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Loss of *HER2* Amplification Following Trastuzumab-Based Neoadjuvant Systemic Therapy and Survival Outcomes

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Abstract

Purpose: To evaluate *HER2* status in residual tumor identified at the time of surgery in patients not achieving a pathologic complete response (pCR) and to determine the effect of alterations in *HER2* status on recurrence-free survival (RFS).

Experimental Design: Clinicopathologic data for patients with HER2-overexpressing breast cancer receiving neoadjuvant therapy with a taxane, anthracycline, and concomitant trastuzumab between 2004 and 2007 were reviewed. Surgical specimens for patients achieving less than a pCR were assessed to determine if there was enough residual tissue to evaluate posttreatment *HER2* status. RFS was determined using the Kaplan-Meier method and compared by the log-rank statistic.

Results: A pCR was achieved in 72 of the 142 (50.7%) patients. Residual tumor was sufficient to assess posttreatment *HER2* status in 25 patients. Fluorescence *in situ* hybridization done on pretreatment specimens confirmed *HER2* amplification before beginning therapy. Eight (32.0%) posttreatment tumors were found to be *HER2*-negative by fluorescence *in situ* hybridization. At a median follow-up of 37 months (range, 8-56 months), the RFS was significantly better for patients with tumors that retained *HER2* amplification (87.5% versus 50%, $P = 0.04$).

Conclusion: High pCR rates are achieved in patients with HER2-positive breast cancer treated with neoadjuvant trastuzumab in combination with anthracyclines and taxanes. One third of patients with significant residual disease loses *HER2* amplification, and this change is associated with poor RFS. Residual tumor identified at the time of surgery should be reassessed for HER2 status, and novel adjuvant therapy strategies need to be studied in this population. (Clin Cancer Res 2009;15(23):7381–8)

The *HER2/neu* (*HER2*) gene is amplified in ~25% of breast cancers (1). Gene amplification results in overexpression of the HER2 protein, which is associated with an aggressive clinical course to include a shorter disease-free interval after adjuvant therapy and decreased overall survival (OS; refs. 2–4). The natural history of HER2-overexpressing breast cancer has been altered, however, by the routine use of trastuzumab, a monoclonal antibody targeting the extracellular domain of the HER2 protein. Trastuzumab has been shown to improve survival in patients with metastatic HER2-positive breast cancer (5, 6) as well as in patients with earlier stage disease. Several large, multicenter adjuvant therapy trials showed that the addition of trastuzumab to systemic chemotherapy reduces recurrence by ~50% and improves OS by 30% (7, 8). Trastuzumab has also been shown to be efficacious when given in the neoadjuvant setting with pathologic complete response (pCR) rates ranging from 7% to as high as 65% in patients with both early and locally advanced breast cancer (9–14). Despite these successes with trastuzumab therapy, not all HER2-positive tumors respond and some patients whose tumors do respond will experience disease recurrence. Investigators from our group recently reported a case of a patient with HER2-positive breast cancer who received adjuvant trastuzumab but relapsed with HER2-negative metastatic disease (15). In a study conducted to evaluate changes in HER2 status in metastatic lesions of patients previously treated with trastuzumab, Pectasides et al. showed that 37% of patients no longer had HER2 expression/amplification, and these patients had significantly shorter time

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Translational Relevance

This study confirmed that patients with HER2-overexpressing breast cancer treated in the neoadjuvant setting with trastuzumab-based systemic therapy achieve a high rate (~50%) of pathologic complete response. Importantly, in patients not achieving a pathologic complete response who had significant residual disease, fluorescence *in situ* hybridization showed that the tumors from one third of these patients no longer had amplification of the *HER2* gene. Those patients with tumors that were no longer *HER2* amplified had a significantly worse recurrence-free survival than those with tumors that retained HER2 amplification. Taken together, these data suggest that residual tumor identified at the time of surgery in patients receiving trastuzumab-based neoadjuvant therapy should be reassessed for HER2 status and that novel adjuvant therapy strategies need to be studied in this population.

to tumor progression than the group who remained HER2-positive (16).

The purpose of the current study was to evaluate *HER2* gene amplification status using fluorescence *in situ* hybridization (FISH) in the residual tumors of patients who received neoadjuvant systemic therapy with paclitaxel and FEC (5-fluorouracil, epirubicin, and cyclophosphamide) with concomitant weekly trastuzumab. We also sought to determine the effect of changes in *HER2* status on recurrence-free survival (RFS).

Materials and Methods

Cell lines and treatments. The BT-474 cell line was purchased from the American Type Culture Collection. Cells were maintained in DMEM/Ham F12 1:1 (DMEM/F12) supplemented with 10% fetal bovine serum and 2 mmol/L L-glutamine (Life Technologies, Inc. Ltd.) at 37°C in 5% CO_2. Trastuzumab (Herceptin; kindly provided by F. Hoffmann, La Roche) was dissolved in sterile apyrogen water and stored at 4°C. Trastuzumab-resistant BT-474 (BT-474R) cells were obtained by culturing the parental BT-474 cells in the presence of increasing concentrations of trastuzumab (up to 500 nmol/L) for >18 mo. Genetic analysis was done using single nucleotide polymorphism (SNP) arrays on the clones and parental cell lines. Protein extraction, Western blot, and immunohistochemistry were done as previously described (17).

