Featured Article

Her2/neu Expression Predicts the Response to Antiaromatase Neoadjuvant Therapy in Primary Breast Cancer: Subgroup Analysis from Celecoxib Antiaromatase Neoadjuvant Trial

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ABSTRACT

Purpose: **Many studies suggest that Her2/neu play an important role in neoadjuvant endocrine therapy. This study aimed to determine whether the level of Her2/neu expression in advanced breast cancer changes after antiaromatase neoadjuvant treatment, as well as to identify the relationship between Her2/neu expression and response to this kind of therapy.**

Experimental Design: **Thirty-six postmenopausal patients with hormonal receptor-positive primary breast cancer were included in a study of three monthly cycles of neoadjuvant endocrine therapy with either Aromasin (25 mg daily) or Femara (2.5 mg daily). Immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH) for Her2/neu were conducted both on pretreatment biopsies and surgical tumors.**

Results: **Using IHC, 5 of 36 (13.9%) of the patients had a Her2/neu overexpression after treatment, as compared with 16 of 36 (44.4%) before. Meanwhile, there was no change in 21 (58.3%) patients, and through FISH, there was a change from amplification to no amplification in 15 (41.7%) patients. The response rate to the treatment was 75% for Her2/neu (+) tumors and 35% for Her2/neu (−) tumors (*P = 0.017*) while FISH was performed. The response rate was also significantly affected by the decrease in Her2/neu status after the treatment, with 73% of the tumors showing decreased Her2/neu expression and with 38% of the tumors showing no change of Her2/neu expression (*P = 0.037*).**

Conclusions: **Using both IHC and FISH, advanced breast cancers show statistical evidence of decreasing incidence of Her2/neu expression after antiaromatase neoadjuvant treatment. Our data also suggest that Her2/neu expression and its change during the treatment might be predictive markers for this kind of therapy.**

INTRODUCTION

Human epidermal growth factor receptor-2 is a proto-oncogene encoding a cell-surface glycoprotein designated the Her2 or c-erbB-2 receptor that belongs to the tyrosine kinase receptor family. The *Her2/neu* gene is amplified and/or its protein is overexpressed in 15–25% of breast cancers (1–4). Her2/neu status is a prognostic marker for poor clinical outcome (2, 3) and possibly a predictive marker for tamoxifen resistance (5–9). Although experimental data suggest an important role for Her2/neu in primary and acquired resistance to endocrine therapy using tamoxifen, early data from the neoadjuvant setting indicate that response to aromatase inhibitors may be maintained in patients with Her2/neu overexpression (10–12).

The goal of neoadjuvant therapy is shrinkage of locally advanced and unresectable primary breast tumors, permitting their successful surgical removal (13, 14). It has been used more recently in patients with large operable breast cancers that would require mastectomy but in whom tumor shrinkage can permit breast-conserving surgery (15, 16). Agents used have been mainly limited to cytotoxins used in other forms of chemotherapy. However, endocrine treatment is becoming an attractive alternative in hormone receptor-positive postmenopausal women.

It has been widely demonstrated that various endocrine agents (including tamoxifen and the aromatase inhibitors) can reduce the tumor volume over a 3–4-month treatment in post-menopausal estrogen receptor (ER) (+) patients (10, 11, 17–20). Aromatase inhibitors have most recently been shown to be superior to tamoxifen as initial therapy and are being extensively tested in the neoadjuvant setting instead of tamoxifen. In this setting, aromatase inhibitors not only show enhanced efficacy but also overcome tamoxifen resistance (10, 11). Although studies as yet have failed to show any survival advantage in patients receiving neoadjuvant compared with adjuvant chemotherapy (16, 21), there could nevertheless be benefits from neoadjuvant endocrine therapy provided there was more appropriate patient selection. When selecting patients for endocrine treatment, ER (+) status and, to a lesser extent, progesterone receptor (+) status are important determinants of response (10).

