

HER-2/neu Overexpression and Response to Oophorectomy Plus Tamoxifen Adjuvant Therapy in Estrogen Receptor-Positive Premenopausal Women With Operable Breast Cancer

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Purpose: Studies evaluating the relationship of *HER-2/neu* breast tumor status and response to adjuvant endocrine therapy have reached conflicting conclusions about resistance of *HER-2/neu*-positive tumors to this treatment. We studied 282 patients participating in a randomized controlled trial of adjuvant oophorectomy and tamoxifen or observation who had estrogen receptor-positive tumors and whose tumors were evaluated for *HER-2/neu* overexpression by immunohistochemistry.

Patients and Methods: Univariate and multivariate Cox proportional hazards regression models and Kaplan-Meier disease-free and overall survival estimate methods were used.

Results: *HER-2/neu* overexpression was a negative prognostic factor for overall survival. In univariate analyses, in *HER-2/neu*-positive patients, the hazard ratio (HR) for disease-free survival (DFS) with adjuvant endocrine therapy was 0.37 (95% confidence interval [CI], 0.26 to 0.89);

for *HER-2/neu*-negative patients, the corresponding HR for DFS was 0.48 (95% CI, 0.31 to 0.71). The overall survival (OS) data were HR=0.26 (95% CI, 0.07 to 0.92) and HR=0.68 (95% CI, 0.32 to 1.42) for *HER-2/neu*-positive and *HER-2/neu*-negative patients, respectively. In multivariate models, the *P* values for tests of interaction of *HER-2/neu* status and response to adjuvant endocrine therapy were 0.18 and 0.07 for DFS and OS, respectively. Kaplan-Meier DFS and OS curves and 3-year DFS estimates were consistent in showing greater benefit to the *HER-2/neu*-positive subgroup given adjuvant treatment.

Conclusion: *HER-2/neu* overexpression does not adversely and may favorably influence response to adjuvant oophorectomy and tamoxifen treatment in patients with estrogen receptor-positive tumors.

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THE RELATIONSHIP of overexpression of the proto-oncogene *HER-2/neu* (*c-erbB-2*) to response to adjuvant endocrine therapy has been of concern because of the major role of such treatments in women with early-stage breast cancer and because preclinical laboratory data and some clinical data have indicated that patients with *HER-2/neu*-positive (ie, overexpressed) tumors may not benefit from this treatment.¹⁻⁴ In the only randomized controlled study,⁴ an initial report indicated that adjuvant tamoxifen was associated with worse overall survival in *HER-2/neu*-positive cases (*P* = .03) and better overall survival in *HER-2/neu*-negative cases (*P* = .9). In the follow-up study of the same trial⁵, a multivariate analysis supported a conclusion that *HER-2/neu*-positive cases were unresponsive to tamoxifen.⁵ Although limited by nonrandom assignment of tamoxifen therapy and other confounding factors, a recent analysis of experience in a large, cooperative group study indicated an opposite conclusion: Disease-free survival (DFS) risk reduction associated with adjuvant tamoxifen was 25% in a *HER-2/neu*-negative group and 44% in a *HER-2/neu*-positive group.⁶ Taken together, other studies have indicated an adverse interaction of *HER-2/neu* positivity and endocrine (usually tamoxifen) therapy.⁷⁻¹¹ In a recent review of the status of *HER-2/neu* as a predictive factor in breast cancer, Yamauchi et al³ concluded that, with respect to adjuvant endocrine therapy, no study has been reported that “was specifically designed to clarify the interaction between endocrine therapy in ER [estrogen receptor]-positive patients and *c-erbB-2* status.” Furthermore, these authors concluded that “ideally a study that can clarify the association . . . must include patients with ER-positive tumors randomly assigned to endocrine therapy versus no treatment.”³

PATIENTS AND METHODS

The rationale, design, treatments, quality control, definitions, ER and progesterone receptor (PR) protein evaluation methods, histologic subtyping and grading, and initial results for the trial have been published, from which a subset of patients are reported on here.¹² Briefly, from 1993 to 1999, 709 Vietnamese (662) and Chinese (47) premenopausal women with operable breast cancer were recruited into a randomized clinical trial of adjuvant surgical oophorectomy and tamoxifen (20 mg orally daily) for 5 years versus observation and this combined hormonal therapy on recurrence. All hormone receptor and *HER-2/neu* studies were performed 2 to 7 years later. The adjuvant oophorectomy and tamoxifen therapy was associated with better DFS and overall survival (OS)¹² primarily because an unexpectedly high percentage (62%) of patients had hormone receptor-positive tumors.

