erbB-2 and Response to Doxorubicin in Patients With Axillary Lymph Node-Positive, Hormone Receptor-Negative Breast Cancer

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Background: **Overexpression of the erbB-2 protein by breast cancer cells has been suggested to be a predictor of response to doxorubicin. A retrospective study was designed to test this hypothesis.** ***Methods:*** **In National Surgical Adjuvant Breast and Bowel Project protocol B-11, patients with axillary lymph node-positive, hormone receptor-negative breast cancer were randomly assigned to receive either L-phenylalanine mustard plus 5-fluorouracil (PF) or a combination of L-phenylalanine mustard, 5-fluorouracil, and doxorubicin (PAF). Tumor cell expression of erbB-2 was determined by immunohistochemistry for 638 of 682 eligible patients. Statistical analyses were performed to test for interaction between treatment and erbB-2 status (positive versus negative) with respect to disease-free survival (DFS), survival, recurrence-free survival (RFS), and distant disease-free survival (DDFS). Reported *P* values are two-sided.** ***Results:*** **Overexpression of erbB-2 (i.e., positive immunohistochemical staining) was observed in 239 (37.5%) of the 638 tumors studied. Overexpression was associated with tumor size (*P* = .02), lack of estrogen receptors (*P* = .008), and the number of positive lymph nodes (*P* = .0001). After a mean time on study of 13.5 years, the clinical benefit from doxorubicin (PAF versus PF) was statistically significant for patients with erbB-2-positive tumors—DFS: relative risk of failure (RR) = 0.60 (95% confidence interval [CI] = 0.44–0.83), *P* = .001; survival: RR = 0.66 (95% CI = 0.47–0.92), *P* = .01; RFS: RR = 0.58 (95% CI = 0.42–0.82), *P* = .002; DDFS: RR = 0.61 (95% CI = 0.44–0.85), *P* = .003. However, it was not significant for patients with erbB-2-negative tumors—DFS: RR = 0.96 (95% CI = 0.75–1.23), *P* = .74; survival: RR = 0.90 (95% CI = 0.69–1.19), *P* = .47; RFS: RR = 0.88 (95% CI = 0.67–1.16), *P* = .37; DDFS: RR = 1.03 (95% CI = 0.79–1.35), *P* = .84. Interaction between doxorubicin treatment and erbB-2 overexpression was statistically significant for DFS (*P* = .02) and DDFS (*P* = .02) but not for survival (*P* = .15) or RFS (*P* = .06).** ***Conclusions:*** **These data support the hypothesis of a preferential benefit from doxorubicin in patients with erbB-2-positive breast cancer. [J Natl Cancer Inst 1998;90:1361–70]**

Many patient and tumor characteristics (e.g., pathologic axillary lymph node status, clinical tumor size, and tumor grade) are prognostic for outcome in operable breast cancer *(1,2)*, but only the expression of estrogen receptors is widely recognized as a clinically useful marker that predicts response to a specific systemic treatment *(1)*. Various reports *(3–8)* have suggested that the overexpression of the erbB-2 protein on breast cancer cells may also serve as a predictive marker of therapeutic response.

This article describes a study designed to test the hypothesis that adding doxorubicin to a chemotherapeutic regimen may provide a preferential benefit to those patients who overexpress erbB-2.

An indication that erbB-2 may be a clinically useful therapeutic response variable was provided by Cancer and Leukemia Group B (CALGB) study 8869 *(3)*. That study was conducted as a companion to CALGB protocol 8541, in which 1550 lymph node-positive patients were randomly assigned to receive one of three different regimens containing cyclophosphamide, doxorubicin (Adriamycin), and 5-fluorouracil (CAF) with dose intensification of all three drugs (300 : 30 : 300 mg/m^2 [cyclophosphamide : doxorubicin : 5-fluorouracil] every 4 weeks for four cycles, 400 : 40 : 400 mg/m^2 every 4 weeks for six cycles, or 600 : 60 : 600 mg/m^2 every 4 weeks for four cycles) *(9)*. In study 8869, 442 patients were randomly selected from those enrolled in study 8541, and their tumors were assayed for expression of erbB-2 by immunohistochemistry. The effect of dose intensification on survival and disease-free survival (DFS) was evident in the erbB-2-positive cohort, but it was absent in the erbB-2-negative patients. An explanation for this interaction may be that overexpression of the erbB-2 protein is associated with increased sensitivity to doxorubicin. The demonstrated association between the expression of erbB-2 and topoisomerase-IIα supports such an argument, since the latter is the molecular target of doxorubicin *(10)*. However, since all three drugs were dose intensified in study 8869, it is difficult to accept such an interpretation without independent confirmation of the hypothesis in a setting in which doxorubicin is the only treatment variable. The National Surgical Adjuvant Breast and Bowel Project (NSABP) protocol B-11 provided such a study setting. Initiated in 1981, protocol B-11 tested the addition of doxorubicin to a regi-

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See ''Notes'' following ''References.''

men of L-phenylalanine mustard and 5-fluorouracil (PF). Findings first reported in 1989 through 6 years of follow-up *(11)* indicated that doxorubicin plus the PF regimen (PAF) resulted in both extended DFS ($P = .003$) and extended survival ($P = .05$). We hypothesized that, in the B-11 protocol population of patients, the superiority of the PAF regimen over the PF regimen would be most evident among erbB-2-positive patients and would be minimal or even nonexistent among patients whose tumors did not overexpress erbB-2.

