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**CLINICAL INVESTIGATION** **Breast**

# HER2/NEU-POSITIVE DISEASE DOES NOT INCREASE RISK OF LOCOREGIONAL RECURRENCE FOR PATIENTS TREATED WITH NEOADJUVANT DOXORUBICIN-BASED CHEMOTHERAPY, MASTECTOMY, AND RADIOTHERAPY

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**Purpose: Preclinical data suggest that overexpression of Her2/neu confers cellular radioresistance. We retrospectively studied whether Her2/neu-positive disease was associated with locoregional recurrence (LRR) after postmastectomy radiotherapy (RT) for breast cancer.**

**Methods and Materials: Data from 337 patients treated in four institutional prospective clinical trials neoadjuvant doxorubicin-based chemotherapy, mastectomy, and RT were reviewed. The trials were conducted between 1989 and 2000. Of the 337 patients, 108 (32%) had tumors that were tested for Her2/neu, with positivity defined by 3+ immunohistochemistry staining or gene amplification detected by fluorescence *in situ* hybridization. RT was delivered to the chest wall and draining lymphatics (median dose, 50 Gy) followed by a chest wall boost (median dose, 10 Gy).**

**Results: Thirty-two patients had Her2/neu-positive disease and 76 patients had Her2/neu-negative disease. The Her2/neu-positive tumors were associated with a greater rate of estrogen receptor-negative disease ($p = 0.03$), the presence of supraclavicular disease at diagnosis ($p = 0.027$), and a greater number of positive lymph nodes after chemotherapy ($p = 0.026$). Despite these adverse features, the actuarial overall LRR rate was roughly equivalent for the patients with Her2/neu-positive tumors vs. those with Her2/neu-negative tumors (5-year rate 17.5% vs. 13.9%, respectively; 10-year rate 17.5% vs. 18.9%, respectively; $p = 0.757$). On Cox regression analysis of LRR adjusted for N stage and estrogen receptor status, the hazard ratio for Her2/neu positivity was 0.89 (95% confidence interval, 0.31–2.59; $p = 0.83$).**

**Conclusion: Her2/neu overexpression does not appear to predispose to LRR after neoadjuvant doxorubicin-based chemotherapy, mastectomy, and RT.** **© 2004 Elsevier Inc.**

**Breast cancer, Mastectomy, Her2/Neu, Radiation, Locoregional recurrence.**

## INTRODUCTION

Achievement of locoregional control is an important component of cure for breast cancer patients. The addition of radiotherapy (RT) has been shown to reduce the probability of locoregional recurrence (LRR) for many subsets of breast cancer patients treated with mastectomy and systemic therapy (1–4). Furthermore, randomized trials investigating postmastectomy RT have shown that the reduction in LRR is associated with an overall survival benefit (1–3).

We recently investigated the efficacy of postmastectomy RT in 500 patients treated with mastectomy and adjuvant chemotherapy (4). Although our data clearly indicated that RT is associated with an improved locoregional control rate, some patients developed LRR despite the use of RT. One potential reason for this is intrinsic resistance of tumor cells to the effects of RT. Because preclinical data have suggested that overexpression of Her2/neu confers cellular radioresistance, the purpose of this report was to investigate whether overexpression of the Her2/neu receptor or Her2/neu gene amplification was associated with RT resistance in

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breast cancer patients treated with mastectomy, chemotherapy, and postmastectomy RT.

Her2/neu is a member of the epidermal growth factor family of cellular receptors (5). The gene encodes a 185-kD transmembrane glycoprotein whose internal domain acts as a tyrosine kinase that activates a variety of downstream signaling events (6, 7). Some consequences of activation of the Her2/neu receptor are to increase the metastatic potential of tumor cells and decrease their susceptibility to apoptosis (8, 9). Approximately 30% of breast cancers overexpress the Her2/neu receptor, with the vast majority of these cases also exhibiting gene amplification (10, 11).

Previous clinical studies have found overexpression of Her2/neu to be associated with resistance to certain types of chemotherapy and hormonal therapy for breast cancer (12–16). Preclinical data have suggested that Her2/neu overexpression may also confer cellular radioresistance in breast cancer cells (17). These data have been supported by a case–control study that found that breast tumors that recurred after breast-conserving therapy were more likely to overexpress Her2/neu than a matched set of cases without recurrence (18). However, no data have been published regarding the effect of Her2/neu overexpression on recurrence rates after postmastectomy RT.