Each participant gave written informed consent. The study was reviewed and approved by institutional review boards at the institution of the principal investigator (R.R.L), by the Office for Protection of Research Risk of the United States National Institutes of Health, by the Scientific and Technical Council of the Ministry of Health of Vietnam, and by institutional review committees in China. A data monitoring committee of five experts reviewed the trial conduct and results periodically.

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STUDY SAMPLES

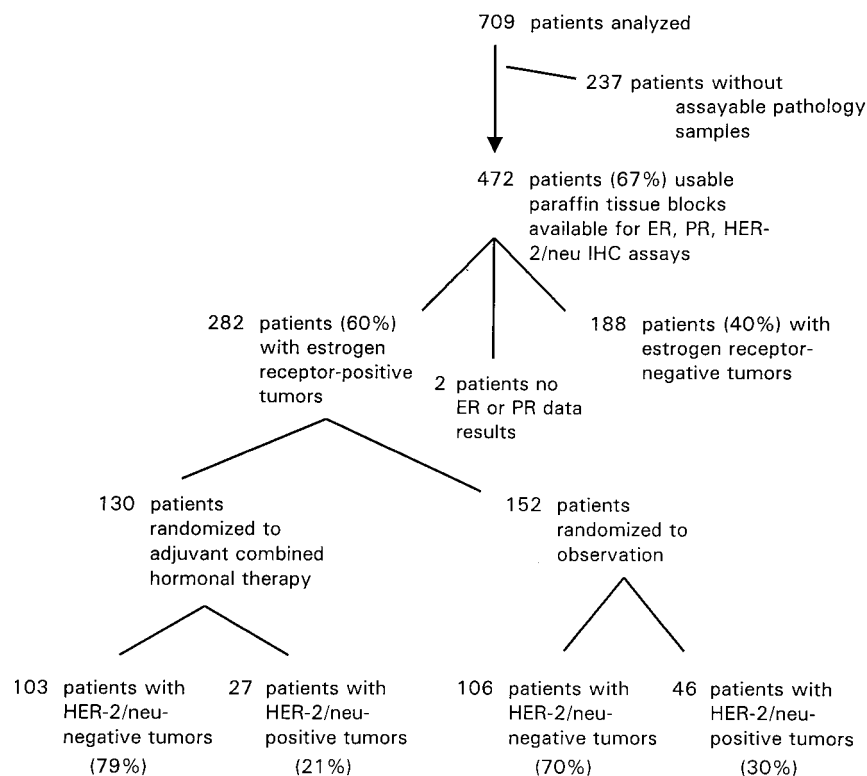


Fig 1. Subsets of trial participants

HER-2/neu expression was measured and scored by permanent-section immunohistochemistry using standardized and previously described methodology.¹³⁻¹⁵ Briefly, 3- μ m histologic sections on adhesive glass slides (Fisher PLUS; Fisher Scientific, Houston, TX) were deparaffinized (xylene), dehydrated (graded alcohols), blocked for endogenous peroxidase activity (3% hydrogen peroxide), blocked for endogenous biotin (Vector A/B Blocking Kit; Vector Laboratories, Burlingame, CA), incubated with primary antibody (Zymed; Tab-250 at 1:200), incubated with biotinylated rabbit antimouse linking antibody (DAKO, Carpinteria, CA; at 1:200), incubated with peroxidase-conjugated streptavidin (DAKO at 1:200), colorized with DAB + Chromogen Solution (DAKO), enhanced with osmium tetroxide (0.2%), counterstained with methyl green, dehydrated, cleared, and over-slipped for visualization of the dark-brown membranous signal under the microscope. The signal was quantified by assigning a proportion score (representing the estimated proportion of positive tumor cells on the slide: 0 = none; 1 < 1/100; 2 = 1/100 to 1/10; 3 = 1/10 to 1/3; 4 = 1/3 to 2/3; and 5 > 2/3) and an intensity score (representing the estimated average staining intensity of positive tumor cells: 0 = none; 1 = weak; 2 = intermediate; and 3 = strong). The proportion and intensity scores were then added to give a total score ranging from zero to eight. Data analysis was based on total score.