MATERIALS AND METHODS

Subjects

Primary tumor specimens were examined from patients enrolled on NSABP protocol B-11, in which patients with axillary lymph node-positive and estrogen receptor (ER)-negative and/or progesterone receptor (PR)-negative tumors were randomly assigned to receive either PF or PAF as adjuvant therapy *(11)*. Total accrual to the trial from June 1, 1981, to September 30, 1984, was 707 patients, of whom 682 met all protocol eligibility requirements.

Treatment regimens. Patients randomly assigned to either arm of protocol B-11 received L-phenylalanine mustard at a dose of 4 mg/m^2 given by mouth on days 1–5 every 6 weeks for17 cycles and 5-fluorouracil at a dose of 300 mg/m^2 given intravenously on days 1–5 every 6 weeks for 17 cycles. In addition, patients randomly assigned to the PAF arm received doxorubicin at a dose of 30 mg/m^2 administered intravenously every 3 weeks up to a total dose of 300 mg/m^2. The median delivered dose of doxorubicin in the PAF arm was 297 mg/m^2 (close to the intended dose of 300 mg/m^2), whereas the intensity was lower than targeted at 31.3 mg/m^2 per month. Dosing and intensity of the other drugs were similar in the two treatment arms.

Selection of the study subset. Although there were approximately 200 protocol B-11 patients for whom paraffin-embedded tumor blocks were available, this cohort was not of sufficient size to provide an adequate test of erbB-2 expression as a therapeutic response marker. To include almost all eligible protocol B-11 patients in the analysis, we used archived precut, unstained sections and hematoxylin–eosin (H&E)-stained sections, which had been collected as part of the quality-assurance program for protocol B-11. Of 682 eligible patients, 638 (93.5%) were available for erbB-2 assay. In the 44 cases for which the assay could not be performed, the reasons included the following: 1) failure of the accruing institution to submit appropriate slides, 2) absence of sections containing invasive tumor among submitted slides, and 3) use of non-formalin-based fixatives. Of the 638 cases, 512 were assayed by use of precut, unstained sections stored at room temperature. The remaining 126 cases were assayed by use of H&E-stained slides.

Immunohistochemistry for erbB-2

All immunohistochemical analyses were performed at the NSABP Pathology Section laboratory. Use of non-ideal materials required a sensitive method for immunostaining that recognizes stable epitopes. Immunohistochemical staining for erbB-2 was done by use of a cocktail of two antibodies (mAb-1 at 1 : 40 dilution and pAb-1 at 1 : 150 dilution; Zymed Laboratories Inc., South San Francisco, CA) as described previously *(12)*. The mAb-1 is a mouse monoclonal antibody that binds to an epitope on the extracellular domain of erbB-2, and pAb-1 is a polyclonal rabbit antiserum raised against a peptide derived from the intracellular domain of erbB-2. The specificity of these antibodies and cocktail preparation has been described previously *(12)*. Use of mAb-1 alone resulted in a heterogeneous staining pattern when old unstained sections were used. A more homogeneous staining pattern was achieved when pAb-1 was added. The immunostaining procedure was performed with the use of an avidin–biotin–peroxidase complex reaction as described previously by use of an OptiMax 1.5 automated immunostainer (BioGenex Laboratories, San Ramon, CA).

Data Analysis and Statistical Methods

Scoring of erbB-2 expression. Results were scored as positive or negative. The reaction was scored as positive if any tumor cell showed definite membrane staining resulting in a so-called fishnet appearance. Ambiguous staining with cytoplasmic background was scored as negative. Cases in which noninvasive carcinoma components stained positively were scored as negative if the invasive component did not stain. Cases were scored by two of the authors (S. Paik and C. Park), both of whom were blinded to both treatment assignment and clinical outcome; using a double-headed microscope, these investigators examined slides together.

Comparison of staining sensitivity. The assays of 51 cases were replicated by use of both archived precut, unstained sections and H&E-stained sections. Similarly, 60 cases were stained by use of both fresh-cut sections and archived precut, unstained sections. Results of these assays were cross-tabulated to compare the sensitivity of the staining obtained from the various materials.