We have studied the expression of Her2/neu before and after neoadjuvant endocrine therapy in patients with breast cancer in an attempt to obtain more information on the effect of endocrine treatment on these patients. Her2/neu status was assessed by both immunohistochemistry (IHC) and fluorescence *in situ* hybridization (FISH). It was also examined in relation to clinicopathological variables and clinical response.

MATERIALS AND METHODS

Patients. Thirty-six pathologically proven, post-menopausal patients with hormonal receptor positive breast cancer were included between October 2001 and July 2003 as part of the Celecoxib Antiaromatase Neoadjuvant Trial (22). The third generation aromatase inhibitor either letrozole (Femara; Novartis Pharma AG, Basel, Switzerland) or exemestane (Aromasin; Pharmacia & Upjohn Company, Kalamazoo, MI) was given daily in a 2.5 or 25 mg dose over the monthly cycle after pathological confirmation from core biopsy of the primary tumor. Eighteen patients were admitted into each group. Each patient was treated for three cycles, and surgery was performed within 7 days after the last cycle.

Physical examination and ultrasound examination were repeated every cycle. Response categories were defined according to the standard Union International Contre Cancer criteria as complete remission, partial remission, no change, progressive disease and not assessable.

All core biopsy and surgically excised tumors of the above patients were fixed in formalin and embedded in paraffin wax. Her2/neu expression was determined using IHC and FISH simultaneously.

IHC. The Hercep Test (Dako Corp., Carpinteria, CA) was performed according to the approved protocol as described by the manufacturer. Tissue sections were cut, mounted on plus slides, heat-treated for antigen retrieval, and immunostained. The sections were counterstained with H&E and then mounted in Permount. Immunostaining was interpreted with a bright-field Olympus microscope according to the scoring system of the manufacturer as 0, 1+, 2+, and 3+ (Dako Corp.). Controls without primary antibody and positive control tissues were included in all experiments to ensure the quality of staining.

FISH. FISH was performed according to the PathVysion (Vysis, Inc., Downers Grove, IL) protocol, described in the package insert as approved by the United States Food and Drug Administration. In brief, the PathVysion protocol involves re-
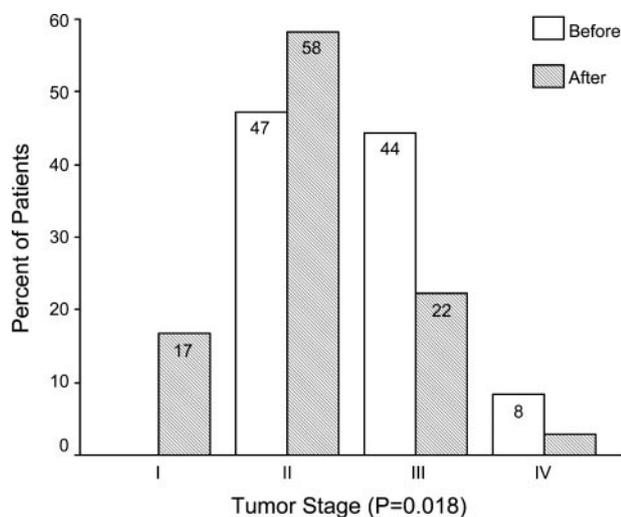

Fig. 1 The comparison of tumor stage before and after neoadjuvant endocrine therapy. Most of the tumors are down-staged significantly after the treatment ($P = 0.018$). □, before; ▦, after.
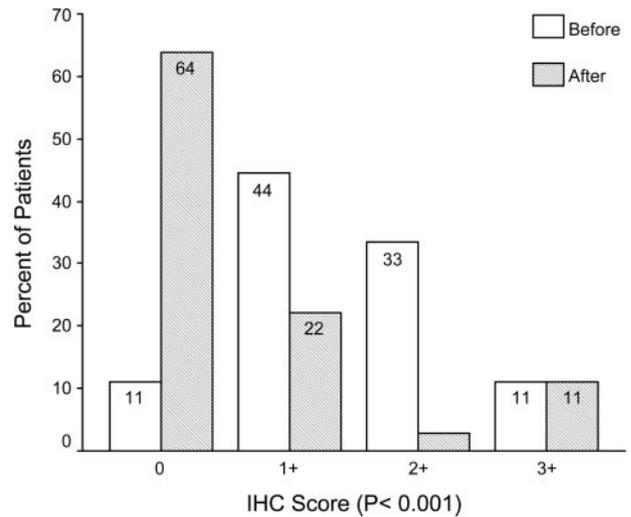