## METHODS AND MATERIALS

The institutional surveillance committee of our institution approved this retrospective review of clinical data and the immunostaining and fluorescence *in situ* hybridization testing (FISH) of archival tissue for Her2/neu. For the purpose of this study, we reviewed the data concerning patients treated in our four most recent institutional prospective clinical trials that investigated neoadjuvant chemotherapy. Specificically, we were interested in the patients treated with neoadjuvant chemotherapy, mastectomy, and postmastectomy RT. We identified 337 such patients, and found that 108 (32%) of these had tumors that had been tested for Her2/neu expression. Six of these patients had had Her2/neu testing performed on the primary tumor at disease recurrence and the remainder were tested either at diagnosis ($n$ = 22) or during a study assessing biomarkers and chemotherapy response ($n$ = 80) (19). An attempt was made to obtain pretreatment tissue blocks from all 337 cases. A total of 244 patients were diagnosed with fine needle aspiration only before chemotherapy, and in the remaing cases we were unable to obtain the blocks. We did not have data concerning Her2/neu status for the patients treated in our institutional adjuvant chemotherapy trials because this test was not routinely performed during the years of our adjuvant trials.

Her2/neu expression was considered to be either positive or negative. Immunohistochemistry (IHC) was performed using commercially available monoclonal antibody AB-8 (neomarker). Scoring was based on the membrane reactivity using the Dako HercepTest. Cytoplasmic staining and weak or no membrane staining was considered to be negative. FISH was performed with dual-color interphase analysis using combined Her2/neu and chromosome 17-∞ satellite DNA probes from Uysis. A score of >2 was considered to indicate amplification. Her2/neu-positive tumors were defined by the same guidelines used in clinical practice within our institution as either 3+ receptor overexpression on IHC staining or gene amplification found on FISH testing. In general, FISH was performed only the tumors with 1+ or 2+ positivity on IHC staining.

None of the patients had evidence of systemic metastases at the time of initial treatment. All patients underwent neoadjuvant combination chemotherapy that included doxorubicin, with 53% also receiving a taxane. None of these patients received trastuzumab as a component of their primary treatment. A total of 52 patients were treated with a median of four cycles (range, two to six) of neoadjuvant 5-fluorouracil, doxorubicin, and cyclophosphamide (500–600 mg/m$^2$ 5-fluorouracil given on Days 1 and 4 or 8, 50–60 mg/m$^2$ doxorubicin given as a Day 1 bolus or 72-h continuous infusion, and 500–1000 mg/m$^2$ cyclophosphamide given on Day 1). Four of these patients also received four cycles of single-agent paclitaxel. The remaining 56 patients were treated with six cycles of 60 mg/m$^2$ doxorubicin and 60 mg/m$^2$ docetaxel given as i.v. boluses. All patients underwent modified radical mastectomy after chemotherapy, with a median of 14 (range, 0–36) axillary lymph nodes recovered from the specimen. All patients were treated with postmastectomy RT to the ipsilateral chest wall and draining lymphatics. The median dose to these initial fields was 50 Gy in 25 fractions and was typically followed by a 10-Gy chest wall boost (median total dose to this site was 60 Gy).

The primary outcome of this study was the development of LRR. LRR was defined as recurrence on the ipsilateral chest wall or in the ipsilateral draining lymph nodes (axillary, supraclavicular, or internal mammary). All LRR were considered independent of their timing relative to a distant metastasis. The Kaplan-Meier method was used to estimate LRR (20), with time 0 defined as the date of diagnosis. Kaplan-Meier curves were compared using two-sided log–rank tests. Demographic and disease characteristics were compared using chi-square tests. A Cox proportional hazards model was used to perform a multivariate analysis (21).

To assess whether a study much larger than the present study would be likely to show a statistically significant difference between the LRR rates in the Her2/neu-positive and Her2/neu-negative subgroups, we performed a predictive analysis (22). This method is similar to that used by statisticians to determine whether a clinical trial should be stopped early because the available data suggest a high probability that the final results will show that the hypothesis is either true or false. Using the LRR data from this study and a resampling procedure, we simulated the LRR data for hypothetical sets of 1000 patients with similar disease and outcome characteristics (23). The resampling trial was repeated 1000 times. For each of these trials, the resulting data either did or did not show a statistically significant interaction between Her2/neu status and LRR.

