 tested positive by immunohistochemistry. Both assays predicted shorter patient survival on multivariate analysis, confirming that both HER2/neu gene amplification and overexpression are correlated with survival independently of other variables. However, on univariate analysis, although there was a direct correlation between survival and gene copy number as determined by FISH, patients stratified by immunohistochemistry demonstrated this relationship only in the highest (3+) immunostaining group. Therefore, the ability to define high-risk groups differed significantly between the two techniques. Further analysis demonstrated that neither the 2+ nor 3+ immunohistochemistry groups completely corresponded to tumors containing the highest gene copy number.

**Mass et al Study**[42] At the 2001 ASCO meeting, Mass et al presented data from a cohort of 451 breast cancer specimens that were tested for HER2 overexpression using FISH and two separate immunohistochemistry assays performed with the monoclonal antibodies 4D5 or CB11 (Clinical Trial Assay).[43] Immunohistochemical overexpression was scored as either 2+ or 3+. Gene amplification was detected in 89% of 3+ cases and 24% of 2+ cases. Consistent with other reports, a high concordance was found between FISH and immunohistochemistry for immunohistochemistry cases scored as 0 and 3+, and a low concordance for immunohistochemistry cases scored as 1+ and 2+ (Table 3).[32,43-45]

**Tubbs et al Study**[44] Tubbs et al tested the hypothesis that HER2 immunohistochemistry discrepancies, as compared with gene copy enumeration by FISH, are actually false-positive results and not enhanced expression of a growth factor in the absence of genomic amplification of the oncogene.[32] In this report, the authors tested 400 breast carcinoma specimens by immunohistochemistry using monoclonal (CB11) and polyclonal (HercepTest) antibodies after antigen retrieval. Specimens were also evaluated for HER2 gene amplification by FISH and by detection of mRNA overexpression via autoradiographic RNA-RNA in situ hybridization. Overall HercepTest/CB11 discordance was 12%.

Expression of mRNA was highly concordant with FISH gene amplification and CB11. Immunohistochemically false-positive cases (no HER2 gene amplification) occurred with both HercepTest (23%) and CB11 (17%), and the majority of false-positive results (34 of 44) were scored as 2+. All 2+ false-positive cases were mRNA-negative. The combined results of HercepTest and CB11 showed that 79% (38 of 48) of 3+ cases were HER2 gene amplified, but only 17% (7 of 41) of 2+ cases had increased gene copy. Therefore, the authors concluded that discordant HercepTest/FISH results are most commonly immunohistochemically false-positive with a score of 2+, and recommended FISH as a more reliable method for assessing HER2.

**Perez et al Study**[45] Perez et al reached similar conclusions in a recently published experiment. The authors tested HER2 status using immunohistochemistry (HercepTest) on 1,556 consecutive breast tumor biopsy specimens received at the Mayo Medical Laboratories in Rochester, Minn, over a
5-month period.[46] Of these, 216 specimens (14%) were scored as 2+ HER2-overexpressing and were further evaluated by FISH; only 26 (12%) demonstrated high-level HER2 gene amplification, 54 (25%) demonstrated duplication of HER2, 4 (2%) showed deleted HER2 and/or chromosome 17 centromere (CEP 17), and 123 (57%) had no apparent HER2 and/or CEP 17 anomaly. The authors recommended that all specimens with a 2+ HercepTest result be evaluated further by FISH prior to any recommendation for anti-HER2 therapy.

Seidman et al Study
Two of the authors of this manuscript (Andrew Seidman and Monica Fornier) have reported a higher level of concordance between HercepTest and FISH: In their analysis, 50% of the specimens that showed 2+ HER2 overexpression by HercepTest also showed gene amplification.[45]

Therapeutic Application of HER2 Testing

Preclinical Studies
Strategies to antagonize the abnormal function of overexpressed HER2 were developed with the creation of antibodies directed against the rat neu receptor and the HER2 receptor on human cell lines. The 4D5 murine monoclonal antibody, directed against the extracellular domain of the HER2/neu gene product, is a potent inhibitor of growth in vitro and in xenograft models of HER2-overexpressing breast cancer.[47,48] As murine antibodies are limited clinically because they are immunogenic, a recombinant humanized monoclonal antibody that binds specifically to the extracellular domain of the p185HER2 protein (trastuzumab) was produced. This agent has shown antitumor activity as a single agent in phase I and II trials in patients with metastatic breast carcinoma overexpressing HER2.

