Advances in Brief

HER-2 Expression and Response to Tamoxifen in Estrogen Receptor-positive Breast Cancer: A Southwest Oncology Group Study

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Abstract

HER-2/neu is a growth factor receptor, the expression of which has been associated with a more aggressive breast tumor biology and resistance to some types of chemotherapy. Preliminary laboratory and clinical data have led to claims that HER-2/neu expression is also associated with resistance to tamoxifen. Therefore, to test the hypothesis that HER-2/neu expression is associated with a poorer response to tamoxifen, a shorter time to treatment failure (TTF), and worse survival in estrogen receptor (ER)-positive metastatic breast cancer, we examined 205 paraffin-embedded blocks of tumors from patients enrolled on Southwest Oncology Group 8228 for HER-2/neu expression. Tumors were ER positive (ER level >3 fmol/mg cytosolic protein in either primary tumors or metastases), and patients had not received any prior therapy for metastatic disease. All patients were treated with daily tamoxifen. The study began in 1982, and median follow-up of patients who are still alive is now 9 years. Membrane staining for HER-2/neu was evaluated by immunohistochemistry using antibody TAB 250 and was scored according to the proportion of cells staining positive; tumors were deemed positive if >1% of the cells stained for HER-2/neu. HER-2/neu positivity was associated with lower ER values (P = 0.04) and low bcl-2 (P = 0.01). HER-2/neu positivity was not significantly associated with response rate (negative versus positive, 57 versus 54%; P = 0.67), TTF (median, 8 versus 6 months; P = 0.15), or survival (median, 31 versus 29 months; P = 0.36). There was no significant evidence of a progressive relationship between an increasing proportion of cells expressing HER-2/neu and a shorter TTF or survival.

Introduction

Overexpression of the growth factor receptor HER-2, also known as erbB-2, has been associated with a worse outcome in breast cancer patients. Evidence has accumulated that this worse outcome may be due to resistance to some types of systemic therapy, especially to cytotoxic combinations such as cyclophosphamide, methotrexate, and 5-fluorouracil (1, 2).

Because tamoxifen, at least in part, exerts its therapeutic effect through growth factor pathways, changes in HER-2 expression might also affect response to tamoxifen. There is a preclinical and clinical evidence that has been taken to support this notion. Overexpression of HER-2 in MCF-7 cells, an ER-positive breast cancer cell line, resulted in tamoxifen resistance, but only in one of the three clones that were tested, and in that single clone, HER-2 expression was 45 times that of the parental control (3). Results of retrospective clinical studies are mixed: some investigators claim significant resistance (4–6), and others do not (7). Heterogeneity in patients and treatment, small sample size, and subset analyses further complicate the interpretation of existing data. In particular, because HER-2 overexpression is strongly correlated with ER negativity (8), it might be expected that overexpressing tumors would be less sensitive to tamoxifen because they are more likely to be ER poor or

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3 The abbreviations used are: ER, estrogen receptor; TTF, time to treatment failure; SWOG, Southwest Oncology Group; PgR, progesterone receptor; CR, complete response; PR, partial response; SD, stable disease; IHC, immunohistochemical; OS, overall survival.
negative, and ER-negative tumors were not always rigorously excluded from the previous studies. Therefore, to test the hypothesis that HER-2 overexpression is associated with resistance to tamoxifen in ER-positive breast cancer, we measured HER-2 expression in 205 patients with evaluable or measurable ER-positive metastatic breast cancer, all of whom received tamoxifen as initial metastatic therapy in a prospective study (9, 10). Results were compared with patient and tumor characteristics, response, TTF, and survival. The trial began in 1982 and closed in 1987; follow-up of those still alive is 9 years. This study design seeks to minimize the confounding effect of ER status and also tests the hypothesis in a relatively homogeneous population with a high event rate.

Patients and Methods

Eligibility. To be eligible for SWOG 8228 (10) patients must have had the following characteristics: metastatic breast cancer; ER level of >3 fmol/mg of cytosolic protein in the primary or metastatic specimen; no prior treatment for metastatic disease; prior adjuvant chemotherapy or tamoxifen therapy allowed if it was completed more than 3 months prior to relapse; PgR ligand-binding assay performed; no massive liver involvement; and a signed and approved informed consent.