Statistical Methods

The results reported here are from exploratory post hoc analyses of subsets of trial participants as defined in Fig 1. The χ^2 test and the Wilcoxon test were used to analyze differences in categorical and ordinal variables, respectively.¹⁶ The Cox proportional hazards model was used to perform univariate and multivariate models on the data.¹⁷ DFS and OS curves were calculated using Kaplan-Meier methods,¹⁸ and the differences in survival curves were assessed with a log-rank test.¹⁹ Interactions between *HER-2/neu* and treatment status were tested in two ways. The first method was by adding an interaction term to the models containing both terms and testing the significance of the interaction. The second method was the Gail Simon test for interaction.²⁰ The interaction tests did not differ substantially in *P* value, and values for the first type of test are reported. All computations were

performed with SAS software (Version 6.12, SAS Institute, Cary, NC). All *P* values were calculated with two-sided tests of significance.

RESULTS

Figure 1 describes the selection of the patient population for this report. Of 709 patients entered onto the study, usable primary breast cancer paraffin blocks were available for 472 (67%). Demographic and pathologic characteristics of the 472- and 237- patient groups were similar for mean age and percentages of patients younger than 40 years, axillary lymph node-positive patients, and with histologic grade 3 tumors. Kaplan-Meier DFS and OS curves in both treatments for these two groups of patients were also not significantly different.

Table 1. Patient and Disease Characteristics and *HER-2/neu* Status (all patients with estrogen receptor-positive tumors)

Variable	<i>HER-2/neu</i>		<i>P</i>
	Negative (n = 209)	Positive (n = 73)	
Age (mean, years)	41.3	40.9	NS (Wilcoxon)
Age <40	34.3%	41.1%	NS (χ^2)
Axillary node-positive	50.0%	62.5%	.068 (χ^2)
No. of positive axillary nodes (in N+)	4.3	4.9	.14 (Wilcoxon)
Histologic grade, percentage grade III	10.1%	11.7%	NS (χ^2)
Pathologic size (cm)	3.13	3.54	NS (Wilcoxon)
Received adjuvant combined hormonal therapy	49.3%	37.0%	.070 (χ^2)

Abbreviation: NS, not significant.

Table 2. Proportional Hazards Models for DFS and OS for Interaction of Oophorectomy/Tamoxifen Adjuvant Therapy and HER-2/neu Status in Estrogen Receptor-Positive Patients: Multivariate Analyses

Variable	DFS			OS		
	Risk Ratio	95% CI	P	Risk Ratio	95% CI	P
Adjuvant oophorectomy/tamoxifen	0.59	0.31 to 1.13	.11	0.76	0.34 to 1.68	.50
Square root of number of positive axillary lymph nodes*	1.30	1.03 to 1.64	.03	1.48	1.10 to 1.99	.01
Pathologic tumor size	1.24	1.04 to 1.49	.02	1.14	0.92 to 1.42	.24
Histologic grade 3	1.91	0.98 to 3.73	.06	2.19	0.98 to 4.86	.05
HER-2/neu-positive status	1.45	0.75 to 2.80	.27	2.20	1.00 to 4.85	.05
Interaction of HER-2/neu status and adjuvant treatment†	0.38	0.09 to 1.56	.18	0.21	0.04 to 1.13	.07

*This variable provides a better fit to the data in this study than other single or combination of variables used to describe axillary nodal status.

†Risk ratio = 1 if data indicate that the calculations being made are valid without including considerations of HER-2/neu and treatment status.

Abbreviations: DFS, disease-free survival; OS, overall survival; CI, confidence interval.