Fig. 2 The comparison of immunohistochemistry (IHC) scores of Her2/neu status before and after neoadjuvant endocrine therapy. The overexpression of Her2/neu are decreased significantly after the treatment ($P < 0.001$). □, before; ▦, after.

hydration of paraffin-embedded, 4-μm thick, multitumor tissue sections. The sections were air-dried, pretreated, and digested with protease before being hybridized with fluorescent-labeled probes for Her2/neu gene and α-satellite DNA for chromosome 17. The nuclei were routinely counterstained with an intercalating fluorescent counterstain, 4′,6-diamidino-2-phenylindole. For each tumor, 20 tumor cell nuclei were identified and scored for both *Her2/neu* and chromosome 17 centromere numbers. *Her2/neu* gene amplification was defined as a Her2-to-chromosome 17 ratio > 2.0 as required by the manufacturer.

Statistical Methods. Statistical analysis was performed using the SPSS 11.0 (SPSS, Inc., Chicago, IL). Associations between clinicopathological variables and Her2/neu status before and after treatment were evaluated using the χ^2 test. The paired samples *t* test was performed to compare the *Her2/neu*-to-chromosome 17 ratios before and after treatment. All *P* values reported were two-sided with $P < 0.05$ considered to be statistically significant. κ was estimated to evaluate concordance among Her2/neu assay methods.

RESULTS

The median age of the patients at initial diagnosis was 66 years, ranging from 48 to 84 years. Histology was as follows: invasive ductal carcinoma in 31 (86%) patients; mixed invasive ductal carcinoma and mucinous in 2 (5.6%) patients; and invasive lobular carcinoma, ductal carcinoma *in situ*, and mucinous in 1 (2.8%) patient each. The invasive ductal carcinoma was calculated as grade 1 (Bloom and Richardson grade) in 11 (35.5%) patients, grade 2 in 15 (48.4%) patients, and grade 3 in 5 (16.1%) patients. There was no significant correlation between Her2/neu and any of the clinicopathological variables, including histological type, grade, or disease.

All 36 enrolled patients were fully assessable for response. The overall response rate in the intent-to-treat population of 36 patients was 53%, with 3 complete responses (8%) and 16

Fig. 3 Her2/neu overexpression (3+) determined by immunohistochemistry, original magnification, ×200.
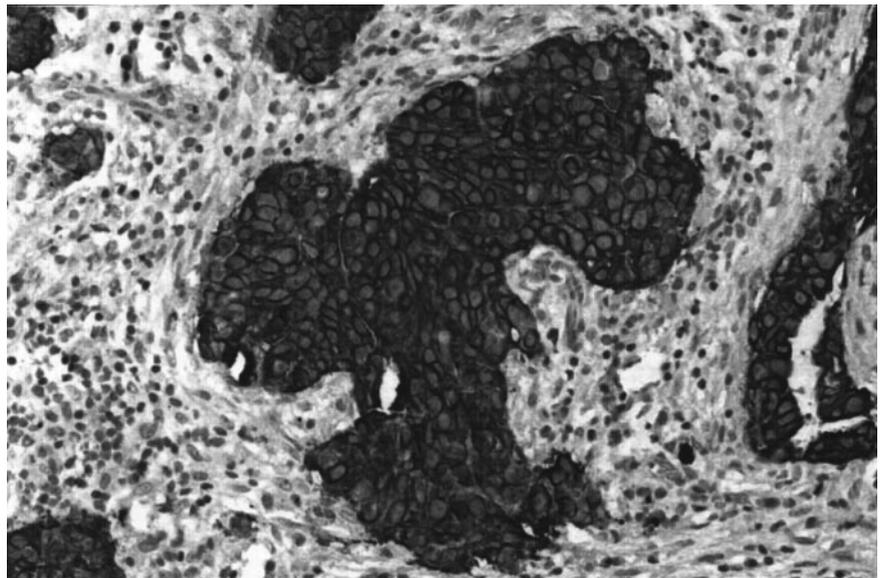
partial responses (45%). No changes were observed in 47% of assessable patients. Over the study period, none of the patients had direct disease progression without a period of stable disease. The percentage of patients with changes in tumor staging before and after the neoadjuvant therapy were compared (Fig. 1).