Patient selection. The Department of Breast Medical Oncology database was queried to identify patients with histologically confirmed, HER2-overexpressing (defined as immunohistochemical 3+) or amplified (FISH positive), nonmetastatic, invasive breast cancer who received the neoadjuvant systemic chemotherapy-based regimen with concomitant trastuzumab described below. Patient and tumor characteristics including age at diagnosis, presenting clinical stage, histology, nuclear grade, estrogen receptor (ER) and progesterone receptor (PR) status, presence or absence of lymphovascular invasion, type of surgery, and pathologic response in the breast and axilla were recorded. Follow-up data was updated through January 2009. The University of Texas M.D. Anderson Cancer Center Institutional Review Board approved this study.

Pathology. The breast cancer diagnosis was confirmed by a review of core biopsy material by dedicated breast pathologists. The histologic subtype of all tumors was defined according to the WHO classification system (18), and the modified Black's nuclear grading system was used (19). Immunohistochemical analysis was done to determine ER and PR status. Nuclear staining of ≥10% was considered positive. HER2 status was evaluated by immunohistochemistry and further confirmed by FISH in tissue obtained before initiation of neoadjuvant chemotherapy. Interpretations of these assays were based on the most recent American Society of Clinical Oncology/College of American Pathologists guidelines (20).

FISH analysis of breast carcinoma was done using the PathVysion HER-2 DNA probe kit (Vysis, Inc.). Briefly, this assay uses two directly labeled fluorescent DNA probes that specifically target the *HER2* locus and CEP17, the α-satellite DNA sequence at the centromeric region of the chromosome. For the pretreatment biopsy specimens, all areas of invasive tumor were screened under a fluorescent microscope to evaluate the possibility of heterogeneity among tumor cells. No heterogeneity was identified. Sixty tumor cells (versus 20 cells as per the manufacturer's recommendation) in each case were then scored for HER2 and CEP17 signals. Among the posttreatment specimens, we scored all tumors cells identified up to 60 when present. For cases with reduced residual tumor cell density due to treatment response, we scored a minimum of 20 tumor cells for HER2 and CEP 17 signals. A FISH ratio (*HER2* gene signals to chromosome 17 signals) was determined and if >2.2 was considered positive.

A pCR was defined as no residual invasive disease in the breast and axilla on final pathologic assessment. For patients achieving less than a pCR who had enough residual tumor tissue, a dedicated breast pathologist (Y.W.) reassessed HER2 status in the pretreatment biopsy specimen and in the posttreatment residual tumor using FISH (described above) to determine if *HER2* gene amplification was present.

Treatment. Paclitaxel was given weekly for 12 wk at a dose of 80 mg/m^2/wk i.v. This was followed by four cycles of FEC_{75} (fluorouracil 500 mg/m^2 epirubicin 75 mg/m^2, cyclophosphamide 500 mg/m^2 i.v., given the first day of each cycle) given every 3 wk. Trastuzumab was given as a loading dose of 4 mg/kg i.v. on the first day and then subsequently given weekly at a dose of 2 mg/kg concomitantly with both the anthracycline and taxane chemotherapy. After completion of neoadjuvant systemic therapy, patients underwent appropriate surgery with either a segmental or total mastectomy. The axillary lymph nodes were assessed with sentinel lymph node biopsy for patients who presented initially with node-negative disease and with axillary lymph node dissection for patients who were documented to have axillary lymph node metastasis before beginning neoadjuvant systemic therapy. Surgery was followed by radiation therapy when indicated and appropriate endocrine therapy for patients with hormone receptor–positive disease. Trastuzumab was continued to complete 1 y of therapy.

Statistical analysis. Patient characteristics were tabulated or described by their median and range overall, by pCR group, and by post-neoadjuvant chemotherapy *HER2* status group. The χ^2 test or Wilcoxon rank sum test was used as appropriate to determine associations between patient characteristics. Median follow-up time was calculated as the median observation time among all patients. Recurrence was defined as recurrence of disease in either local, regional, or distant sites. RFS was defined as the time from diagnosis to the time of first recurrence or last follow-up. Survival distributions were estimated with the Kaplan-Meier method, and the log-rank statistic was used to compare the differences between groups.

Results

Between June 2003 and May 2007, 142 HER2-positive patients were treated with the concomitant trastuzumab and neoadjuvant systemic therapy regimen. Table 1 lists patient characteristics overall and by whether they experienced a pCR. Seventy-two (50.7%) patients achieved a pCR. From the 70 patients with residual disease, 61 (43.0%) had a partial response to neoadjuvant chemotherapy, 6 (4.2%) had stable disease, and 3 (2.1%) had progression of disease. Compared with patients with residual disease, patients who had a pCR were more likely to have ductal histology (versus lobular or mixed ductal/lobular;

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Table 1. Patient characteristics overall and by pCR