Patient outcome end points. The role of erbB-2 as a predictor of treatment effect was investigated with respect to both DFS and survival; as secondary end points, we also examined recurrence-free survival (RFS) and distant disease-free survival (DDFS). DFS was defined as the time from surgery prior to recurrent breast cancer, occurrence of contralateral breast cancer, occurrence of other second primary cancer, or death from any cause. Survival was defined as the time from surgery to death from any cause. RFS was defined as the time from surgery to first local, regional, or distant tumor recurrence—with second primary cancers, contralateral events, and deaths without evidence of disease treated as censoring events. DDFS was defined as the time from surgery to tumor recurrence at distant sites, second primary cancers, and contralateral breast cancers. Distant failures of treatment were included regardless of whether they occurred as first events or subsequent to local or regional failures.

Inclusion of data. All reported analyses are based on the cohort of eligible patients for whom erbB-2 assay data are available (n = 638). Patients were analyzed as randomly assigned to treatment, regardless of noncompliance or crossovers. Similar results were obtained when all patients were analyzed regardless of eligibility or when analysis was restricted to those patients accepting their assigned treatments. The findings presented in this article are based on follow-up information received as of June 30, 1996, at which point the average time on study in either treatment arm was 13.5 years. Ten-year DFS status is known for 622 (97.5%) of the 638 patients; i.e., 622 patients are known to have had an event prior to 10 years or have been followed event free for at least 10 years.

Association of erbB-2 overexpression with other patient characteristics. The frequency of erbB-2 overexpression was associated with patient and tumor characteristics, including patient's age at surgery (≤39 years old, 40–49 years old, or ≥50 years old), self-reported menopausal status (premenopausal/perimenopausal versus postmenopausal), clinical tumor size rounded to the nearest 0.1 cm (≤2.0 cm, 2.1–4.0 cm, or ≥4.1 cm), pathologic lymph node status (one to three, four to nine, or 10 or more), and ER and PR expression (≤9 fmol/mg or >9 fmol/mg). The associations of erbB-2 overexpression with each of these covariates were tested individually by use of the chi-squared test; logistic regression was used to model the frequency of overexpression with each of these variables simultaneously.

Assessing the role of erbB-2 as a predictive marker of therapeutic response. For both erbB-2-negative and erbB-2-positive patients, survival curves and curves for DFS, RFS, and DDFS were estimated for the PF and PAF treatment regimens by use of the Kaplan–Meier method. Within erbB-2-negative and erbB-2-positive cohorts, treatment relative risks were estimated by fitting Cox proportional hazards models, and treatments were tested for equality by use of the logrank test. The erbB-2-negative and erbB-2-positive cohorts were examined with regard to differences in the magnitude of treatment effect on patient outcome by a comparison of survival curves, relative risks, and *P* values. The treatment relative risks associated with patients whose tumors were erbB-2 negative and erbB-2 positive were tested for equality by adding an interaction term to a Cox proportional hazards model and testing its significance with the Wald test. Treatment relative risks were estimated and tested for equality by both ignoring and adjusting for other patient characteristics that were prognostic for outcome, including patient's age at surgery (≤39 years old, 40–49 years old, or ≥50 years old), clinical tumor size rounded to the nearest 0.1 cm (≤2.0 cm, 2.1–4.0 cm, or ≥4.1 cm), number of pathologically positive axillary lymph nodes (one to three, three to nine, or 10 or more), and ER status (≤9 fmol/mg or >9 fmol/mg). This set of additional prognostic variables was selected on the basis of preliminary Cox proportional hazards models for DFS, survival, RFS, and DDFS, which were determined with the use of backward elimination, starting with the following list of potential covariates: treatment arm, patient's age at surgery, menopausal status, clinical tumor size, pathologic lymph node status, ER status, and PR status. Each selected covariate was found to be a statistically significant predictor at the .05 level for at least one of the four end points. All reported *P* values are two-sided.

Correction for multiple testing. Statistical tests of the hypothesis that the effectiveness of the addition of doxorubicin differs in patients with erbB-2-positive and erbB-2-negative tumors were performed by computing treatment-by-erbB-2 interaction tests for each of four end points, i.e., DFS, survival, RFS, and DDFS. The first two of these end points were considered primary, whereas the remaining two were analyzed to determine whether consistent results would be obtained. Nevertheless, there was concern that multiple testing would inflate the overall type I error rate. The use of Bonferroni's method to adjust the minimum achieved *P* value to account for the multiple tests (multiply minimum *P* value by the number of tests) is overly conservative here, since the four standardized test statistics are highly correlated. Instead, correlations were estimated by use of bootstrap resampling, and the *P* value associated with the maximum absolute *Z* value was computed by numerical integration. This quantity was also checked by use of the distribution of bootstrap replications.