A total of 36 pairs of core biopsy and surgically excised tumors were available for IHC and FISH assays. Change in the distribution of Her2/neu IHC expression scores before and after therapy is shown in Fig. 2. Before therapy, 4 (11.1%), 16 (44.4%), 12 (33.4%), and 4 (11.1%) patients achieved scores of 0, 1+, 2+, 3+ (Fig. 3), respectively, yielding overexpression in 16 of the 36 patients (55.6%). In general, the IHC scores decreased after operation, except for 9 patients (Table 1). Thus, 5 of 36 (13.9%) of the patients had a Her2/neu expression of 2+ and 3+ after treatment, as compared with 16 of 36 (44.4%) before. Fig. 2 depicts the change in percentage of patients scored at each staining level before and after treatment by IHC assay. Using FISH, the *Her2/neu*-to-chromosome 17 ratios varied from 0.68 to 13.07. The ratios decreased significantly after the treatment. (t = 4.947, $P < 0.001$). *Her2/neu* amplification (Fig. 4) was determined in 20 patients (55.6%) before neoadjuvant therapy compared with 5 patients (13.9%) during surgery (Fig. 5). Concordance between the IHC and FISH results for the patients in whom data from both assays were available is listed in Table 2. The κ value of 0.875 suggests that there was excellent agreement between IHC and FISH in our population.

As shown in Table 3, the response rate to the treatment was significantly influenced by initial Her2/neu status, which was confirmed by FISH, with a response rate of 75% for Her2/neu (+) tumors and 35% for Her2/neu (−) tumors ($P = 0.017$). In addition, the response rate was also significantly affected by the decrease in Her2/neu status after the treatment, with a response rate of 73% for tumors showing decreased Her2/neu expression and 38% for tumors showing no change in Her2/neu expression ($P = 0.037$). There was no significant difference in either response rate ($P = 0.52$) or the change of Her2/neu expression ($P = 0.50$) between the Femara group and the Aromasin group.

DISCUSSION

Her2/neu positivity of breast cancer has been suggested that may be indicative of resistance to hormonal (predominantly tamoxifen) therapy, but the data are by no means conclusive (23–27). The heterogeneity of the published data may in part result from the ER status of the tumor not being considered. Much of the reported hormonal insensitivity of Her2/neu (+) tumors could result from ER (−) rather than Her2/neu (+) *per se* (27). So in this study, we had an attempt to solve this problem by selecting an entirely ER (+) group of patients.

Currently, no single assay is globally accepted as the gold standard for Her2/neu testing. Of a wide range of techniques, two technologies are now predominant in the routine clinical practice: determination of Her2/neu protein overexpression by IHC; and *Her2/neu* gene amplification by FISH (28). After directly comparing parallel IHC and FISH assessment of the same samples (29–37), some studies suggest the combination of these two assays provide comprehensive and valuable information on both Her2/neu protein concentrations and gene amplification (29–31). We also conducted both of these methods to help us make crucial management decisions. The variables produced by clonal selection using IHC can be overcome by detection of *Her2/neu* copy number. Although there is high

Table 1 Change of Her2/neu status before and after treatment

	Change of Her2/neu status	No. of patients (%)
IHC[a]	No change	8 (22.2)
	1+ → 0	15 (41.7)
	2+ → 0	4 (11.1)
	2+ → 1+	7 (19.4)
	2+ → 3+	1 (2.8)
	3+ → 2+	1 (2.8)
FISH	No change	21 (58.3)
	Amplification → nonamplification	15 (41.7)