	Overall	pCR		*P*
		No	Yes	
	n	*n* (%)	*n* (%)	
	142	70	72	
Race				
Black	23	11 (15.7%)	12 (16.7%)	
Spanish/Hispanic	29	15 (21.4%)	14 (19.4%)	
White	84	43 (61.4%)	41 (56.9%)	
Asian/Pacific Islander	6	1 (1.4%)	5 (6.9%)	0.480
Age at diagnosis, y				
Median (range)	50 (21-81)	48 (25-74)	52 (21-81)	0.0954
Histology				
Ductal	133	64 (91.4%)	69 (95.8%)	
Other*	9	6 (8.6%)	3 (4.2%)	<0.0001
Clinical T stage				
T1	23	7 (10.0%)	16 (20.8%)	
T2	71	34 (48.6%)	37 (51.4%)	
T3	23	15 (21.4%)	8 (11.1%)	
T4	25	14 (20.0%)	11 (15.3%)	0.138
Clinical N stage				
N0	45	20 (28.6%)	25 (34.7%)	
N1	60	32 (45.7%)	28 (38.9%)	
N2	4	1 (1.4%)	3 (4.2%)	
N3	33	17 (24.3%)	16 (22.2%)	0.633
Clinical stage				
I	5	1 (1.4%)	4 (5.6%)	
II	75	38 (54.3%)	37 (51.4%)	
III	62	31 (44.3%)	31 (43.0%)	0.513
Nuclear grade				
II	33	20 (28.6%)	13 (18.1%)	
III	106	49 (70.0%)	57 (79.2%)	
Not reported	3	1 (1.4%)	2 (2.8%)	0.214
LVI				
Positive	28	21 (30.0%)	7 (9.7%)	
Negative	114	49 (70.0%)	65 (90.3%)	0.005
ER				
Positive	68	40 (57.1%)	28 (38.9%)	
Negative	74	30 (42.9%)	44 (61.1%)	0.045
PR				
Positive	50	31 (44.3%)	19 (26.4%)	
Negative	91	39 (55.7%)	52 (72.2%)	
Not reported	1	0	1 (1.4%)	0.046

Abbreviation: LVI, lymphovascular invasion.
*Includes lobular (n = 3) and mixed ductal/lobular (n = 6) histology.

$P < 0.0001$), absence of lymphovascular invasion ($P = 0.005$), and hormone receptor–negative tumors ($P = 0.045$ for ER; $P = 0.046$ for PR).

The majority of patients who did not achieve a pCR had a near complete response with only minimal residual disease, such as scattered tumor cells in the primary tumor site or lymph node or minimal cellularity in the surgical specimens. In these patients, *HER2* status could not be reassessed. However, in 25 patients achieving less than a pCR, enough residual tissue was available at the time of surgery to reassess *HER2* status by FISH. Eight (32.0%) of these patients had tumors that lost *HER2* amplification. To confirm that these patients had *HER2* gene amplified tumors before receiving the concomitant trastuzumab and neoadjuvant chemotherapy regimen, FISH was repeated on their pretreatment biopsy specimens and homogeneous *HER2* amplification was confirmed in all cases (Table 2; Fig. 1). Twenty patients had enough residual disease to reassess ER status to compare with pretreatment ER status. Four (20%) patients had tumors that converted from ER-negative to ER-positive disease. When comparing patients with tumors that lost *HER2* gene amplification (n = 8) with those with tumors that remained *HER2*-amplified (n = 17), there were no significant differences in clinicopathologic features associated with conversion of *HER2* status (Table 3).

The median follow-up for the entire population was 33.5 months (range, 8-65 months). Patients achieving a pCR had significantly better RFS compared with patients who did not achieve a pCR ($P = 0.0175$; Fig. 2A). The 3- and 5-year RFS estimate for all patients and the 3-year RFS estimate for those who achieved a pCR versus those who did not achieve a pCR are listed in Table 4. The median follow-up for the patients who achieved less than a pCR and had enough residual tumor tissue to reassess *HER2* status was 37 months (range, 8-56 months). Analysis of these patients showed that patients who retained *HER2* gene amplification had significantly better RFS compared with patients whose tumors lost *HER2* gene amplification

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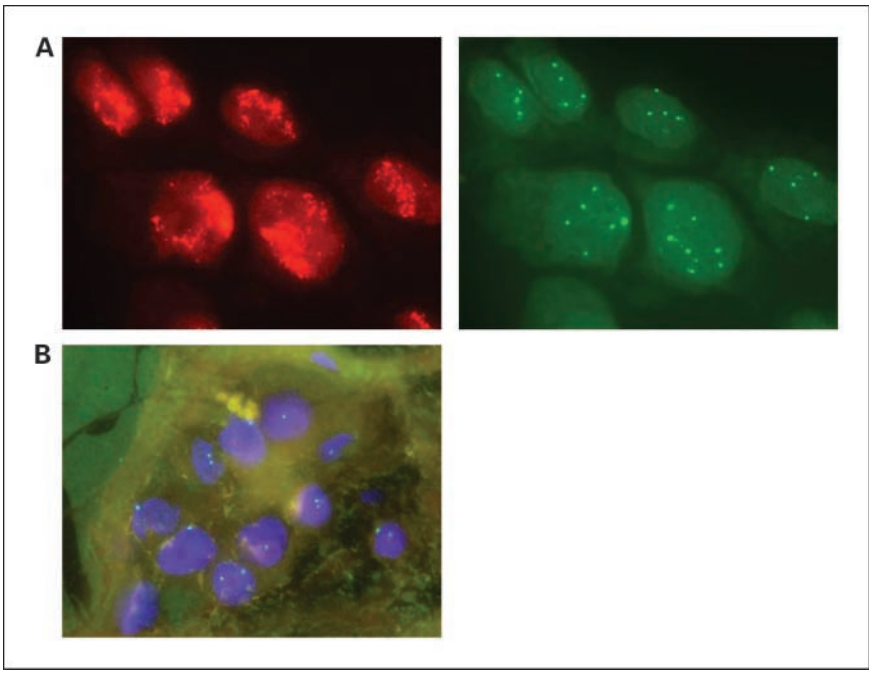

Fig. 1. FISH was done to assess *HER2* status. *A,* FISH done on biopsy specimen before treatment with a trastuzumab containing neoadjuvant chemotherapy regimen. Red, HER2 gene; green, CEP17 (centromere of chromosome 17). HER2/CEP17 = 6.22. Due to the intensity of HER2 staining, merged images were not obtained. *B,* FISH done on residual disease identified at the time of surgery from the same patient after completion of neoadjuvant chemotherapy. Image shown is a merged image of staining for HER2 and CEP17. HER2/CEP17 = 1.1.