Treatment of missing covariates. In the cohort of eligible patients with known erbB-2 status, complete data are available for patient's age at surgery and lymph node status. Percentages of missing values for the remaining characteristics are as follows: menopausal status, 0.5%; clinical tumor size, 7.4%; ER status, 2.5%; and PR status, 4.7%. In univariate analyses, patients with missing characteristics were omitted; in multivariate analyses, patients with missing menopausal status were assumed to be postmenopausal if their age was 50 years or greater and were assumed to be premenopausal/perimenopausal otherwise. Patients with missing clinical tumor size, ER status, or PR status were included in the corresponding modal category (2.1–4.0 cm, ⩽9 fmol/mg, and ⩽9 fmol/mg, respectively.

RESULTS

Patient Characteristics

The distribution of characteristics among eligible patients for whom erbB-2 status was ascertained is shown in Table 1. Accrual was stratified by patient's age at surgery and lymph node status; therefore, these factors were well balanced between treatments. Other factors were also generally well balanced, although there were slightly more small (⩽2 cm) tumors on the PF arm ($P = .051$) and more PR-positive women on the PAF arm ($P = .02$). There were also slightly more erbB-2 overexpressors on the PF arm, but this difference was not statistically significant ($P = .12$). Since tumor samples from 93.5% of all eligible patients were assayed to determine erbB-2 status, the distribution of characteristics shown in Table 1 and the patients' DFS and survival distributions are very similar to those seen in the entire patient population.

Table 1. Patient and tumor characteristics: eligible patients with erbB-2 assay data

Patient or tumor characteristic	Treatment arm: PF* (n = 316), %	Treatment arm: PAF† (n = 322), %
Age at surgery, y		
⩽39	23.7	24.2
40–49	32.9	31.1
⩾50	43.4	44.7
Menopausal status		
Premenopausal/perimenopausal	55.7	50.0
Postmenopausal	44.0	49.4
Unknown	0.3	0.6
Race		
White	82.0	84.2
Black	13.0	9.9
Other	4.4	4.7
Unknown	0.6	1.2
Clinical tumor size,‡ cm		
⩽2.0	28.2	20.2
2.1–4.0	42.4	49.4
⩾4.1	22.2	23.0
Unknown	7.3	7.4
No. of positive axillary lymph nodes		
1–3	49.1	53.1
4–9	33.5	29.2
⩾10	17.4	17.7
Estrogen receptor, fmol/mg		
⩽9	73.1	73.3
>9	25.3	23.3
Unknown	1.6	3.4
Progesterone receptor, fmol/mg		
⩽9	83.5	73.0
>9	13.9	20.2
Unknown	2.5	6.8
erbB-2 overexpression		
Negative	59.5	65.5
Positive	40.5	34.5

*PF = L-phenylalanine mustard + 5-fluorouracil.
†PAF = L-phenylalanine mustard + doxorubicin + 5-fluorouracil.
‡Tumor size was rounded to the nearest 0.1 cm.

Comparability of Materials and Staining Sensitivity

Of 638 primary tumor specimens assayed for erbB-2 expression, 239 (37.5%) exhibited positive immunohistochemical staining. There was no difference in the percentage of positively stained specimens according to the source of material (36.3% of unstained specimens versus 42.1% of H&E-stained specimens; $P = .23$). Assays were replicated for 51 cases by use of both unstained sections and H&E-stained sections to compare the sensitivity of the staining. There was agreement in 50 (98.0%) of 51 cases, with agreement on all 22 positive cases. One of 29 cases that were classified as negative on the basis of the assay of unstained slides was scored as positive by use of H&E-stained slides. On the basis of these data, inclusion of both unstained sections and H&E-stained sections in the study cohort appeared to be justified.

Paraffin blocks were also available for some cases, thereby permitting a comparison study. Sixty cases were stained by use of both freshly cut sections and archived precut, unstained sections. Thirty-five of these cases were negative on the basis of both freshly cut sections and archived, unstained sections. While 25 of 60 cases were positive when freshly cut sections were assayed (with three having focal and 22 having homogeneous staining patterns), all three focal positive cases stained negatively when archived, unstained sections were used. In addition, four of the 22 homogeneously stained cases showed only focal staining based on archived, unstained sections.

Association of erbB-2 Overexpression With Other Patient and Tumor Characteristics

The association of erbB-2 overexpression with patient's age at surgery, menopausal status, clinical tumor size, pathologic lymph node status, ER expression, and PR expression is summarized in Table 2. On a univariate basis, increasing numbers of involved lymph nodes ($P = .0001$) and larger clinical tumor sizes ($P = .02$) were associated with increased rates of erbB-2 overexpression, whereas ER expression was negatively associated with erbB-2 overexpression ($P = .008$). A logistic regression model relating erbB-2 overexpression with all covariates in Table 2 gave similar results.

