Featured Article

HER-2 Amplification, HER-1 Expression, and Tamoxifen Response in Estrogen Receptor-Positive Metastatic Breast Cancer: A Southwest Oncology Group Study

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ABSTRACT

Purpose: **Preclinical data indicate that expression of the ErbB family of receptors, such as HER-2 and HER-1 (EGFR) may be involved in endocrine resistance. Evidence of resistance from clinical studies has been inconsistent. The present study examined whether HER-2 gene amplification or HER-1 expression predicted response to tamoxifen.**

Patients and Methods: **Three hundred and forty nine patients had estrogen receptor (ER)-positive breast cancer and received daily tamoxifen as initial therapy for advanced disease. HER-2 gene amplification, detected by fluorescence *in situ* hybridization, and HER-1 expression, evaluated by immunohistochemistry, was determined on 136 and 204 patients, respectively.**

Results: **HER-2 amplification was correlated with lower ER ($P = 0.02$), HER-1 positivity ($P = 0.004$), and HER-2 protein overexpression ($P < 0.00001$). The response rate was 56% for HER-2 non-amplified *versus* 47% for HER-2 amplified tumors ($P = 0.38$), and 58% for HER-1–negative *versus* 36% for HER-1–positive ($P = 0.05$). Time to treatment failure (TTF) was 7 months for non-amplified HER-2 tumors and 5 months ($P = 0.007$) for amplified HER-2 tumors, and there was a trend toward a better overall survival (OS) in patients with non-amplified HER-2 tumors (median 31 *versus* 25 months, respectively, $P = 0.07$). For positive *versus* negative HER-1 tumors, TTF was 4 *versus* 8 months ($P = 0.08$) and median survival was 24 *versus* 31 months ($P = 0.41$). Combining HER-1 expression and HER-2 gene status, patients with both negative HER-1 expression and non-amplified HER-2 had longer TTF ($P = 0.001$) and OS ($P = 0.03$) than if either were positive. In multivariate analysis, HER-2 was not an independent factor for TTF and OS, although HER-1 was significant for TTF only ($P \leq 0.001$).**

Conclusion: **Patients with HER-2 amplification and HER-1 expression had lower ER levels and were modestly less responsive to tamoxifen, suggesting that molecular events in addition to those involving the ErbB receptors are important in determining the endocrine-resistant phenotype.**

INTRODUCTION

Tamoxifen has been the most commonly prescribed hormonal therapy for breast cancer, and for over two decades, its role has expanded from treatment for advanced disease to established adjuvant therapy after surgery for early breast cancer (1) and to breast cancer prevention (2). Currently, estrogen receptor (ER) and progesterone receptor (PgR) are the standard biological factors that most accurately predict response to tamoxifen and other types of hormonal therapy. Unfortunately, despite the presence of ER, many patients experience primary (*de novo*) resistance to tamoxifen, and all patients with advanced disease eventually acquire resistance to therapy. The potential mechanisms for either primary or acquired endocrine resistance are still poorly understood, but they may include ER co-regulatory proteins and cross-talk between ER pathways and other growth factor networks.

Evidence from preclinical models shows that the HER-1/HER-2 signaling network may play an important role in the development of antiestrogen resistance in human breast cancer (3–5). A variety of clinical investigations suggest that HER-2–positive tumors are less likely to respond to endocrine therapy, specifically tamoxifen (6–10), but data from other trials have not supported this notion (11–13). Heterogeneity in patients and treatment, small sample size, inclusion of ER-negative patients, and the lack of standard reproducible methods to measure these two markers complicate the interpretation of existing data.

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This study was undertaken to test the hypothesis that HER-2 amplification and HER-1 protein expression may be associated with tamoxifen resistance in ER-positive breast cancer. The status of these molecules was evaluated in tumors from 205 patients with ER-positive metastatic breast cancer. These patients had all been enrolled onto the Southwest Oncology Group (SWOG) protocol 8228, a prospective trial originally designed to investigate the independent significance of PgR levels in predicting response to tamoxifen in patients with ER-positive metastatic breast cancer.