Sixty percent of patients in this study had ER-positive tumors (n = 282). Of these, 26% (n = 73) had HER-2/neu-positive tumors (Fig 1). Table 1 describes the demographic and pathologic characteristics in the HER-2/neu-negative and HER-2/neu-positive groups of patients. Although no single variable is statistically different between the two groups, there is a slightly higher percentage of patients who are axillary lymph node positive, and the mean number of involved axillary lymph nodes is greater in the HER-2/neu-positive subgroup (Table 1). The median follow-up at the time of this report was 3 years, 8 months.

In univariate analyses of 152 patients in the observation group who had ER-positive tumors, HER-2/neu-positive status was associated with poorer DFS and OS (risk ratio (RR): DFS, 1.66, P = .09; OS, 2.49, P = .01). In a multivariate model including the variables listed in Table 2 (except for treatment) with ER-positive observation patients, HER-2/neu-positive status was similarly associated with poorer DFS and OS (RR: DFS, 1.53, P = .22; OS, 2.33, P = .044).

In univariate analyses of all 282 ER-positive patients, risk reductions associated with adjuvant oophorectomy and tamoxifen therapy occurred in both HER-2/neu-positive and HER-2/neu-negative subgroups, with a suggestion of greater benefit in the HER-2/neu-positive subgroup (Table 3). In multivariate analyses (Table 2), models with HER-2/neu status and no interaction term gave risk ratios consistent with results in the literature. Table 2 shows the results of models that indicate interactions between HER-2/neu status and treatment that are not statistically significant. The suggested interaction is quantitative, not qualitative. This can be seen from the model because the estimated relative risks for adjuvant oophorectomy/tamoxifen (0.59 for DFS and OS 0.76) and the interactive terms (0.38 and 0.21) are all less than 1. It can also be seen in Fig 2, because the

results for the no-adjuvant-treatment groups are below those for the treated group in both HER-2/neu subgroups. The difference is simply larger for the HER-2/neu-positive patients. The Kaplan-Meier DFS and OS curves support this conclusion (Fig 3). Whereas the adjuvantly treated group curves for DFS and OS for the HER-2/neu-positive and HER-2/neu-negative groups are similar, the DFS and OS survival curves are much worse for the observation patients who are HER-2/neu positive than for those who are HER-2/neu negative (DFS, P = .09; OS, P = .01). The 3-year DFS by treatment, in the 113 patients observed for this duration, showed that HER-2/neu-positive patients received greater absolute benefit from the adjuvant treatment (HER-2/neu-positive observation patients 52% DFS, treated 81%; HER-2/neu-negative observation patients 73% DFS, treated 85% [Fig 2]). A multivariate analysis in the 177 ER-negative HER-2/neu patient groups demonstrated no significant evidence of a treatment effect (as have other analyses),¹² RRs usually observed for known prognostic factors, and no evidence of interaction of treatment and HER-2/neu status (Table 4).

Our data are quite inconsistent with those reported by Carlomagno et al.⁴ If there were truly a negative interaction of the magnitude they described (RR = 4.13), there is only a 0.017 chance of observing the positive interaction (RR = 0.38) that we observed in our study. Analyses in which the cutoffs for HER-2/neu positivity and ER positivity are increased from more than 0 to more than 2 (on a 0 to 7 scale)¹³ involved the change of eight cases from HER-2/neu positive to HER-2/neu negative and had no measurable effect on the results presented. Results in axillary lymph node-positive and lymph node-negative patient groups were similar.

DISCUSSION

This study is well suited to address the ER/HER-2/neu status interaction because the evaluable cases come from a randomized

Table 3. Proportional Hazards Models for DFS and OS for Interaction of Oophorectomy/Tamoxifen Adjuvant Therapy and HER-2/neu Status in Estrogen Receptor-Positive Patients: Univariate Analyses

Group	n	DFS			OS		
		Adjuvant Therapy Risk Ratio	95% CI	P	Adjuvant Therapy Risk Ratio	95% CI	P
HER-2/neu negative	209	0.48	(0.31-0.71)	.019	0.68	(0.32-1.42)	.300
HER-2/neu positive	73	0.37	(0.26-0.89)	.047	0.26	(0.07-0.92)	.038
All	282	0.43	(0.25-0.72)	.001	0.48	(0.26-0.91)	.023

Abbreviations: DFS, disease-free survival; OS, overall survival; CI, confidence interval.