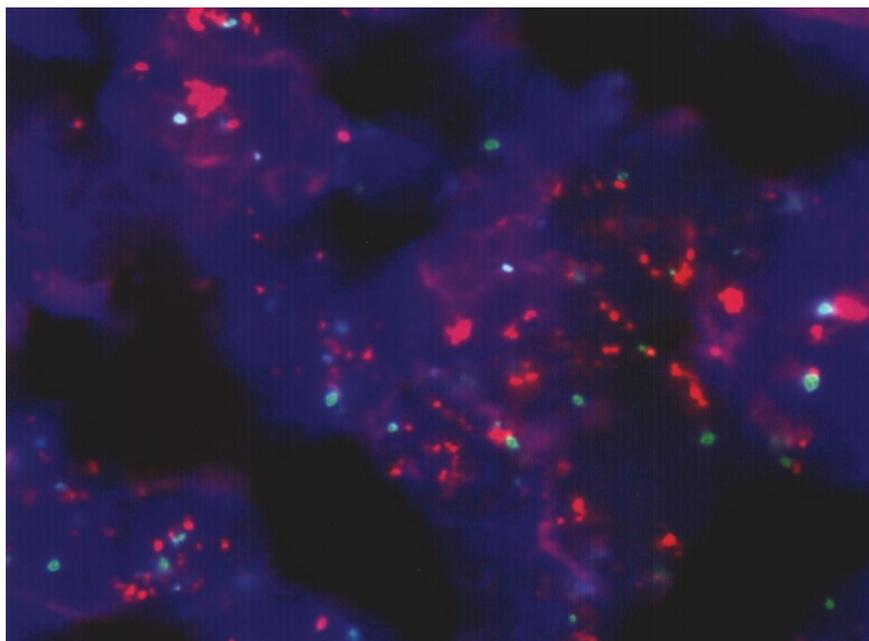[a] IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization.

Fig. 4 *Her2/neu* amplification determined by fluorescence *in situ* hybridization, ×400.

concordance of IHC and FISH results in our group ($\kappa = 0.875$), discrepancies are still known to occur because of the transcriptional or posttranscriptional regulation for increased surface receptor expression in the absence of gene amplification (32).

We have evaluated 36 primary breast cancers to determine whether Her2/neu status changes after the neoadjuvant endocrine therapy. Overexpression and amplification of Her2/neu was 44.4 and 55.6%, respectively. Our findings indicate a higher level of overexpression of Her2/neu in other groups with the primary diagnosis of breast cancers (1–4), which is possibly due

Table 2 Concordance of Her2/neu assay results: IHC *versus* FISH[a]

	FISH (No. of patients)		
IHC score	Nonamplified	Amplified	Total (no. of patients)
0–1+	46	4	50
2+–3+	0	22	22
Total	46	26	72

Note. Kappa = 0.875.
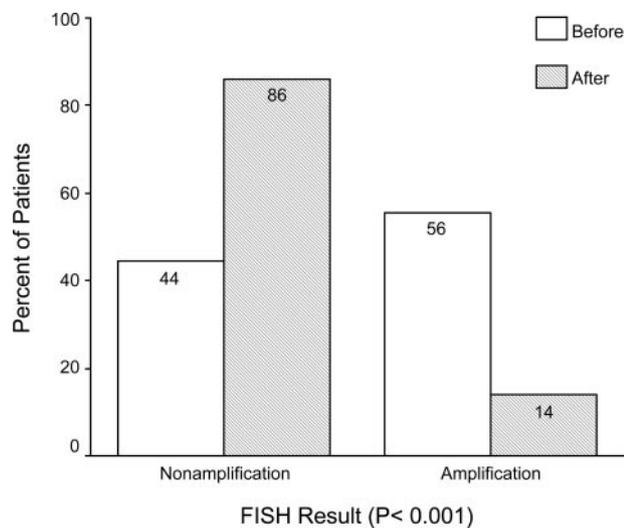[a] IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization.