($P = 0.041$; Fig. 2B). The 3-year RFS estimates for patients whose tumors retained *HER2* amplification was 87.5% [95% confidence interval (95% CI), 72.7-100%] versus 50.0% (95% CI, 25.0-100%) for those that did not (Table 4).

There have been eight deaths in the entire cohort, two in the group of patients who achieved a pCR versus six in the group achieving less than a pCR ($P = 0.137$). In the group of 25 patients that had enough residual disease to reassess *HER2* status,

Table 2. HER2 gene amplification and hormone receptor status following trastuzumab containing neoadjuvant chemotherapy in patients with enough residual disease identified at the time of surgery to reassess HER2 status

Patient no.	HER2 FISH ratio pretreatment	HER2 FISH ratio posttreatment	ER* pretreatment	ER posttreatment
1	**3.17**	**1.96**	**NEG**	**NEG**
2	6.06	4.78	POS	POS
3	POS (Aneuploid)†	POS (Aneuploid)†	POS	POS
4	7.19	6.22	NEG	POS
5	**3.70**	**1.94**	**NEG**	**NEG**
6	**2.88**	**1.24**	**POS**	**N/A**
7	5.06	5.02	POS	POS
8	5.26	5.26	POS	POS
9	5.48	4.46	POS	POS
10	**5.41**	**1.32**	**NEG**	**POS**
11	**5.04**	**1.26**	**NEG**	**N/A**
12	13.79	6.23	POS	POS
13	4.70	4.25	NEG	POS
14	11.63	9.63	POS	POS
15	2.39	2.42	POS	POS
16	**6.22**	**1.23**	**NEG**	**NEG**
17	4.26	4.22	NEG	NEG
18	**11.65**	**1.28**	**POS**	**N/A**
19	8.74	6.56	POS	POS
20	6.52	4.26	POS	N/A
21	3.87	3.56	NEG	NEG
22	2.56	2.61	NEG	POS
23	6.82	7.12	POS	POS
24	**2.96**	**1.29**	**NEG**	**NEG**
25	2.78	2.38	POS	N/A

NOTE: Patients who lost *HER2* amplification are identified in bold.
Abbreviations: POS, positive; NEG, negative; N/A, not enough residual tumor available to assess.
*ER status was determined by immunohistochemical analysis. Nuclear staining ≥ 10% was considered positive.
†Due to marked aneuploidy of tumor cells and clustering of signals, HER2/neu and CEP17 signals could not be accurately counted; however, there was at least a 2-fold increase in the number of signals for HER2/neu compared with CEP17.

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Table 3. Patient characteristics by *HER2* status following trastuzumab containing primary chemotherapy

	***HER2* not amplified**	**HER2 amplified**	***P***
	***n* (%)**	***n* (%)**	
	8	17	
Race			
Black	1 (12.5%)	3 (17.7%)	
Spanish/Hispanic	2 (25.0%)	17.7%	
White	5 (62.5%)	11 (64.7%)	1
Age at diagnosis, y			
Min	40 (–)	30 (–)	
Median	49 (–)	50 (–)	
Max	67 (–)	61 (–)	0.777
Histology			
Ductal	7 (87.5%)	14 (82.3%)	
Other	1 (12.5%)	3 (17.7%)	1
Clinical T stage			
T1	1 (12.5%)	3 (17.7%)	
T2	3 (37.5%)	7 (41.2%)	
T3	0 (0.0%)	5 (29.4%)	
T4	4 (50.0%)	2 (11.8%)	0.143
Clinical N stage			
N0	2 (25.0%)	6 (35.3%)	
N1	2 (25.0%)	9 (52.9%)	
N3	4 (50.0%)	2 (11.8%)	0.186
Clinical stage			
I	0 (0.0%)	1 (5.9%)	
II	3 (37.5%)	9 (52.9%)	
III	5 (62.5%)	7 (41.2%)	0.774
Nuclear grade			
II	2 (25.0%)	5 (29.4%)	
III	6 (75.0%)	12 (70.6%)	1
LVI			
Positive	3 (37.5%)	5 (29.4%)	
Negative	5 (62.5%)	12 (70.6%)	1
ER			
Positive	2 (25.0%)	12 (70.6%)	
Negative	6 (75.0%)	5 (29.4%)	0.081
PR			
Positive	2 (25.0%)	11 (64.7%)	
Negative	6 (75.0%)	6 (35.3%)	0.097

Abbreviation: LVI, lymphovascular invasion.

there has been one death, which occurred in a patient whose tumor had lost *HER2* amplification.