Table 1. Patient characteristics

| Factor | Her2/neu-positive disease | Her2/neu-negative disease | *p* |
|---|---|---|---|
| Patients (*n*) | 32 | 76 | |
| Age | | | |
| <40 | 22 | 15 | 0.400 |
| ≥40 | 78 | 85 | |
| T stage | | | |
| T1 | 3 | 4 | 0.562 |
| T2 | 16 | 15 | |
| T3 | 19 | 32 | |
| T4 | 62 | 49 | |
| N stage | | | |
| N0 or N1 | 37 | 46 | 0.273 |
| N2 or N3 | 63 | 54 | |
| Supraclavicular disease | | | |
| No | 63 | 83 | 0.027 |
| Yes | 37 | 17 | |
| Estrogen receptor* | | | |
| Positive | 28 | 51 | 0.051 |
| Negative | 72 | 49 | |
| Progesterone receptor* | | | |
| Positive | 26 | 36 | 0.363 |
| Negative | 74 | 64 | |
| Pathologic primary size (cm) | | | |
| 0–2 | 65 | 50 | 0.337 |
| 2–5 | 22 | 35 | |
| >5 | 13 | 15 | |
| No. of positive lymph nodes | | | |
| 0 | 6 | 24 | 0.026 |
| 1–3 | 25 | 33 | |
| ≥4 | 69 | 43 | |

Data presented as percentage of patients, unless other noted.
* Cases with unknown values excluded.

The proportion of the former was the predictive probability of statistical significance had the study included a total of 1000 patients.

## RESULTS

Thirty-two patients had tumors with Her2/neu overexpression, and 76 patients had tumors in which the Her2/neu test was considered negative for overexpression. Of the 32 patients with Her2/neu-positive tumors, 15 had confirmation with FISH testing for gene amplification. Table 1 shows the demographic and disease characteristics of these two patient populations. In general, the population of this study presented with advanced disease, with 89% of patients having Stage III disease or involvement of the supraclavicular lymph nodes without systemic metastases. As shown in Table 1, the patients with Her2/neu-positive tumors more commonly had estrogen receptor-negative disease (72% vs. 49%, respectively, $p = 0.051$). In addition, the patients with Her2/neu-positive tumors presented with more advanced nodal disease ($p = 0.012$) and had a greater median number of positive lymph nodes after chemotherapy ($n = 6$ vs. $n = 3$, $p = 0.002$).

During a median follow-up of 3.75 years (range, 1.5–11.6 years) for surviving patients, 16 (14.8%) of the 108 patients developed LRR. The 5-year and 8-year actuarial LRR rate for the entire population was 14.8% ± 3.6% and 18.9% ± 5.2%, respectively. Despite the excess in poor prognostic features in the patients with Her2/neu-positive tumors vs. those with Her2/neu-negative disease, the actuarial overall LRR rate was roughly equivalent for the two groups. Figure 1 shows the actuarial LRR curves for the patients divided according to Her2/neu status. The LRR rates for the patients with Her2/neu-positive tumors compared with those with Her2/neu-negative tumors were 17.5% ± 7.3% vs. 13.9% ± 4.1%, respectively, at 5 years and 17.5% ± 7.3% vs. 18.9% ± 6.2%, respectively, at 8 years ($p = 0.757$).

Table 2 shows the data concerning how the various other factors were associated with LRR. Clinical N2 or N3 disease, supraclavicular nodal involvement at presentation, and estrogen receptor-negative disease were positively associated with greater rates of LRR.

On a forward logistic Cox regression analysis, N2 or N3 disease and estrogen receptor-negative disease were independent predictors of LRR. When a Cox analysis was performed with clinical N stage, estrogen receptor status, and Her2/neu status in the model, the hazard ratio for LRR associated with Her2/neu positivity was 0.89 (95% confidence interval, 0.31–2.59; $p = 0.83$).

The predictive analysis used to model the probability of finding the probability that a sample size of 1000 patients would show an interaction between LRR and Her2/neu positivity revealed an 8.6% probability of finding a statistically significant interaction in this much larger study.

## DISCUSSION

We found no association between Her2/neu overexpression and LRR for a cohort of patients with locally advanced breast cancer who were treated with neoadjuvant doxorubicin-based chemotherapy, mastectomy, and postmastectomy RT. Specifically, in a multivariate analysis, the hazard ratio associated with Her2/neu positivity was <1. In addition, as shown in Table 1, patients with Her2/neu-positive tumors more commonly had estrogen receptor-negative disease and more commonly had supraclavicular nodal involvement at presentation than patients with Her2/neu-negative disease. These imbalances would be predicted to improve the LRR outcome for patients with Her2/neu-negative tumors. That no improved outcome was noted strengthens the evidence that Her2/neu positivity was not independently associated with greater rates of LRR.