PATIENTS AND METHODS

Eligibility. To be eligible for SWOG 8228 (14) patients must have had the following characteristics: (*a*) diagnosis of metastatic breast cancer; (*b*) ER level of >3 fmol/mg cytosolic protein in the primary or metastatic specimen; (*c*) no prior treatment for metastatic disease; (*d*) prior adjuvant tamoxifen or chemotherapy allowed, provided it was completed >3 months before relapse; (*e*) PgR ligand-binding assay performed; and (*f*) a signed institutional review board 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 the present 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. One tumor was not evaluable for immunohistochemistry (IHC) because the section that was cut from the paraffin block repeatedly washed off of the slide during the staining. Thus, the IHC staining for a total of 204 patients was technically evaluable for both HER-1 and HER-2 protein status, and full clinical follow-up was available. Nearly all tumor blocks from this study were >10 years old at the time the assays were performed, with some blocks being >20 years old.

Of the 205 paraffin blocks available at the beginning of the study, 151 specimens were available for fluorescence *in situ* hybridization (FISH) analysis to detect HER-2 amplification. The remainder of formalin-fixed paraffin blocks were consumed by previous studies or had been lost or damaged in a flood. For technical reasons most likely attributable to inadequate tissue fixation, FISH could not be adequately performed and interpreted in 15 patients, and thus a total of 136 cases could be analyzed for HER-2 by FISH.

Clinical and disease characteristics for the 205 patients in the present study were similar to those in the total group in SWOG 8228: PgR level of <10 fmol/mg, 30 *versus* 30%; ER level of <50 fmol/g, 35 *versus* 34%; premenopausal, 10 *versus* 11%; age of <65 years, 60 *versus* 61%; visceral disease, 32 *versus* 31%; prior adjuvant therapy 22 *versus* 22%; metastatic at presentation 42% *versus* 48%, respectively.

Treatment. In the initial phase of the SWOG 8228 study, the first 87 patients were treated with tamoxifen, 10 mg/m^2/day twice a day. The tamoxifen dose was changed to 10 mg twice a day for the remaining 255 patients. For those evaluated in SWOG 9314, 56 patients received tamoxifen, 10 mg twice a day, and 149 patients received 10 mg/m^2 twice a day.

Response Criteria. Details of response criteria have been published previously (13). Response was defined as a complete response, partial response, or stable disease for ≥6 months.

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 and FISH analysis. Details of IHC have been published previously (13). For HER-2 immunostaining, specimens were incubated for 1 hour in monoclonal antibody TAB 250 (1:500 dilution, Triton, Alameda, CA); for HER-1 determination, specimens were incubated for 1 hour in monoclonal antibody 31G7 (1:50 dilution, Zymed Lab, Inc, San Francisco, CA).

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: 0 = none; 1 < 1%; 2 = 1–10%; 3 = 10–33%; 4 = 33–66%; 5 >67%. For both HER-2 and HER-1 only membrane staining of tumor cells was evaluated. Prospectively, tumors that had an IHC score ≥2 were deemed positive for HER-1 overexpression. Tumors with a score of ≥3, based on data from prior studies and HercepTest cutoffs (≥10%) were deemed positive for HER-2 (15). Slides were scored by the study pathologist (D. C. A.). A complete analysis of results of HER-2 by IHC has been published previously (13).

Fluorescence *In situ* Hybridization. FISH was performed using a PathVysion HER-2/*neu* DNA probe kit (Vysis Inc. Downers Grove, IL) according to the manufacturer's protocol. A formalin-fixed paraffin-embedded tissue block was cut into 5 μm-thick sections and mounted on silane-coated slides (Dako A/S, Grostrup, Denmark). One of the sections was stained with H&E and used for the microscopic confirmation of the invasive part of the carcinoma tissue, and other sections were used for the FISH assay. Hybridization was performed at 37°C for 14 to 18 hours with a denatured DNA probe (190 kb) that was localized on 17q11.2-q12 and labeled with Spectrum Orange (Vysis) as well as a CEP17α satellite DNA probe (5.4 kb), which was localized on 17p11.1-q11.1 and labeled with SpectrumGreen (Vysis). FISH was considered to be successful if the tissue section met the following criteria: (*a*) no detectable DNA loss had occurred as determined by localization of 4′,6-diamidino-2-phenylindole staining; (*b*) hybridization was uniform throughout the tissue section; and (*c*) single copy status could be identified and was not assumed to be a consequence of the lack of signal (16). The HER-2 and the CEP17 signals were counted using a fluorescent microscope through appropriate filters. Only invasive carcinoma components were counted. The total number of the HER-2 and CEP17 signals in 60 interphase tumor cell nuclei were counted by two observers (G.A. and C.G.). Each observer independently identified tumor cells and calculated the HER-2/CEP17 ratio by dividing the total counts of CEP17 signals. The ratio of ≥ 2 was prospectively defined as HER-2 gene amplification. The cutoff value was established as a HER-2/CEP17 ratio ≥ 2 as described in the protocol of PathVision kit (17). When the score of the two observers coincided, that score was adopted as HER-2 gene status of the

tumor. When the score differed, the signals were enumerated again, and the HER-2/CEP17 ratio was reevaluated, and a consensus was reached.