To investigate the hypothesis that trastuzumab treatment could play a causative role in selecting *HER2*-negative (without gene amplification) cells within a population of HER2-positive (with gene amplification) cells, we cultivated HER2 positive BT-474 breast cancer cells in the presence of increasing concentrations of trastuzumab for >18 months isolating several independent subclones. After this period of time, we found that two independent clones treated continuously with trastuzumab (BT-474R) had lost both HER2 overexpression and *HER2* gene amplification (Fig. 3) and had acquired resistance to the antiproliferative activity of trastuzumab *in vitro* (data not shown).

Discussion

Patients with HER2-overexpressing breast cancer treated with trastuzumab-based neoadjuvant systemic therapy achieve a high rate of pCR. In the current study, the pCR rate was 51% after treatment with a neoadjuvant regimen that included taxane and anthracycline-based chemotherapy used concurrently with weekly trastuzumab for 24 weeks. The majority of patients not achieving a pCR had very minimal residual disease (near complete response), with only a third of patients having enough tumor tissue identified at the time of surgery to reassess *HER2* status. Importantly, one third of patients who had enough residual disease to repeat *HER2* testing had lost amplification of the *HER2* gene. Patients who had enough residual disease to reassess *HER2* status and had lost *HER2* gene amplification had a significantly decreased RFS compared with patients whose tumors remained *HER2* amplified.

Other investigators have evaluated HER2 expression in paired samples of pretreatment and posttreatment tissue from patients treated with trastuzumab in the neoadjuvant setting. Burstein et al. reported on HER2 status in patients with residual tumor after treatment with 12 weeks of paclitaxel and trastuzumab (9). Their trial enrolled 40 patients, 23 of whom had residual tissue available for HER2 testing by immunohistochemistry. In six (26.1%) cases, all of whom were immunohistochemical 3+ before treatment, the HER2 status changed to 2+ in two patients and 0 in four patients. In a phase II study of 48 patients treated with 12 weeks of neoadjuvant trastuzumab and vinorelbine, Harris et al. reported a HER2 conversion rate of 12% in 18 patients with enough residual tissue to repeat HER2 testing by immunohistochemistry (12). Although the concordance between HER2 overexpression detected by immunohistochemistry and *HER2* gene amplification by FISH has been shown to be statistically significant (21–23), there are issues regarding consistency in immunohistochemistry testing that may affect results, including variable fixation, antigen retrieval methods, and observer analysis (24). In addition, FISH has been shown to be more reproducible than immunohistochemistry between central and peripheral laboratories (22, 25). Because we used FISH to determine *HER2* gene amplification status pretreatment and posttreatment, we are confident that the changes in *HER2* status are not due to artifact or inconsistent testing. Consistent with our findings, Hurley et al. showed that 43% of tumors that had *HER2* gene amplification by FISH before treatment with neoadjuvant trastuzumab, docetaxel, and cisplatin became FISH-negative after therapy (13).

It is unclear whether this change reflects response to therapy or a mechanism of resistance. It is possible that a change in *HER2* status could reflect the heterogeneity of HER2 expression within the tumor, suggesting that trastuzumab eliminated HER2-overexpressing clones leaving only HER2-negative tumor cells upon completion of therapy. The results obtained with our preclinical model based on BT-474 cells that acquired resistance to trastuzumab support this possibility. It seems likely that the change in HER2 status reflects treatment of HER2-overexpressing clones, and one could speculate that the trastuzumab therapy was effective in treating the HER2-amplified cells in over 65% of tumors, the 50% that achieved a pCR, and the 15% that became HER2 negative.

Another interesting finding from our analysis is that four patients whose tumors were ER-negative pretreatment were found to be ER positive when residual tumor tissue was examined. Previous reports have described cross talk between the ER and the HER2 pathways, and studies have suggested an association between HER2 signaling and resistance to antiestrogens in

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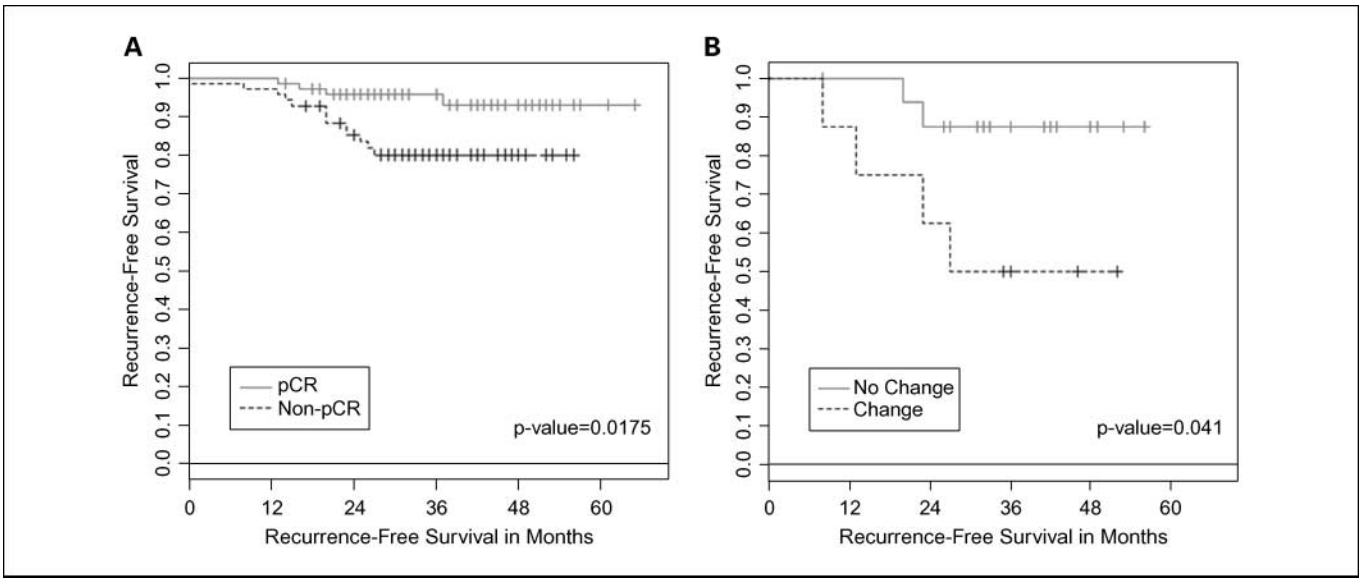