Patients and Tumor Specimens. SWOG 8228 was opened in 1982 and closed in 1987. There were 349 patients who were eligible for SWOG 8228. In this ancillary study, SWOG 9314, formalin-fixed paraffin blocks from the primary or metastatic tumor were collected on 215 of these patients. For the remainder of the patients, the blocks had been previously discarded or could not be located. Blocks on five patients could not be further analyzed because of poor fixation. Additionally, for one patient, the submitted specimen contained no invasive cancer. Four patients were not evaluable for response in SWOG 8228. Thus, a total of 205 patients were analyzed. Patient and disease characteristics for patients in the present study were similar to those in SWOG 8228 who were not registered in this study: PgR level of <10 fmol/mg, 29 versus 32%; PgR level of ≥100 fmol/mg, 33 versus 31%; ER level of <50 fmol/mg, 34 versus 37%; premenopausal, 11 versus 9%; age of <65 years, 61 versus 58%; visceral disease, 31 versus 34%; no prior adjuvant therapy, 78 versus 77%; prior adjuvant tamoxifen, 3 versus 4%, respectively. Only disease-free interval was statistically different for those in SWOG 9314 as compared to those patients from SWOG 8228 who were not included in SWOG 9314, with fewer patients having a disease-free interval of >3 years (19 versus 26%) and a larger portion having metastatic disease at presentation (47 versus 34%; \( \chi^2, P = 0.05, 2 \) degrees of freedom).

Tissue was analyzed from the following anatomical sites: 162 primary tumor, 15 skin/soft tissue, 18 lymph node, 4 lung, 5 bone, and 1 ovary.

Treatment. In the initial phase of the SWOG 8228 study, the first 87 patients were treated with tamoxifen, 10 mg b.i.d. The tamoxifen dose was changed to 10 mg/m² b.i.d. for the remaining 255 patients. For those evaluated in SWOG 9314, 56 patients received tamoxifen, 10 mg b.i.d., and 149 patients received 10 mg/m² tamoxifen b.i.d.

Response Criteria. CR in patients without osseous involvement was defined as the disappearance of all evidence of measurable or assessable disease for ≥4 weeks. In patients with osseous disease, CR was defined as the disappearance of all evidence of nonosseous cancer, bone scans, or skeletal radiographs that showed no evidence of progression or new lesions, a return of alkaline phosphatase to normal, and disappearance of bone pain. In patients with only osseous disease, CR required complete normalization of radiographs and scans. PR in patients without osseous involvement was defined as a reduction of >50% in cross-sectional areas of all measurable lesions for ≥4 weeks. In patients with osseous disease, PR was defined as a >50% reduction in cross-sectional areas of all cancer in nonosseous sites, bone scans, or skeletal radiographs that showed no evidence of progression or new lesions, a reduction in alkaline phosphatase, and improvement of bone pain. If only osseous disease was present, PR required a reduction in alkaline phosphatase, with evidence of healing of lytic lesions and/or improvement in the bone scan. SD was defined as a steady state or a response less than partial remission. Progression was defined as the appearance of new lesions or an increase in >25% of the cross-sectional area of all measurable tumor or as a worsening of tumor-related symptoms in a patient with otherwise SD. Quality control of response evaluation was assured by the study and data coordinator’s review of the submitted data.

Response to treatment was defined as a patient having either CR, PR, or prolonged SD (a TTF of more than 6 months). Prolonged SD was included as a response to treatment because patients with prolonged disease stabilization in response to tamoxifen clearly benefited clinically and because objective benefit is difficult to assess in patients with osseous disease. TTF was defined as the time from registration to first occurrence of progression, discontinuation of treatment, or death. Physicians were informed that responses to tamoxifen might take 6–12 weeks to become clinically evident. The study design required tamoxifen to be continued for at least 4 weeks, even if there was an initial progression or tumor flare, and for at least 8 weeks in patients with SD.

IHC Analysis. One 5-µm section of each submitted paraffin block was first stained with H&E to verify that adequate numbers of invasive tumor cells were present and that fixation quality was sufficient for IHC analysis.

Additional 5-µm sections mounted on adhesive coated slides were then heated to 60°C for 10 min, cooled, deparaffinized, and hydrated through three changes of xylene and graded alcohols. Slides were rinsed with PBS, quenched in 0.1% sodium azide in 3% H₂O₂ and autobody for 30 min, rinsed in PBS and autobody, and blocked with 10% ovalbumin for 15 min. For HER-2 immunostaining, specimens were incubated for 1 h in monoclonal antibody TAB 250 (Triton, Alameda, CA; 1:500 dilution) for 1 h. In a comparison study of various HER-2 antibodies, TAB 250 was reported to be one of the best (11). After rinsing with PBS, a secondary biotinylated antimouse antibody (Dako) was applied for 30 min, and slides were rinsed with PBS, incubated with streptavidin-horseradish peroxidase 1:100 for 30 min, rinsed with PBS, exposed to 3,3’-diaminobenzidine chromogen for 10 min, rinsed with autobody and PBS, intensified with 0.2% osmium tetroxide for 30 s, rinsed with PBS, counterstained with 1% methyl green, rinsed with deionized water, and then mounted.