Statistical Analyses. χ^2 tests were used to test the association of HER-2 FISH and HER-1 IHC results with dichotomized patient and tumor characteristics and with response. Estimation of time to treatment failure (TTF) and overall survival 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 test for trend over ordered categories used (for binomial data) was the Kruskal-Wallis test. All reported *P* values were two-sided.

RESULTS

Correlation of HER-2 and HER-1 and Patient Characteristics. The status of HER-2 and HER-1 proteins was compared with other tumor and patient characteristics. First, 136 tumors were assessable for HER-2 amplification. Thirty-two (24%) of these tumors had HER-2 gene amplification using a cut-point of a FISH score of ≥2, and 15 (11.0%) were highly amplified using a cut-point of ≥4. HER-2 amplification was inversely related to ER levels and directly related to HER-1 positivity (Table 1). Fifty percent of tumors with HER-2 amplification had ER levels of <50 fmol/mg protein, compared with 28% of tumors without amplification ($P = 0.02$). The median level of ER was nearly double in HER-2 non-amplified tumors compared with HER-2 amplified tumors, 106 fmol/mg *versus* 55 fmol/mg, respectively. The proportion of PgR-positive tumors was also lower in HER-2-amplified tumors compared with HER-2 non-amplified tumors, but the difference was not statistically significant. Median PgR was more than three times higher in non-amplified tumors compared with HER-2 amplified, 58 fmol/mg *versus* 15 fmol/mg. Additionally, as found by other investigators (18), HER-2 amplification correlated with HER-1 positivity ($P = 0.004$) and with HER-2 protein overexpression ($P < 0.00001$). There was no consistent, significant relationship between HER-2 and menopausal status, site of disease, prior adjuvant therapy, or disease-free interval (Table 1).

Table 1 Correlation of HER-2 FISH status with patient and tumor characteristics ($N = 136$)

	HER-2 FISH Score			
	<2 (non-amplified) (N = 104)		≥2 (amplified) (N = 32)	
Characteristics	*n*	%	*n*	%
ER				
<50 fmol/mg*	29	28	16	50
Median ER level	106 fmol/mg		55 fmol/mg	
PgR				
<10 fmol/mg	29	28	12	37
10–99 fmol/mg	34	33	13	41
≥100 fmol/mg	41	39	7	22
Median level	58 fmol/mg		15 fmol/mg	
Negative	100	96	25	78
Positive†	4	4	6	19
Premenopausal	13	12	5	16
Age < 65 years	59	57	21	66
Visceral disease	33	32	12	37
No prior adjuvant therapy	79	76	25	78
Disease-free interval				
0 year	50	48	12	38
0–3 year	32	31	18	56
>3 year	22	21	2	6

* $P = .02$.
† $P = .004$.

Table 2 Correlation of HER-1 expression score with patient and tumor characteristics ($N = 204$)

	IHC Score			
	<2 (negative) (N = 182)		≥2 (positive) (N = 22)	
Characteristics	*n*	%	*n*	%
ER				
<50 fmol/mg*	53	29	16	73
Median ER level	95 fmol/mg		31 fmol/mg	
PgR				
<10 fmol/mg	67	37	10	45
10–99 fmol/mg	53	29	7	32
≥100 fmol/mg	62	34	5	23
Premenopausal	18	4	4	18
Age <65 yrs	107	59	17	77
Visceral disease	59	32	4	18
No prior adjuvant therapy	143	79	16	73
Disease-free interval				
0 yrs	85	47	13	59
>0–3 yrs	63	35	5	23
>3 yrs	34	19	4	18

* $P < .00001$.