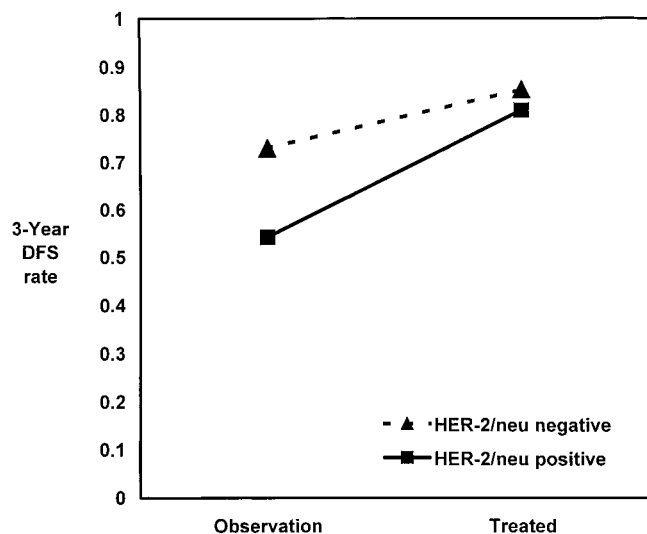


Fig 2. Disease-free survival in 113 estrogen receptor-positive tumor patients followed for at least 3 years according to *HER-2/neu* status and treatment status.

controlled trial in which half of the entered patients were not treated with adjuvant therapy. Although only two thirds of the participants entered onto the study had primary cancer tissue usable for hormone and *HER-2/neu* assays, these patients appeared to have demographic and prognostic characteristics and DFS and OS that did not differ from the one third of patients without assayable tissue. Additional strengths of this study are uniform rigorous methods of ER and *HER-2/neu* assessments.^{12,21} The internal consistency of the results supports the general conclusion that in ER-positive patients, *HER-2/neu* status assessed by primary tumor immunohistochemical staining does not adversely, and may favorably, interact with combined adjuvant hormonal therapy.

The results of this study are in concordance with those from the analysis of Cancer and Leukemia Group B (CALGB) 8541⁶ and a recent Danish report²² but show an opposite trend from those of the smaller Italian GUN 1 trial.^{4,5} The Italian trial used 2 years of tamoxifen (only) treatment, has reported 15-year median follow-up data, and included 145 axillary lymph node-negative patients, 37% of whom were premenopausal. ER status was known for 108 (74%); of these patients; 37 were ER negative. Thus, for analyses of tamoxifen efficacy in ER positive patients, the total effective sample size is 70 or 71 cases. The latest data from this study reported evaluating eight biologic