Fig. 2. Kaplan-Meier plots of RFS by (*A*) pCR and (*B*) status of *HER2* gene amplification in patients with residual tissue identified at the time of surgery.

human breast cancer (26–28). Whereas we acknowledge that the current study reports a small number of patients, the findings suggest that, in some patients with HER2-overexpressing, ER-negative breast cancer treatment with trastuzumab may facilitate sensitivity to antiestrogen therapy by upregulating ER expression. This finding requires further confirmation in a larger cohort of patients, but given the potential therapeutic implications, we recommend that residual tumor tissue identified in patients treated with concurrent trastuzumab and neoadjuvant chemotherapy be reassessed for HER2 and ER status.

Studies incorporating trastuzumab into neoadjuvant chemotherapy regimens have reported pCR rates ranging from 17% to 65% (9–14). One explanation for the high pCR rates using such regimens is the use of two potentially non–cross-resistant chemotherapy agents given sequentially in combination with trastuzumab. This concept is supported by data from the NOAH (NeOAdjuvant Herceptin) trial, which randomized women with HER2-overexpressing locally advanced breast cancer or inflammatory breast cancer to receive doxorubicin, paclitaxel, and cyclophosphamide, methotrexate, and fluorouracil (CMF)-based neoadjuvant systemic therapy with or without concomitant trastuzumab. This trial enrolled 327 women, and the pCR rates were significantly higher in trastuzumab-treated patients (39% versus 20%, $P = 0.002$; ref. 14). This lower pCR rate compared with our patient cohort may be due to differences in presenting disease stage. An earlier study also focusing on locally advanced and inflammatory HER2-positive disease given 12 weeks of docetaxel, cisplatin, and trastuzumab in 48 patients and reported a pCR rate of 23% in the breast and 17% in the breast and axilla (13). It is difficult to compare pCR rates between trials due to differences in presenting clinical stage, regimens used, and duration of therapy, as well as differing definitions of pCR. However, because the NOAH trial and the trial reported by Hurley et al. enrolled similar patient populations, the differences in the pCR rates are interesting and suggest that the duration of therapy and the use of an anthracycline may be important determining factors for pCR. Currently, the American College of Surgeons Oncology Group is leading a large, multicenter trial (ACOSOG Z1041) comparing a neoadjuvant regimen of FEC_{75} followed by paclitaxel plus trastuzumab with a neoadjuvant regimen of paclitaxel plus trastuzumab followed by FEC_{75} plus trastuzumab in patients with HER2-overexpressing breast cancer. Results from this trial should provide conclusive data regarding the utility of administering trastuzumab concurrently with an anthracycline in the neoadjuvant setting.

Table 4. Kaplan-Meier estimates of RFS among all patients by pCR and by *HER2* status in patients with residual tissue identified at the time of surgery

Status	No. patients	No. events	Median follow-up time (mo)	3-y estimates		5-y estimates		*P*
				%	95% CI	%	95% CI	
Overall	142	17	33.5	87.8	82.4-93.6	86.20	80.1-92.8	
pCR			33.5					
Yes	72	4		95.7	91.0-100	92.90	86.0-100	
No	70	13		80.1	70.8-90.5	—		0.0175
HER2 Status in Residual Tissue	25	6	37.0	74.9	59.4-94.5	—	—	
Amplified	17	2		87.5	72.7-100	—	—	
Not Amplified	8	4		50.0	25.0-100	—	—	0.041