Selection of HER2-positive patients for trastuzumab therapy is supported by preclinical studies, which demonstrated that the antibody has little or no effect on HER2-negative cells.[49] In fact, preclinical observations suggest that HER2-receptor density in excess of 100,000 receptors per cell would be necessary to achieve maximal trastuzumab benefit.[50]

Clinical Trials

Baselga et al Trial
The efficacy and safety of single-agent trastuzumab was first evaluated by Baselga et al in a phase II trial in 46 patients with extensive metastatic breast cancer, whose tumors overexpressed HER2.[51] Most patients (97.8%) had received previous chemotherapy; 43.5% had received more than two previous regimens for metastatic disease.

Trastuzumab was administered intravenously at a loading dose of 250 mg, followed by 10 weekly doses of 100 mg each. Of the 43 patients evaluable for response, 5 achieved a complete or partial remission, for an overall response rate of 11.6%. In particular, one patient achieved a pathologically proven complete remission of chest wall lesions, which has persisted for over 80 months.

Cobleigh et al Trial
A pivotal phase II multicenter study was conducted in 222 women with HER2-overexpressing metastatic breast cancer that had progressed after treatment with one or two chemotherapy regimens.[52] In this study, the majority of patients (78%) had metastatic disease at multiple sites and had undergone extensive prior treatment with either anthracyclines (94%) or taxanes (67%). Treatment with trastuzumab was initiated at 4 mg/kg, followed by a weekly dose of 2 mg/kg for the remainder of the study.

HER2 testing to establish eligibility for trial entry was performed at a central testing laboratory using the clinical trial assay. This comprised two separate immunohistochemistry assays performed with either of the monoclonal antibodies 4D5 or CB11. All patients shown to have 2+ or 3+ HER2 overexpression were enrolled into the trial. Of note, the clinical trial assay was demonstrated to have a 79% concordance with HercepTest.[53]

Response to trastuzumab was assessed by a blinded, independent response evaluation committee. For the 222 patients entered into the trial, the overall response rate on intent-to-treat analysis was 15% (95% confidence interval [CI]: 11%-21%): there were 8 complete responders (4%) and 26 partial responders (12%). Median duration of response was 9.1 months (range: 1.6 to 26+ months). Median survival was 13 months (range: 0.5 to 30+ months). Most importantly, response to therapy correlated with HER2 overexpression: Patients whose disease overexpressed HER2 at a 3+ level had a response rate of 18%, compared with 15% in the overall patient population.

A retesting of patients’ tissues was performed in a retrospective analysis using FISH, thus enabling a correlation between efficacy and gene amplification. A 20% response rate was reported for patients whose disease showed HER2 gene amplification by FISH, compared with a 0% response rate in patients with FISH-negative tumors.[54] Significantly, all the patients in the immunohistochemistry
3+ (n = 107) and 2+ (n = 35) subgroups who responded to trastuzumab tested positive on FISH analysis.[55]

**Vogel et al Trial**[5] Vogel et al recently reported the phase II experience with trastuzumab as first-line therapy for patients with metastatic breast cancer.[56] A total of 114 patients with HER2-overexpressing metastatic breast cancer were randomized to receive either standard-dose trastuzumab (4 mg/kg loading dose, followed by 2 mg/kg weekly) or a higher dose of the agent (8 mg/kg initial dose, followed by 4 mg/kg weekly). An overall response rate of 26% was observed. Response rates were similar regardless of the dose of trastuzumab (24% and 28%, respectively). The median time to disease progression was also similar for the two groups. The median survival duration for all enrolled patients was 24.4 months, which, again, did not differ by dose of trastuzumab.

A retrospective analysis of tumor gene amplification was performed on archived pathology slides. Significantly, the response rate was 34% for patients whose tumors amplified HER2 and 7% for those whose tumors did not. These findings again strengthen the argument that FISH is a superior method for selecting patients who will likely benefit from trastuzumab therapy.