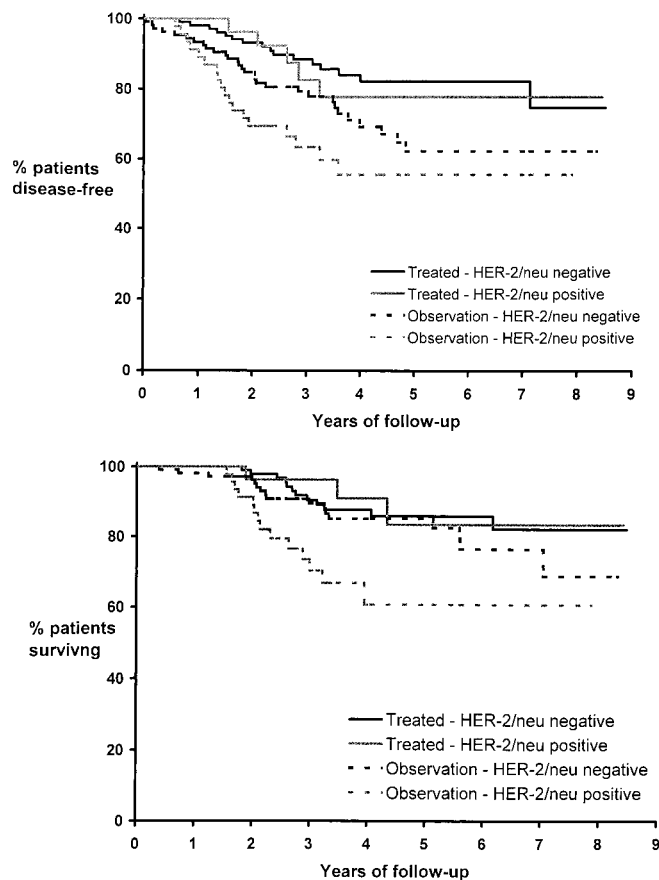


Fig 3. Disease-free and overall survival in 282 estrogen receptor-positive patients according to *HER-2/neu* status and treatment with adjuvant surgical oophorectomy and tamoxifen or observation. For overall survival, the *HER-2/neu*-positive adjuvant patient group experience is significantly better than that of the *HER-2/neu*-positive control group ($P = .026$).

markers, and therefore, this study has multiple comparisons being made on a small data set.⁵

The results of this study are perhaps most understandable when considered together with those of Ellis et al,¹⁰ who found that in postmenopausal patients with hormone receptor-positive *ErbB-1* or *ErbB-2*-positive tumors, neoadjuvant treatment with the aromatase inhibitor letrozole was associated with much higher response rates than was tamoxifen. Ellis et al's result indicates that in this study, the estrogen deprivation from the surgical oophorectomy is the dominant

Table 4. Proportional Hazards Models for DFS and OS for Interaction of Oophorectomy/Tamoxifen Adjuvant Therapy and *HER-2/neu* Status in Estrogen Receptor-Negative Patients: Multivariate Analyses

Variable	DFS			OS		
	Risk Ratio	95% CI	P	Risk Ratio	95% CI	P
Adjuvant oophorectomy/tamoxifen	0.58	0.27 to 1.25	.17	0.91	0.37 to 2.22	.84
Square root of number of positive axillary lymph nodes*	1.40	1.10 to 1.79	.01	1.30	1.00 to 1.68	.05
Pathologic tumor size	1.08	0.92 to 1.28	.34	1.16	0.98 to 1.37	.08
Histologic grade 3	1.55	0.75 to 3.24	.24	1.48	0.68 to 3.24	.32
<i>HER-2/neu</i> -positive status	0.84	0.40 to 1.78	.65	1.45	0.62 to 3.40	.39
Interaction of <i>HER-2/neu</i> status and adjuvant treatment†	1.38	0.48 to 4.00	.55	0.96	0.30 to 3.09	.94

*This variable provides a better fit to the data in this study than other single or combination of variables used to describe axillary nodal status.

†Risk ratio = 1 if data indicate that the calculations being made are valid without including considerations of *HER-2/neu* and treatment status.

Abbreviations: DFS, disease-free survival; OS, overall survival; CI, confidence interval.

therapeutic event. Ellis et al have highlighted preclinical data that support a role for estrogen deprivation in *ErbB-2*-positive tumors. Benz et al²³ demonstrated that MCF-7 breast cancer cells transfected with an *ErbB-2* expression vector grow rapidly as xenografts in nude mice supplemented with estrogen. No *ErbB-2*-positive tumors were formed in the absence of estrogen, indicating that estrogen dependence was maintained despite *ErbB-2* overexpression.^{10, p. 3,814} If estrogen deprivation is a useful part of therapy for hormone receptor-positive, *ErbB-2*-positive premenopausal patients, and tamoxifen is not useful in such patients, then the overall benefits and risks of combined therapy we investigated would need to be

re-evaluated in this subgroup. Although a specific prospective study is probably not possible, analysis of other studies may help to clarify this issue.

In summary, we find strong evidence that *HER-2/neu* overexpression does not adversely affect response to an adjuvant endocrine treatment of surgical oophorectomy and tamoxifen in ER-positive women with breast cancer. We also find suggestive evidence that, in fact, response to adjuvant oophorectomy and tamoxifen is greater in *HER-2/neu*-positive than in *HER-2/neu*-negative, ER-positive patients. We find no evidence of a qualitative interaction of this adjuvant hormonal therapy and *HER-2/neu* status in ER-negative cases.

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