Table 3 Association Between IHC and FISH for HER-2 Evaluation ($N = 136$)

	IHC− (N = 107)		IHC+ (N = 29)		
	%	(*n*)	%	(*n*)	*P* value
FISH− (N = 104)	70	(95)	7	(9)	0.00001
FISH+ (N = 32)	9	(12)	15	(20)	

For HER-1, 204 tumors were assessable (Table 2). Ten percent of tumors were positive for HER-1 using a cut-point of an IHC score of ≥2. In several previous studies, HER-1 expression was strongly correlated with lower ER level (19–22). Consistent with this, in the present study, in which all tumors were ER-positive, tumors with ER levels lower than 50 fmol/mg protein were more likely to be HER-1–positive (Table 2). Median level of ER was 95 fmol/mg in HER-1–negative tumors and 31 fmol/mg in HER-1–positive tumors, respectively. There were no other significant associations between HER-1 and other patients or disease characteristics, except as noted above.

Comparison of HER-2 FISH and IHC. The results of HER-2 by both FISH and IHC assays were directly comparable in 136 cases. Results of these two assays were concordant in 115 cases (84.6%, $P < 0.00001$; Table 3). Of the 32 cases amplified by FISH, 12 were negative by IHC (Table 4). Seven of these 12 cases that were negative for IHC and amplified by FISH had a FISH score ≤2.2 with two additional cases having scores of

Table 4 Discordant Cases Between FISH and IHC for HER-2

FISH+/IHC− cases		FISH−/IHC+ cases	
FISH score	IHC score	FISH score	IHC score
2.01	0	< 2	3
2.08	0	1.94	3
2.14	0	< 2	3
2.15	0	< 2	3
2.15	0	< 2	3
2.21	0	1.16	4
2.22	0	1.88	4
2.64	0	1.43	5
2.84	0	1.75	5
6.64	0		
7.36	0		
9.52	0		

<3 (Table 4). Thus 9 of 12 "discordant" FISH-positive/IHC-negative tumors had borderline or low amplification. Of the nine cases that were FISH-negative and IHC-positive, five had an IHC score of 3 (Table 4). Thus, in 14 of 21 "discordant" cases, values lay close to the interface of cut-points.

Response and TTF. Complete remission, partial remission, or stable disease for 6 months was deemed a response. Using a FISH cut-point of ≥2 to define HER-2 amplification, there was no significant difference in the rate of response (47% *versus* 56%) for tumors with scores of ≥2 *versus* <2 (P = 0.38), respectively. Although a trend is apparent, there was also no significant response relationship for tumors with scores of <2, ≥2, but <4 and ≥4, with response rates of 55.7% *versus* 52.9% *versus* 40% (P = 0.33, Kruskal-Wallis test), respectively, for each category. To investigate whether the response rate in HER-2-amplified tumors might be driven by the ER level of these tumors, we compared the median ER level in all HER-2-amplified tumors according to response category. As expected, the median level of ER was lower in HER-2-amplified non-responder tumors compared with HER-2-amplified responder tumors (35 fmol/mg *versus* 62 fmol/mg).

For patients with cancer, TTF is perhaps as important as the probability of response, because the durability of a response and tolerability of treatment contributes to quality and quantity of life. By univariate analysis, TTF was significantly different according to HER-2 status (P = 0.007; Fig. 1*A*). Median TTF was 5 months for those with FISH score ≥2 *versus* 7 months for those with a score <2.

Response was also examined for HER-1. On the basis of previous studies, an IHC cut-point of ≥2 was prospectively chosen for this HER-1 analysis (23). Patients with HER-1–positive tumors had a lower response rate to tamoxifen (36% *versus* 58%, P = 0.05). Similar to HER-2 amplified tumors, HER-1–positive tumors that responded to tamoxifen treatment had median ER levels much higher than resistant tumors (70 fmol/mg *versus* 25 fmol/mg).

TTF was also observed to be shorter in HER-1–positive patients, but given the relatively small numbers, this difference was not statistically significant. The median TTF was 4 months for patients with HER-1–positive tumors compared with 8 months for patients with HER-1–negative tumors (P = 0.08).

Several studies have demonstrated that the interaction of HER-2 and HER-1 may be important for the growth of some breast cancers (24). In our study, an analysis that combined HER-2 and HER-1 status showed that tamoxifen was modestly more effective for HER-2 non-amplified and HER-1–negative tumors than for tumors with either one of the two markers positive (response rates of 57% *versus* 43%, respectively), but this difference was not statistically significant (P = 0.15). In the same combined analyses, the TTF was significantly shorter for patients with HER-2 amplified and/or HER-1–positive tumors than for patients with tumors negative for both markers (P = 0.001; medians 4 *versus* 9 months; Fig. 2*A*).