Table 2. Association of patient and tumor characteristics with erbB-2 overexpression in eligible patients (n = 638)

Patient or tumor characteristic*	No. of patients	% erbB-2 overexpressors	*P* value†	
			Univariate analysis	Multivariate analysis
Age at surgery, y				
⩽39	153	35.3	.32	.50
40–49	204	41.7		
⩾50	281	35.6		
Menopausal status				
Premenopausal/perimenopausal	337	38.9	.44	.53
Postmenopausal	298	35.9		
Clinical tumor size,‡ cm				
⩽2.0	154	38.3	.02	.046
2.1–4.0	293	32.4		
⩾4.1	144	46.5		
No. of positive axillary lymph nodes				
1–3	326	29.4	.0001	.0002
4–9	200	46.5		
⩾10	112	44.6		
ER expression, fmol/mg				
⩽9	467	40.3	.008	.015
>9	155	28.4		
PR expression, fmol/mg				
⩽9	499	36.3	.13	.52
>9	109	44.0		

*ER = estrogen receptor; PR = progesterone receptor. Some categories do not add up to total because of missing information.
†All *P* values are two-sided.
‡Tumor size was rounded to the nearest 0.1 cm.

erbB-2 as a Predictor of Response to Doxorubicin

We compared the outcomes of patients treated with PF and PAF separately within the erbB-2-negative (n = 399) and erbB-2-positive (n = 239) cohorts. Fig.1 shows Kaplan–Meier plots for DFS, survival, RFS, and DDFS for each treatment arm (PF or PAF) among patients with erbB-2-negative and erbB-2-positive tumors. In patients with erbB-2-negative tumors, there was little separation between the Kaplan–Meier curves corresponding to the PF and PAF arms for any of the four outcome end points. However, for patients overexpressing erbB-2, the PAF arm was superior on the basis of each end point. Table 3 shows estimates of 10-year DFS, survival, RFS, and DDFS by treatment and erbB-2 status. Ten-year DFS for erbB-2-negative patients did not change when doxorubicin was added to PF (40% versus 41%). In contrast, 10-year DFS for erbB-2-positive patients improved from 26% to 41%, the same level as was seen in erbB-2-negative patients, when doxorubicin was added to PF. Similar trends were apparent for the other clinical outcome measures.

For each cohort, treatment relative risks were estimated by fitting Cox proportional hazards models, and treatments were tested for equality. To assess whether the modulation of the doxorubicin effect by erbB-2 is independent of other variables, we estimated relative risks before and after adjustment for other prognostic variables, including patient's age at surgery, clinical tumor size, pathologic lymph node status, and ER expression. The results of these analyses are shown in Fig. 2. Since the adjusted and unadjusted relative risks and their associated *P* values were similar, reference is made only to the adjusted estimates.

In Fig. 2, the benefit of PAF relative to PF is apparent among patients who were erbB-2 positive. Relative risks of failure (RRs) and 95% confidence intervals (CIs) were as follows: DFS, RR = 0.60 (95% CI = 0.44–0.83), *P* = .001; survival, RR = 0.66 (95% CI = 0.47–0.92), *P* = .01; RFS, RR = 0.58 (95% CI = 0.42–0.82), *P* = .002; and DDFS, RR = 0.61 (95% CI = 0.44–0.85), *P* = .003. Thus, event rates were significantly reduced in the PAF arm for each end point considered. In contrast, the difference between PAF and PF in patients with erbB-2-negative tumors was not statistically significant for any of the four end points. In this cohort, RRs and 95% CIs are as follows: DFS, RR = 0.96 (95% CI = 0.75–1.23), *P* = .74; survival, RR = 0.90 (95% CI = 0.69–1.19), *P* = .47; RFS, RR = 0.88 (95% CI = 0.67–1.16), *P* = .37; and DDFS, RR = 1.03 (95% CI = 0.79–1.35), *P* = .84.

The central hypothesis of this investigation was that the effect of adding doxorubicin to PF is largely confined to the erbB-2-positive subpopulation of patients and is minor or even absent in the erbB-2-negative cohort; i.e., the treatment relative risk among erbB-2-positive patients differs from that among erbB-2-negative patients. Statistical tests for the equality of the treatment relative risks associated with erbB-2-negative and erbB-2-positive patients (tests for interaction) were significant for DFS (*P* = .02) and DDFS (*P* = .02) but not for survival or RFS (*P* = .15 and *P* = .06, respectively). After adjustment for multiple testing, the overall *P* value was .04.