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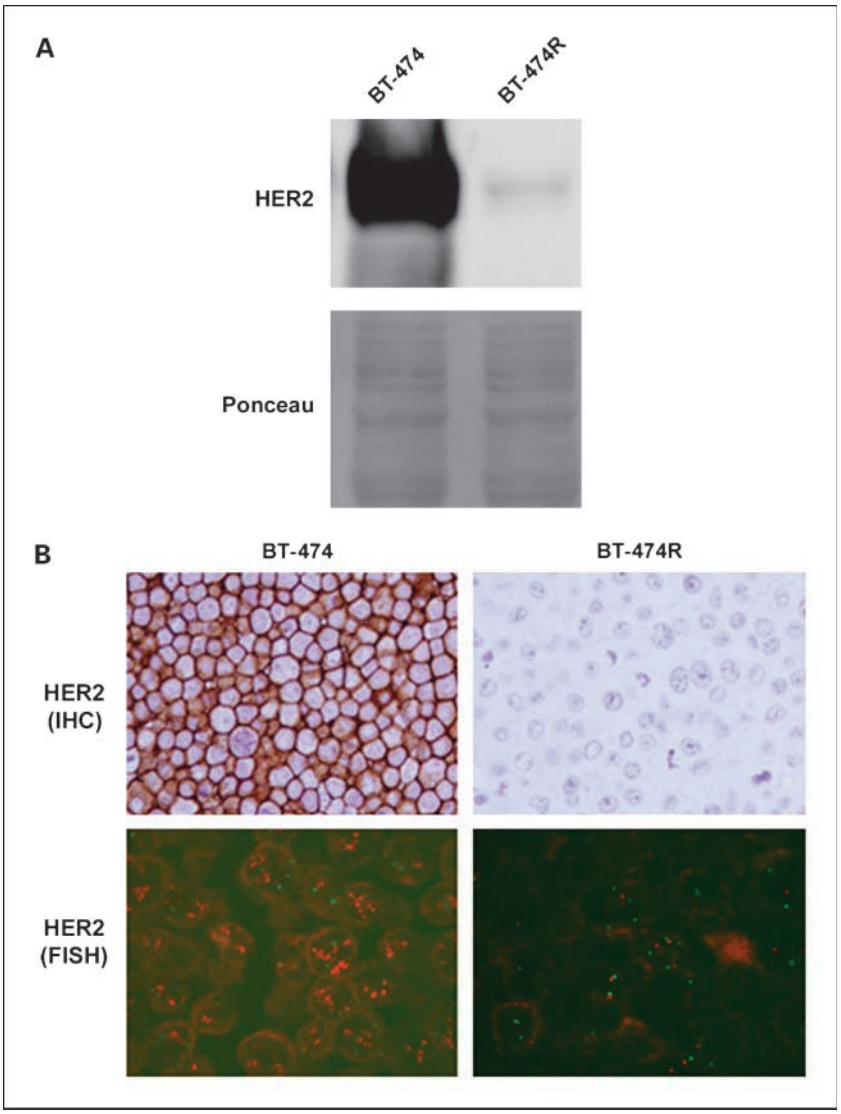

Fig. 3. Loss of HER2 overexpression and amplification in BT-474R cells. *A,* Western blot showing loss of HER2 overexpression in a representative clone of BT-474R cells. Ponceau staining serves as the loading control. *B,* loss of *HER2* overexpression by immunohistochemistry and loss of *HER2* gene amplification by FISH (red, HER2 gene; green, CEP17) of a representative clone of BT-474R cells.

Achieving a pCR is an important end point for patients receiving neoadjuvant systemic therapy, as it has been shown to correlate with long-term outcomes (13, 29). In the current study, we again show that achieving a pCR is associated with improved RFS. There was a trend toward improvement in OS, although this did not reach statistical significance, which we attribute to the relative short median follow-up time of 33.5 months. Importantly, a novel finding in the current study is the effect on RFS of loss of *HER2* gene amplification in patients with measurable residual disease after administration of trastuzumab. Patients whose tumors lost *HER2* gene amplification as determined by FISH analysis had a significantly worse RFS than those whose tumors remained *HER2* amplified.