In a Cox regression analysis that included HER-2 and HER-1 status plus factors that remained important in predicting TTF in SWOG 8228 (ref. 14; menopausal status, disease-free interval, ER level, and PgR level), HER-1 status remained the strongest independent factor in predicting TTF (P = 0.0001; Table 5), but HER-2 was not. This finding suggests that the differences in TTF were not just related to the lower ER levels in HER-1–positive tumors.

Survival. The association between HER-2 amplification and survival has not been extensively examined in the metastatic setting. In this study, we observed shorter overall survival in patients with HER-2-amplified tumors, but the difference was not statistically significant. Median survival was 25 months for HER-2-amplified tumors compared with 31 months for HER-2 non-amplified tumors (P = 0.07; Fig. 1*B*). The median survival for HER-1–positive patients was 24 months compared with 31 months for the HER-1–negative patients, but this difference was

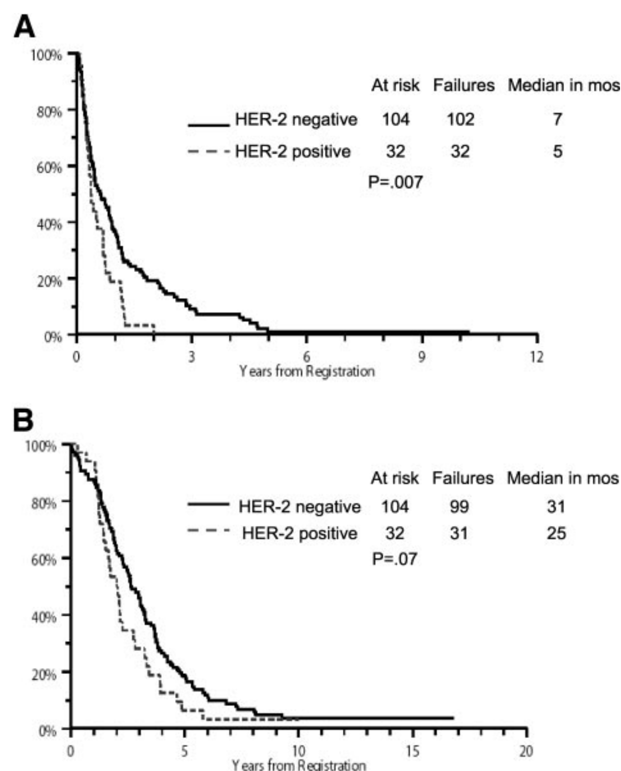

Fig. 1 *A*, time to treatment failure by HER-2 amplification status. *B*, survival by HER-2 amplification status.

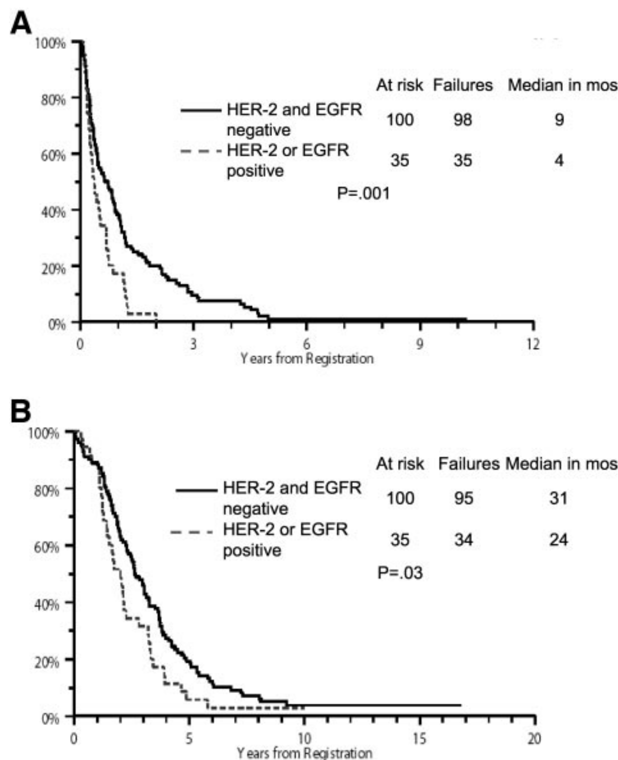

Fig. 2 *A*, time to treatment failure by HER-2 amplification and epidermal growth factor receptor (EGFR) expression status. *B*, survival by HER-2 amplification and EGFR expression.