IHC Scoring. Tumors were scored according to the estimated proportion of tumor cells that were positively stained. Scoring criteria, based on the estimated fraction of positively
staining cells, were as follows: score = 0, none; score = 1, <1/100; score = 2, 1/100–1/10; score = 3, 1/10–1/3; score = 4, 1/3–2/3; and score = 5, >2/3. For HER-2, only membrane staining of tumor cells was evaluated. Prospectively, tumors that had an IHC score of ≥2 were deemed positive for HER-2 overexpression, based on a prior study (1).

Statistical Analysis. χ² tests were used to compare HER-2 IHC results with dichotomized patient and tumor characteristics. Estimation of TTF and OS was performed using the Kaplan-Meier method. Log-rank statistics were used to compare TTF and survival. Multivariate analyses were performed using Cox’s partially nonparametric model for censored survival data. The association of response with HER-2 and other characteristics was analyzed using logistic regression. All reported Ps were two-sided.

Results
The patient and disease characteristics of those entered onto SWOG 9314 are seen in Table 1. A preponderance of the patients were postmenopausal and received no prior adjuvant therapy. Forty-seven % presented with metastatic disease. Thirty % of tumors were positive for HER-2, using a cutoff point of an IHC score of ≥2. A positivity rate of 30% is similar to a published average range of 20–30% for HER-2 overexpression, although it is somewhat higher than was expected in this entirely ER-positive population. In a previous study from San Antonio, 15% of ER-positive patients showed HER-2 overexpression, using Western blotting (8).

The IHC staining for 204 tumors was technically evaluable for HER-2. One tumor was not evaluable because the section that was cut from the tumor would not remain on the slide during staining. HER-2 positivity was associated with a lower level of ER (<50 fmol/mg of protein; P = 0.04) and a lower bcl-2 expression (P = 0.01). There was no significant association between HER-2 positivity and menopausal status, age, presence of visceral disease, prior adjuvant therapy, or p53 accumulation.

CR, PR, or SD for 6 months was deemed a response to tamoxifen. HER-2 status was not associated with response to tamoxifen, using an IHC score of <2 versus ≥2 as a cutoff point (57 versus 54% respectively; P = 0.67) or a higher cutoff point of an IHC score of <3 versus ≥3 (57 versus 5%, respectively; P = 0.78). TTF was not significantly worse for HER-2-positive patients (median, 6 months) than it was for HER-2-negative patients (median, 8 months; P = 0.15; Fig. 1). Similarly, survival was nearly identical for HER-2-positive patients versus HER-2-negative patients (median, 29 versus 31 months; P = 0.36; Fig. 1). When the analyses were restricted to only primary tumors, the results were virtually the same (data not shown). An additional analysis was performed only on tumors that were PgR positive. Response rate was 58 versus 61% (P = 0.76), TTF was 9 versus 8 months (P = 0.19), and survival was 36 versus 31 months (P = 0.4).

Because of issues of reproducibility involving IHC, a second independent observer scored the slides. At a cutoff point of an IHC score of ≥3 for positive, the response rate was 57 versus 47% (P = 0.28), median TTF was 8 versus 5 months (P = 0.04), and median survival was 31 versus 26 months (P = 0.26), negative versus positive, respectively. With a cutoff point of an IHC score of ≥2, response was 58 versus 44% (P = 0.1), TTF was 8 versus 4 months (P = 0.01), and median survival was 34 versus 25 months (P = 0.09), negative versus positive, respectively.

Discussion
The findings of this study do not support the hypothesis that overexpression of HER-2 is associated with tamoxifen unresponsiveness or a more aggressive phenotype of ER-positive breast cancer. It is the largest study to date addressing this issue. Given the parameters observed, this study had a ~75% power, at a level of 0.05, to detect a 20% increase in response rate and a 1.5-fold increase in median TTF and OS in HER-2-negative versus HER-2-positive patients. There are a number of considerations that could explain the apparent discrepancy between the findings in this study and other published reports.

Different antibodies for IHC staining can vary greatly in sensitivity and specificity, thus giving disparate results. The antibody used here was TAB 250, which is a monoclonal antibody that recognizes the external domain of the receptor. Its sensitivity and specificity were found to be relatively high when compared directly with 27 other antibodies to HER-2 protein (11).

Of the tissues analyzed, 161 (79%) were from the primary tumor, 15 (7%) were from skin/soft tissue, 18 (9%) were from...
lymph nodes, and 10 (5%) were from distant sites. Because the majority of specimens were from primary tumors, it is possible that the HER-2 status was different in the metastatic tumor tissue, making it difficult to detect a correlation with tamoxifen response. However, evidence suggests that HER-2 results in the primary tumor and in distant metastases are remarkably consistent (12). Also, results from analyses restricted to primary tumors only were virtually the same as the whole group.