In conclusion, we observed that approximately one third of patients with measurable residual disease after administration of a neoadjuvant systemic therapy regimen that included taxane/anthracycline-based chemotherapy used concurrently with weekly trastuzumab for 24 weeks lost *HER2* gene amplification. Our data show that this change affects RFS. Patients who had measurable residual disease and converted to HER2-negative disease had a significantly shorter RFS than patients who had measurable residual tumor but retained *HER2* gene amplification. This finding could have implications regarding additional adjuvant therapy. Currently, our practice is to administer trastuzumab postoperatively to complete 1 year of therapy based on data from the multicenter adjuvant trials (7, 8). If conversion of HER2 status reflects response to therapy, such that only HER2-negative clones remain, the need to complete 1 year of trastuzumab in the adjuvant setting comes into question. Furthermore, all patients with early-stage HER2-positive disease who relapse after adjuvant or neoadjuvant trastuzumab therapy should have biopsies of their recurrent disease and reassessment of their marker status, as we have shown that a change in marker status correlates with outcome in patients who develop metastatic disease (30). These data suggest that there may be utility in assessing HER2 status in residual disease identified at the time of surgery and that future clinical trials should be designed to investigate the most appropriate strategy for adjuvant therapy in these patients.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Slamon DJ, Godolphin W, Jones LA, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989;244:707–12.
2. Paik S, Hazan R, Fisher ER, et al. Pathologic findings from the National Surgical Adjuvant Breast and Bowel Project: prognostic significance of erbB-2 protein overexpression in primary breast cancer. J Clin Oncol 1990;8:103–12.
3. Press MF, Bernstein L, Thomas PA, et al. HER-2/neu gene amplification characterized by fluorescence *in situ* hybridization: poor prognosis in node-negative breast carcinomas. J Clin Oncol 1997;15:2894–904.
4. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987;235:177–82.
5. Cobleigh MA, Vogel CL, Tripathy D, et al. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 1999;17:2639–48.
6. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001;344:783–92.
7. Piccart-Gebhart MJ, Procter M, Leyland-Jones B, et al. Trastuzumab after adjuvant chemotherapy in HER2-positive breast cancer. N Engl J Med 2005;353:1659–72.
8. Romond EH, Perez EA, Bryant J, et al. Trastuzumab plus adjuvant chemotherapy for operable HER2-positive breast cancer. N Engl J Med 2005;353:1673–84.
9. Burstein HJ, Harris LN, Gelman R, et al. Preoperative therapy with trastuzumab and paclitaxel followed by sequential adjuvant doxorubicin/cyclophosphamide for HER2 overexpressing stage II or III breast cancer: a pilot study. J Clin Oncol 2003;21:46–53.
10. Buzdar AU, Ibrahim NK, Francis D, et al. Significantly higher pathologic complete remission rate after neoadjuvant therapy with trastuzumab, paclitaxel, and epirubicin chemotherapy: results of a randomized trial in human epidermal growth factor receptor 2-positive operable breast cancer. J Clin Oncol 2005;23:3676–85.
11. Buzdar AU, Valero V, Ibrahim NK, et al. Neoadjuvant therapy with paclitaxel followed by 5-fluorouracil, epirubicin, and cyclophosphamide chemotherapy and concurrent trastuzumab in human epidermal growth factor receptor 2-positive operable breast cancer: an update of the initial randomized study population and data of additional patients treated with the same regimen. Clin Cancer Res 2007;13:228–33.
12. Harris LN, You F, Schnitt SJ, et al. Predictors of resistance to preoperative trastuzumab and vinorelbine for HER2-positive early breast cancer. Clin Cancer Res 2007;13:1198–207.
13. Hurley J, Doliny P, Reis I, et al. Docetaxel, cisplatin, and trastuzumab as primary systemic therapy for human epidermal growth factor receptor 2-positive locally advanced breast cancer. J Clin Oncol 2006;24:1831–8.
14. Gianni L, Semiglazov V, Manikhas GM, et al. Neoadjuvant trastuzumab in locally advanced breast cancer (NOAH trial): Feasibility safety and antitumor effects. 2007, p. 131.
15. Dawood S, Resetkova E, Gonzalez-Angulo AM. Trastuzumab administration associated with change in HER2 status. Clin Breast Cancer 2008;8:366–9.
16. Pectasides D, Gaglia A, Arapantoni-Dadioti P, et al. HER-2/neu status of primary breast cancer and corresponding metastatic sites in patients with advanced breast cancer treated with trastuzumab-based therapy. Anticancer Res 2006;26:647–53.
17. Scaltriti M, Verma C, Guzman M, et al. Lapatinib, a HER2 tyrosine kinase inhibitor, induces stabilization and accumulation of HER2 and potentiates trastuzumab-dependent cell cytotoxicity. Oncogene 2009;28:803–14.
18. The World Organization. The World Health Organization histological typing of breast tumors-Second Edition. Am J Clin Pathol 1982;78:806–16.
19. Black MM, Speer FD. Nuclear structure in cancer tissues. Surg Gynecol Obstet 1957;105:97–102.
20. Wolff AC, Hammond ME, Schwartz JN, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. J Clin Oncol 2007;25:118–45.
21. Dybdal N, Leiberman G, Anderson S, et al. Determination of HER2 gene amplification by fluorescence *in situ* hybridization and concordance with the clinical trials immunohistochemical assay in women with metastatic breast cancer evaluated for treatment with trastuzumab. Breast Cancer Res Treat 2005;93:3–11.
22. Perez EA, Suman VJ, Davidson NE, et al. HER2 testing by local, central, and reference laboratories in specimens from the North Central Cancer Treatment Group N9831 intergroup adjuvant trial. J Clin Oncol 2006;24:3032–8.
23. Press MF, Sauter G, Bernstein L, et al. Diagnostic evaluation of HER-2 as a molecular target: an assessment of accuracy and reproducibility of laboratory testing in large, prospective, randomized clinical trials. Clin Cancer Res 2005;11:6598–607.
24. Sauter G, Lee J, Bartlett JM, et al. Guidelines for human epidermal growth factor receptor 2 testing: biologic and methodologic considerations. J Clin Oncol 2009;27:1323–33.
25. Paik S, Bryant J, Tan-Chiu E, et al. Real-world performance of HER2 testing-National Surgical Adjuvant Breast and Bowel Project experience. J Natl Cancer Inst 2002;94:852–4.
26. Kurokawa H, Arteaga CL. Inhibition of erbB receptor (HER) tyrosine kinases as a strategy to abrogate antiestrogen resistance in human breast cancer. Clin Cancer Res 2001;7:4436–42s; discussion 4411-2s.
27. Kurokawa H, Arteaga CL. ErbB (HER) receptors can abrogate antiestrogen action in human breast cancer by multiple signaling mechanisms. Clin Cancer Res 2003;9:511–5S.
28. Liu Y, el-Ashry D, Chen D, et al. MCF-7 breast cancer cells overexpressing transfected c-erbB-2 have an *in vitro* growth advantage in estrogen-depleted conditions and reduced estrogen-dependence and tamoxifen-sensitivity *in vivo*. Breast Cancer Res Treat 1995;34:97–117.
29. Kuerer HM, Newman LA, Smith TL, et al. Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy. J Clin Oncol 1999;17:460–9.
30. Liedtke C, Broglio K, Moulder S, et al. Prognostic impact of discordance between triple receptor measurements in primary and recurrent breast cancer. Annal Oncol. Epub 2009 Jul 12.

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