Fig. 5 The comparison of fluorescence *in situ* hybridization (FISH) results of Her2/neu status before and after neoadjuvant endocrine therapy. The amplification of Her2/neu are decreased significantly after the treatment ($P < 0.001$). □, before; ▦, after.

to the patients included in our group having developed more aggressive tumors (38, 39). Although we didn't find any significant correlation between Her2/neu positivity and clinicopathological factors, which might be due to the small size of our cohort, a statistically significant decrease in positivity for Her2/neu has been shown after neoadjuvant endocrine therapy.

To date, there have been few controlled studies of neoadjuvant endocrine therapy, especially for the use of the new selective third generation aromatase inhibitors. In appropriately selected patients, the Edinburgh group indicated that neoadjuvant endocrine therapy also produces significant responses compared with the preoperative chemotherapy (40). The overall response rates were 78–96%. The Duke group has shown that letrozole produces a superior response rate to tamoxifen (60 *versus* 48%), and the differences in response rates between letrozole and tamoxifen were most marked for Her2/neu (+) tumors (88 *versus* 21%), whereas Her2/neu (−) tumors did not show a statistically significantly higher response rate for letrozole compared with tamoxifen (54 *versus* 42%; Ref. 10). Although our study showed a slightly lower clinical response rate of 53%, we can confirm that Her2/neu (+) tumors show a significantly higher response rate than their Her2/neu (−)

Table 3 Clinical response according to initial and decrease of Her2/neu status

		No. of responders/Total	Response rate (%)	*P*
Initial Her2/neu status	(+)	12/16	75	0.017
(FISH)[a]	(−)	7/20	35	
Decrease of Her2/neu status	(+)	11/15	73	0.037
(before and after therapy)	(−)	8/21	38	

[a] FISH, fluorescence *in situ* hybridization.

counterparts. This remarkable finding suggests that the Her2/neu-activated second messenger pathway mediates estrogen-dependent growth through ER, presumably via ER phosphorylation. Preclinical modeling is consistent with the conclusion that ER (+) and Her2/neu (+) tumors are highly estrogen dependent (41). Additional data also indicate that ER-dependent transcriptional activity in a Her2/neu (+) breast cell line can be impeded by estrogen deprivation caused by aromatase inhibitors (42), which would suggest that a higher sensitivity to these agents might exist in breast cancer.

More interestingly, we found that the amplification of *Her2/neu* decreased in 41.7% of patients after the therapy by FISH. These results suggest that aromatase inhibitors might frequently repress the aggressive nature of breast cancer. The mechanism of aromatase inhibitor-induced Her2/neu down-regulation is unclear and extremely variable (43). A molecular explanation for these findings might be related to inactivation of signal transduction of Her2/neu through the mitogen-activated protein kinase pathway. Additional exploration of the molecular mechanism underlying this phenomenon may prove very useful in explaining and in controlling breast cancer progression in the future. Most importantly, we found that tumors, which show decreasing Her2/neu expression during the treatment, also show a significantly higher response rate than tumors, which show no change of Her2/neu expression. These remarkable observations suggest that positive Her2/neu status and a decrease in Her2/neu expression became significantly sensitive markers for the neoadjuvant endocrine therapy based on aromatase inhibitors. Because it generally takes longer for endocrine therapy than chemotherapy to act, it seems essential to identify nonresponse early in the course of treatment so that the patient can be transferred to alternative therapies. Defining the best way to monitor response is therefore of fundamental importance. The determination of Her2/neu status by repeated biopsy during the therapy may be carried out in the future study. Nevertheless, our study has attempted to explore the role of a decrease in Her2/neu expression as a predictive marker for neoadjuvant endocrine therapy.

In conclusion, we have shown that Her2/neu gene amplification and protein expression decrease after neoadjuvant therapy using aromatase inhibitors. Despite the limited size of the cohort and immature survival data, our findings that both the positive Her2/neu expression and a decrease in Her2/neu expression have a predictive value with respect to the treatment could be clinically relevant.

ACKNOWLEDGMENTS

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