not statistically significant ($P = 0.41$). Because overall survival is influenced by the multiple treatments given after progression on tamoxifen therapy, some of which (*i.e.,* doxorubicin-based chemotherapy) might be more effective in HER-1/HER-2-overexpressing tumors, this end point may not be directly meaningful in addressing initial resistance to tamoxifen.

When patients whose tumors were positive for one of the two growth factor receptors were combined for the survival analyses, patients with HER-2 and/or HER-1–positive tumors had a shorter survival when they were treated with tamoxifen. The median survival in this group was 24 months compared with 31 months in patients with HER-1 and HER-2–negative tumors ($P = 0.03$; Fig. 2*B*). Neither HER-1 nor HER-2 status remained significantly associated with survival in a Cox model that included the same variables as used above for TTF (Table 5).

DISCUSSION

The present investigation studied the association of HER-1 protein expression and HER-2 amplification with a number of parameters including quantitative ER level and response to tamoxifen. The findings of this study do not support the hypothesis that amplification of HER-2 alone is an independent predictor for tamoxifen resistance. HER-1 expression, however, was associated with a poorer outcome in tamoxifen-treated patients. Although ER values tended to be lower in the HER-1–positive subset, a multivariate analysis suggested that the predictive value of HER-1 was independent of ER. In fact, quantitative level of ER was not significant, thus supporting an independent role for HER-1 in tamoxifen resistance.

Several recent papers have suggested that HER-2 positivity of breast carcinomas may be indicative of resistance to hormonal therapy (mainly tamoxifen), but the data are not conclusive (4, 13, 25, 26). There are a number of possibilities that could explain the heterogeneity of the existing data and the apparent discrepancy between the findings of this study and other published reports. ER status is one of the most important confounding factors in the interpretation of results from the available clinical studies. It is well known that there is an inverse relationship between ER-positivity and HER-2 positivity (27–29). In many clinical trials (8, 25, 30), both ER-positive and ER-negative tumors were analyzed together and, when analyzed separately, the actual number of tumors in the ER-positive group was very small (8). In other reports the ER status of the tumor is not considered at all (7). In essence, much of the reported hormonal insensitivity of HER-2–positive tumors could result from ER negativity or low ER rather than HER-2 positivity *per se*.

In the present study we have attempted to minimize this problem by (*a*) selecting an entirely ER-positive group of patients and (*b*) measuring ER and PgR status by ligand binding in central pathology laboratories with extensive experience, assay standardization, and quality control procedures. Our data show that ER levels are one-half to one-third lower in HER-2–positive/ER-positive cancers than HER-2–negative/ER-positive tumors. In both advanced and early disease, the level of ER is very important in determining response or benefit of tamoxifen (1, 31–33). In this study, among the HER-2–amplified tumors, responders had higher ER levels, thus confirming that the amount of ER in the tumor cells is a crucial factor in predicting the benefit from hormone therapy, even in this subset.

HER-2 measurements in breast cancer have been plagued with problems of reproducibility (15, 34, 35). Although it seems that DNA-based assays, such as FISH, may be more accurate in predicting response to trastuzumab (34), it is not clear whether measurements of protein overexpression by IHC or gene amplification by FISH are most appropriate when HER-2 is used to predict endocrine sensitivity. In a previous reported study in which the HER-2 protein expression was measured by IHC and

Table 5 Cox Proportional Hazard Regression Analysis of Factors Predicting Time to Treatment Failure and Survival

	TTF		OS	
	HR	*P* value	HR	*P* value
HER-2 amplified	1.15	.54	.99	.97
HER-1 positive	4.55	.0001	1.10	.81
Premenopausal	1.97	.02	.94	.83
ER*	1.04	.62	1.21	.02
PgR*	1.08	.08	1.10	.04
DFI 0† *vs.* 1–3 yrs	.58	.01	.62	.03
DFI 1–3 yrs vs. >3 yrs	2.5	.001	2.8	.003

Abbreviations: HR, hazards ratio; DFI, disease-free interval.