Immunohistochemistry is not a functional assay, and it is possible that the measured receptor protein is not activated. Indeed, one study demonstrated that only a minority of tumors that overexpressed HER-2, about 30%, contained a phosphorylated form of the receptor, a state that is thought to be indicative of activation (13). However, this does not explain the discrepancy with some other studies because none of them specifically evaluated the activated form of HER-2.

Two laboratory-based studies have observed an effect of HER-2 overexpression on tamoxifen response in MCF-7 breast cells. In the first (3), HER-2 cDNA was introduced by plasmid transfection. In two of three clones tested, tamoxifen resistance was not observed, although resistance was seen in a third clone, in which overexpression was 45 times that of the parental cell line, a phenomenon usually not seen in human tumors. In another publication (14), three separate clones of MCF-7 cells that were virally transduced with two to five copies of HER-2 cDNA clearly showed evidence of tamoxifen resistance. The level of protein expression was not reported. In considering the results of these experiments, it is important to remember that the sudden introduction of multiple copies of HER-2 genes, randomly integrated into the genome and not driven by native promoters, creates a highly artificial system that may not reflect the actual physiology that is present in slowly evolving human

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**Fig. 1.** TTF (A) and survival (B) in ER-positive tamoxifen-treated breast cancer using a prospective, dichotomized cutoff point for HER-2 overexpression.
tumors. Additionally, it is not clear whether the levels of HER-2 protein expression in such experiments approximate those that are actually found in human tumors.

A number of clinical studies have also found evidence of tamoxifen resistance in HER-2-overexpressing tumors (4–6, 15). This may be expected, because HER-2 expression is associated with low or absent ER and, thus, with hormonal unresponsiveness. In a number of these studies, both ER-positive and ER-negative tumors were analyzed together (5, 6, 15), or when analyzed separately, the actual number of tumors in the ER-positive subsets was very small [e.g., in Wright et al. (5), there were five ER-positive, HER-2-positive tumors], such that the results were statistically unstable and no conclusion could be drawn. In a nonrandomized adjuvant study of tamoxifen, HER-2 overexpression appeared to correlate with tamoxifen resistance, primarily in a subset of node-positive, PgR-positive patients but not in PgR-positive node-negative patients (4). When ER was used to define patient groups, evidence of tamoxifen resistance was “less pronounced.” The nonrandomized nature of this trial, together with the fact that significant evidence of tamoxifen resistance was confined to particular subsets, makes definitive conclusions difficult.

Elevated serum levels of the extracellular domain of HER-2 have been associated with resistance to second line hormonal therapy (megestrol acetate or fadrozole; Ref. 16). ER-unknown patients were also included in this population, and cutoff points for elevated circulating protein were derived from a training set of patients, which were then included in the overall study. Interestingly, this same elevated
level of circulating HER-2 was not associated with response to first-line tamoxifen therapy (16). Another study reports resistance to droloxifene with elevated circulating levels of HER-2 in 94 receptor-positive or receptor-unknown patients (17). The difference in response rate was striking (56 versus 9%). However, 37 patients (39%) were ER unknown. When receptor-unknown patients were excluded and only receptor-positive patients were analyzed (n = 54 responses), TTF and OS were “not substantially different” from the group as a whole. Possible reasons for the differing results of the droloxifene study and the present results could be attributed to a number of reasons, including differences in the marker that was measured (HER-2 protein on the cell surface versus a HER-2 protein fragment in the serum), differences in the methods used (IHC versus ELISA), or differences in the type of endocrine agent used (tamoxifen versus droloxifene).

The strengths of the present study include relatively large numbers, a homogeneous patient population receiving a single defined treatment, measurable or evaluable metastatic disease facilitating direct assessment of response, and a long follow-up period.

Because of issues of reproducibility and IHC, a second independent analysis of tumors was performed by a second observer. The results of this analysis were similar to the first, although there were trends indicating a modestly lower responsiveness to tamoxifen in tumors overexpressing HER-2. This again might be due to the lower absolute levels of ER protein found in these HER-2-positive tumors. With a larger sample size these trends might become statistically significant, but the magnitude of the difference is small, such that the findings are not clinically useful.

Although the original clinical study that provided the tumors used here was prospective, the present study was retrospective. Although significant bias was not evident, unrecognized bias cannot be completely excluded because of the retrospective nature of the block collection. Also, immunohistochemistry is a semisubjective method, so that study results can vary somewhat by observer. Despite these limitations, the data from this study are not consistent with the notion that HER-2 overexpression results in significant hormone unresponsiveness in ER-positive patients, suggesting the need for further investigation before HER-2 status is used to make clinical decisions about hormonal therapy. These results also suggest that the poorer prognosis of patients with HER-2 overexpression in adjuvant studies is likely to be due to HER-2’s interaction with cytotoxic chemotherapy rather than with tamoxifen treatment.

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References


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