Since erbB-2 overexpression is associated with lymph node status, we questioned whether its ability to predict responsiveness to doxorubicin may be secondary to a similar interaction of treatment with lymph node status. To test this, we entered treatment-by-lymph node status interaction terms into the Cox proportional hazard models. For none of the four end points did the treatment-by-lymph node status interaction approach statistical significance; moreover, the *P* values for treatment-by-

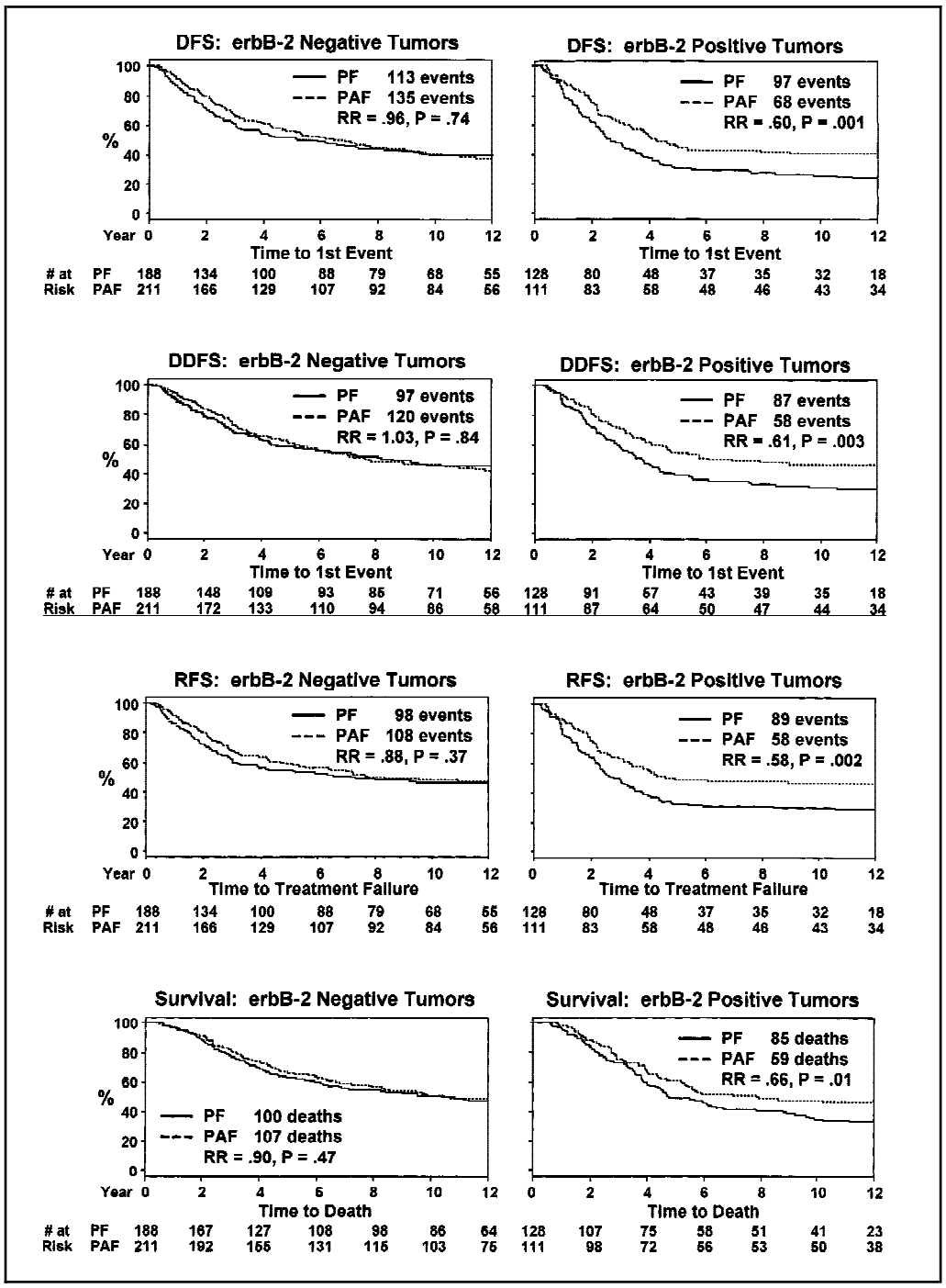

Fig. 1. Kaplan–Meier plots for L-phenylalanine mustard plus 5-fluorouracil (PF) and PF plus doxorubicin (PAF) treatment arms in erbB-2-negative and erbB-2-positive cohorts. Disease-free survival (DFS), survival, recurrence-free survival (RFS), and distant disease-free survival (DDFS) are estimated by the Kaplan–Meier method for patients whose tumors are erbB-2 negative and for patients whose tumors overexpress erbB-2. Relative risks of failure (RR) and *P* values shown on each plot are adjusted (Cox model) for patient's age at surgery, clinical tumor size, pathologic lymph node status, and estrogen receptor expression. All *P* values are two-sided.

erbB-2 interaction shown in Fig. 2 were not significantly changed. We concluded that the ability of erbB-2 to predict response to doxorubicin was not due to its association with lymph node status. Similarly, the association of erbB-2 overexpression with treatment response did not appear to be secondary to other covariates of erbB-2 overexpression, including ER status and tumor size.