These data are important because they are the first information available regarding whether overexpression of Her2/neu increases breast cancer resistance to RT and thus the risk of LRR after postmastectomy RT. The data suggest that previous preclinical studies, which found Her2/neu overexpression to be associated with cellular radioresistance in breast cancer, may not be of clinical relevance for patients treated in a multimo-

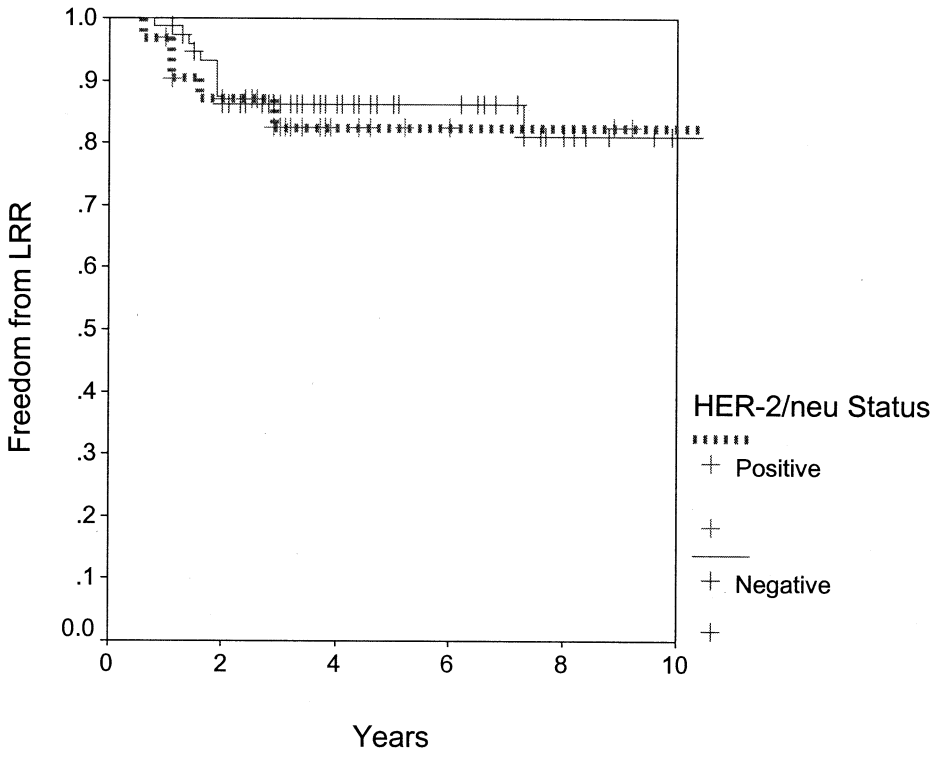


Fig. 1. Actuarial freedom from local-regional recurrence (LRR) for patients categorized according to the Her2/neu status of tumors. Dashed line represents data from patients with Her2/neu-positive disease; solid line represents data from patients with Her2/neu-negative disease. These curves were not significantly different statistically ($p = 0.757$).

dality fashion such as the population we studied. Specifically, preclinical work from Pietras *et al.* (17) showed that MCF-7 human breast cancer cells transfected to overexpress the Her2 receptor were more resistant to radiation-induced death than MCF-7 cells transfected with a control vector. Furthermore, treatment with anti-Her2 antibody overcame this resistance and actually increased cell death above that of the control cell lines. *In vivo* studies of MCF-7/Her2 xenografts further confirmed that combining RT with the anti-Her2 antibody provided a synergistic effect on tumor growth delay. However, the investigators did not compare the efficacy of RT in MCF-7/Her2 xenografts and MCF-7/control xenografts to confirm *in vivo* an association of Her2/neu expression and radioresistance (17). Others have also shown that the strategy of combining RT and blockade of the Her2/neu receptor or its tyrosine kinase activity in a preclinical model allows for synergistic cell killing of breast cancer cells that overexpress Her2/neu (24–27).

Few human data have been published regarding Her2/neu overexpression and radioresistance. One study that investigated this issue compared the rates of Her2/neu overexpression in 16 tumors from patients who subsequently developed breast recurrence after breast surgery and RT with the expression rate in 16 tumors from matched controls without recurrence (18). Her2/neu expression was considered positive if heavy staining was noted on IHC (antibody c-Neu [Ab-3]). This study found a rate of overexpression of 56% (9 of 16) in the recurrent tumors compared with 18% (3 of 16) in the tumors that did not recur.