**Slamon et al Trial**[57] Slamon et al conducted a pivotal phase III trial that examined the activity of trastuzumab in combination with chemotherapy.[57] This four-armed trial enrolled 469 patients with HER2-positive metastatic breast cancer. Women without prior exposure to anthracyclines were randomized to receive doxorubicin (or epirubicin [Ellence]) plus cyclophosphamide (Cytoxan, Neosar), with or without trastuzumab. Women who had previously received adjuvant anthracycline therapy were treated with 3 weeks of paclitaxel as a single agent or paclitaxel plus trastuzumab. Greater clinical benefit was noted among women who received trastuzumab plus chemotherapy, with an improved overall response rate (50% vs 32%), median time to disease progression (7.4 vs 4.6 months), and median overall survival (25 vs 20 months), compared with those who received chemotherapy alone. However, the combination of trastuzumab and an anthracycline-containing regimen resulted in an increased incidence of subclinical and clinical cardiac toxicity.

The investigators also performed a retrospective analysis of patient response on the basis of FISH.[43] Patients whose disease showed HER2 gene amplification by FISH had a higher response rate to the combination regimen, compared to patients whose disease did not show gene amplification (54% vs 38%, respectively). The assay also identified patients who derived a survival benefit from the addition of trastuzumab (relative risk of mortality = 0.71 vs 1.11). Moreover, among patients with tumors lacking HER2 amplification (FISH-negative), the overall response rate was similar regardless of treatment group (38%, with comparable 95% CIs), suggesting that trastuzumab might not confer benefit in this group of patients (Table 4).

**Memorial Sloan-Kettering/M. D. Anderson Trial**[46] In a collaborative trial between Memorial Sloan-Kettering Cancer Center and the M. D. Anderson Cancer Center, 95 patients with metastatic breast carcinoma—both overexpressing and nonoverexpressing HER2—were treated with trastuzumab at an initial dose of 4 mg/kg followed by 2 mg/kg/wk plus weekly 1-hour paclitaxel at 90 mg/m2.[46] The regimen produced significantly higher response rates in HER2-positive patients compared with HER2-negative patients, as determined by tissue analysis with a panel of four antibodies: HercepTest, TAB 250, CB11, and pAb1. For example, response rates were 81% and 43% for HER2-positive and HER2-negative patients, respectively, as determined by TAB 250 (P = .001). In patients with FISH-positive disease, the response rate was 75%, compared with 44% in FISH-negative patients (P = .004).

Of note, immunohistochemistry was as effective as FISH in predicting response to combination therapy with paclitaxel and trastuzumab. The relative utility of immunohistochemistry and FISH assessment of breast carcinomas (as well as the assessment of circulating serum levels of HER2 extracellular domain) as predictors of benefit from paclitaxel plus trastuzumab is also being studied in Cancer and Leukemia Group B (CALGB) 98-40, a large randomized trial in patients with metastatic breast cancer.

**Conclusions**

Since the FDA approved trastuzumab for the treatment of metastatic breast cancer, there has been an increasing need to accurately evaluate the HER2 status of breast cancer specimens, to identify patients who might benefit from this therapeutic approach (particularly considering its potential cardiac toxicity and cost). Data from pivotal trials of the antibody suggest that the benefit is largely limited to patients with the highest levels of HER2 protein overexpression (3+).[58]

Although progress continues, several important issues remain open for further investigation. First,
there is a particularly urgent and vexing need to standardize assays for HER2 testing. Second, optimal use of trastuzumab in the metastatic breast cancer setting is currently being evaluated in several trials that are exploring not only combinations with agents such as docetaxel (Taxotere), vinorelbine (Navelbine), and gemcitabine (Gemzar), but also different scheduling, such as every-3-week administration.[59]
Finally, the adjuvant use of trastuzumab, administered sequentially or in combination with chemotherapy is being evaluated in numerous trials that are actively accruing patients. Germain to all of these efforts is the need to optimally assess HER2 status for patient selection. Compared with the long evolution of assays to quantitate estrogen receptors, much timely progress has been made in the evaluation of HER2.

References:


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