In exploratory analyses, the effect of erbB-2 overexpression was investigated within subsets of patients with either one to three or four or more positive lymph nodes. For each of the four end points, there was a suggestion that erbB-2 overexpression was more predictive of response to doxorubicin in patients with four or more positive lymph nodes, as shown for DFS in Fig. 3. However, the difference in the magnitude of the erbB-2-by-treatment interaction in these two patient subsets was not statistically significant for any end point ($P = .19$; 95% CI for ratio of relative risks = 0.37–1.22).

Analysis of erbB-2 Expression as a Continuous Variable

In the analyses reported above, erbB-2 expression was treated as a dichotomous variable. However, there is no uniform agreement concerning the proper quantification of expression, and some have advocated the use of a continuous scale based on the percentage of stained tumor cells *(3).* In the ''Discussion'' section, we will present our rationale for choosing dichotomous scoring in our primary analyses. However, to facilitate compari-

Table 3. Number of events and 10-year clinical outcome according to erbB-2 status and treatment

	erbB-2-negative patients by treatment arm†				erbB-2-positive patients by treatment arm†			
	PF (n = 188)		PAF (n = 211)		PF (n = 128)		PAF (n = 111)	
End point*	No. of events	10-year estimate ± SD	No. of events	10-year estimate ± SD	No. of events	10-year estimate ± SD	No. of events	10-year estimate ± SD
DFS	113	40% ± 4%	135	41% ± 3%	97	26% ± 4%	68	41% ± 5%
Survival	100	51% ± 4%	107	51% ± 3%	85	35% ± 4%	59	48% ± 5%
RFS	98	46% ± 4%	108	48% ± 4%	89	30% ± 4%	58	47% ± 5%
DDFS	97	46% ± 4%	120	46% ± 4%	87	31% ± 4%	58	47% ± 5%

*DFS = disease-free survival; RFS = recurrence-free survival; DDFS = distant disease-free survival.
†PF = L-phenylalanine mustard + 5-fluorouracil; PAF = L-phenylalanine mustard + doxorubicin + 5-fluorouracil; SD = standard deviation.

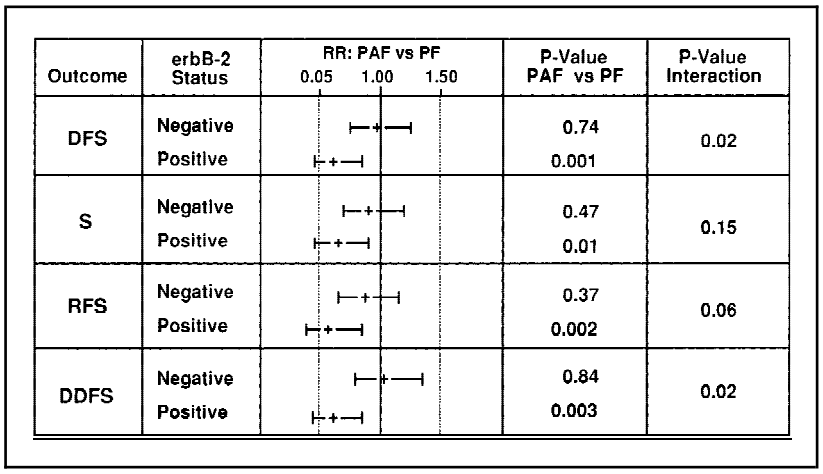

Fig. 2. Risks of patients treated with L-phenylalanine mustard, 5-fluorouracil, and doxorubicin (PAF) relative to those of patients treated with L-phenylalanine mustard plus 5-fluorouracil (PF), according to erbB-2 status. Relative risks of failure (RRs) and *P* values shown on each plot are adjusted (Cox model) for patient's age at surgery, clinical tumor size, pathologic lymph node status, and estrogen receptor expression. Bars indicate 95% confidence intervals. All *P* values are two-sided. DFS = disease-free survival; S = survival; RFS = recurrence-free survival; DDFS = distant disease-free survival.