The only published study to investigate Her2/neu expression in tumors from patients treated with mastectomy evaluated tumors from patients who participated in a clinical trial that compared adjuvant RT against adjuvant nonanthracycline chemotherapy (28). That study found that a greater rate of LRR in those patients with Her2/neu overexpressing tumors who were randomized to chemotherapy compared with those with Her2/neu overexpressing tumors who were randomized to RT. For the patients with Her2/neu-negative tumors, no difference in LRR was found. These data can be interpreted in many ways, but in general, are consistent with our finding that Her2/neu overexpression does not lead to clinically relevant cellular radioresistance that increases rates of LRR after postmastectomy RT.

A unique aspect of our study compared with the limited published data investigating this issue is that all patients in our study received a doxorubicin-containing chemotherapy regimen in addition to surgery and RT. Previous data have indicated that Her2/neu overexpression is associated with resistance to some forms of systemic treatments, but this resistance may be overcome by treatment with an anthracycline (12–15, 29). Whether the prior anthracycline treatment in our patient population played a role in overcoming the radioresistance associated with Her2/neu expression is currently unknown. However, in the previously referenced study that found greater rates of Her2/neu overexpression in tumors that recurred after breast conservation therapy, none of the patients had received any form of systemic treatment (18).

Table 2. Eight-year rate of locoregional recurrence according to various factors

| Factor | Patients (*n*) | LRR rate (%) | *p* |
|---|---|---|---|
| Patient related | | | |
| Age (y) | | | |
| <40 | 18 | 19 | 0.8343 |
| ≥40 | 90 | 19 | |
| Pretreatment stage | | | |
| T stage | | | |
| T1 | 4 | 25* | 0.8832 |
| T2 | 16 | 14 | |
| T3 | 30 | 24 | |
| T4 | 58 | 14 | |
| N stage | | | |
| N0 or N1 | 47 | 11 | 0.0120 |
| N2 or N3 | 61 | 23 | |
| Supraclavicular disease | | | |
| No | 83 | 15 | 0.0199 |
| Yes | 25 | 29 | |
| Pretreatment biomarkers | | | |
| Estrogen receptor† | | | |
| Positive | 44 | 15 | 0.0243 |
| Negative | 57 | 24 | |
| Progesterone receptor† | | | |
| Positive | 33 | 18 | 0.7854 |
| Negative | 67 | 27 | |
| Her2/neu | | | |
| Positive | 32 | 17 | 0.7568 |
| Negative | 76 | 19 | |
| Posttreatment disease extent | | | |
| Primary size (cm) | | | |
| 0–2 | 59 | 14 | 0.9900 |
| 2–5 | 33 | 24 | |
| >5 | 15 | 14 | |
| Positive lymph nodes (*n*) | | | |
| 0 | 20 | 21 | 0.8299 |
| 1–3 | 33 | 20 | |
| >4 | 54 | 14 | |
| Pathologic complete response | | | |
| Yes | 5 | 20 | 0.8188 |
| No | 103 | 19 | |

*Abbreviation:* LRR = locoregional recurrence.
* Case with longest follow-up censored before 8-y point.
† Cases with unknown values excluded.

The overall rate of LRR after postmastectomy RT in this study was greater than that in a previously published report from our institution (4). This was probably because the current population had more advanced disease than those included in our prior report.

A limitation of our study was the small sample size, as well as the corresponding number of LRR events. In particular, our study did not have high enough power to detect modest differences in LRR rates if such exist. However, we did not find even a suggestion of a greater LRR rate associated with Her2/neu positivity. Moreover, in our predictive modeling, we estimated that the probability of detecting an increased risk of LRR with a sample size of 1000 patients would be 8.6%.

A final concept to consider when investigating radioresistance in breast cancer is that most patients who undergo postmastectomy RT do not have disease in the locoregional area at the time of treatment. On the basis of previous data, the predicted 10-year rate of LRR for a population of patients with advanced disease treated with neoadjuvant chemotherapy, mastectomy, and no RT would be approximately 30% (30). In this study, we report a 15% rate with RT. An intrinsic molecular factor that reduced the efficacy of RT by 50% would, therefore, be predicted to only increase the absolute rate of LRR for such a population by 7–8%, and a reduction in efficacy of 25% would only lead to an absolute increase in LRR of 3–4%. Therefore, molecular changes that cause a modest change in cellular radiosensitivity may not result in a statistically significant clinical effect.

## CONCLUSION

We found no evidence that breast cancer patients whose tumors overexpress Her2/neu are at increased risk of LRR after doxorubicin-based chemotherapy, mastectomy, and RT. Whether anti-Her2/neu antibody therapy increases RT efficacy for patients treated for Her2/neu-positive tumors is currently unknown. However, a number of ongoing Phase III trials investigating adjuvant trastuzumab are designed to give this treatment concurrently with postmastectomy RT. The data from these trials may provide further insights into this question.

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