* ER and PgR considered as continuous variables, log (receptor level 1).

† Patients who presented with metastatic disease did better than those with DFI of >0 but <3 years.

the ER level by ligand binding, we found that HER-2 status did not predict tamoxifen resistance (14). Because of the issue of the reproducibility surrounding IHC, in the present investigation we assessed the HER-2 status by gene amplification on all of the available specimens and compared it to the IHC results that we published previously (14). Results from this analysis show that, regardless of the method used, HER-2 status alone is not a strong independent predictive factor for tamoxifen resistance.

We compared DNA-based FISH-derived results with protein-based IHC-derived results. Concordance was 85% overall. Concordance with IHC was particularly high for FISH-negative cases, although not as good for FISH-positive cases. A closer inspection showed that the majority of disagreement with IHC occurred with low levels of amplification (FISH score between 2 and 3). Substantial discrepancies occurred in only 9% of tumors.

Many studies that have evaluated the predictive role of HER-2 have included patients who received different types of endocrine therapy (8, 36). There may be differences between specific endocrine therapies that work by different molecular mechanisms and their interaction with HER-2 (18, 37). In addition, small sample size or low event rate, short follow-up, differences in clinical features of patients, and pathological features of tumors may have contributed to different conclusions concerning the predictive impact of HER-2 status. We attempted to avoid some of these confounding factors by studying a population with a relatively larger number of events, one that was homogeneous and prospectively defined in the original trial, received a single defined treatment, and had a follow-up that was very long. Additionally and importantly, methods for the measurements of molecular markers, FISH for HER-2 and ligand binding assay for ER and PgR are relatively objective, quantitative, and reproducible, and they were performed in a single, experienced laboratory.

HER-1 is another member of ErbB receptor family and it has similar, although not identical, downstream signals as HER-2. In metastatic breast cancer patients, HER-1 overexpression has predicted tamoxifen resistance, especially in ER-positive tumors (24, 38). In the present study, higher HER-1–expressing tumors were less likely to respond to tamoxifen, and these patients had a significantly shorter TTF. When ER and PgR levels were taken into consideration, HER-1 remained predictive of a less sustained response despite lower ER levels; supporting the hypothesis that growth factor signaling may be implicated in tamoxifen resistance.

Although preclinical models strongly suggest a role for the HER-1/HER-2 proteins in the development of tamoxifen resistance, the data from this study are not consistent with the notion that growth factor receptor measurements alone result in significant hormone unresponsiveness in ER-positive patients. However, our study does not exclude a role for HER-1/HER-2 in tamoxifen resistance. In the present report, we measured total HER-1/HER-2 status, and it is possible that more meaningful correlations might be obtained using phospho-specific antibodies that monitor the activation state of these proteins. Furthermore, other proteins in addition to ER, HER-2, and HER-1 might be necessary for the development of endocrine resistance in some patients. Another group of proteins that could contribute to tamoxifen resistance in association with ErbB receptors is the ER-interacting proteins that function as coactivators or corepressors (39, 40). One such co-activator is AIB1, a protein that is phosphorylated and activated together with ER by HER-1/HER-2 signaling (41). We have reported recently that when both HER-2 and AIB1 were overexpressed together, the disease-free survival of patients treated with tamoxifen-adjuvant therapy was strikingly worse than tumors with lower levels of one or both proteins (42). HER-2 by itself, as in the current study, had no predictive value for tamoxifen benefit. Thus tamoxifen resistance may require high AIB1 levels as well as high HER-2 or HER-1 activity to alter the activity of tamoxifen-bound ER, a possibility that needs confirmation by additional studies. Alternatively, other molecules implicated in the growth factors pathway such as downstream effectors like AKT or mitogen-activated protein kinase (43) might play a more important role in determining response to endocrine therapy and thus could be used to better refine predictive assessments.

Breast cancer is a highly heterogeneous disease, and the same molecular event may have differing effects based on the presence of additional abnormalities or combinations of abnormalities. Because the interplay of several events probably determines the final outcome, measurement of more molecules simultaneously by high throughput genomics or proteomics may be necessary to gain insight into whether or how these molecules and pathways are involved in endocrine resistance. Clearly a better understanding of molecular biology and more clinical data are necessary before HER-1 and HER-2 measurements should be used to select endocrine therapy in routine practice.

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