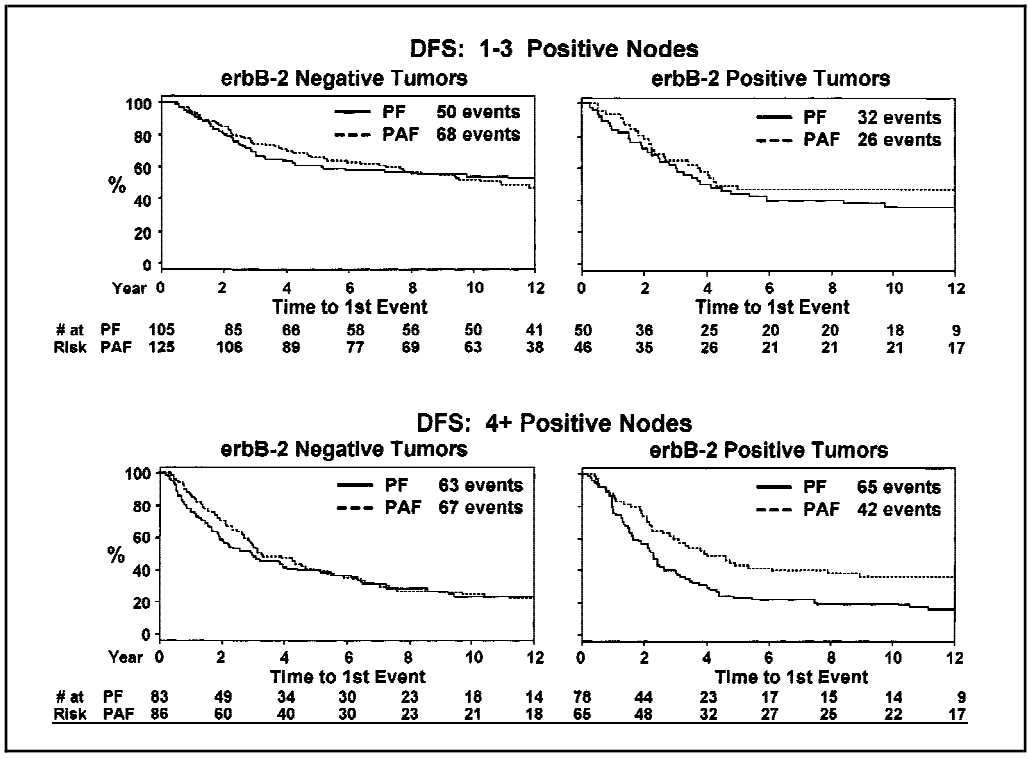

Fig. 3. Kaplan–Meier plots for L-phenylalanine mustard plus 5-fluorouracil (PF) and PF plus doxorubicin (PAF) treatment arms in cohorts defined by lymph node status and erbB-2 status. Disease-free survival (DFS) is estimated by the Kaplan–Meier method for patients with one to three positive lymph nodes and erbB-2-negative tumors, with one to three positive lymph nodes and erbB-2-positive tumors, with four or more positive lymph nodes and erbB-2-negative tumors, and with four or more positive lymph nodes and erbB-2-positive tumors.

son with other studies, we also present results based on a continuous scale of measurement.

Fig. 4 shows the distribution of patients according to the percentage of positively stained cells. Three hundred ninety-nine (62.5%) of the 638 patients had no tumor cells that stained positively and 86 (13.5%) had all cells that stained positively; the remaining patients had tumor cells that showed heterogeneous staining in varying degrees.

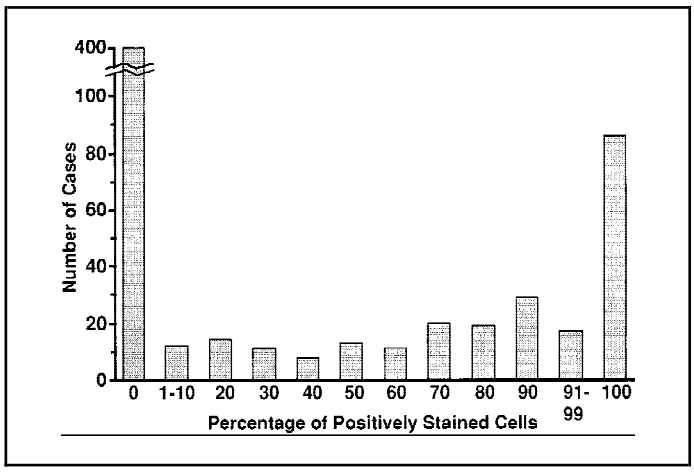

Fig. 4. Distribution of patients according to the percentage of tumor cells stained positively for erbB-2 (n = 638).

Fig. 5 summarizes the modulation of doxorubicin's effectiveness by erbB-2 expression, regarded as a continuous variable. These results were obtained by fitting Cox proportional hazards models, each containing a term representing the comparison of treatment arms, a continuous term corresponding to the level of erbB-2 expression (0%–100%), terms representing the effect of patient's age at surgery, clinical tumor size, lymph node status, and ER status, and an interaction term allowing for the possibility of a graduated treatment effect as a function of the level of erbB-2 expression. Fig. 5 displays estimates of the treatment relative risk (PAF versus PF) for patients who did not express erbB-2 at any level (0% expression) and for patients all of whose tumor cells stained positively (100% expression).

The results shown in Fig. 5 are qualitatively similar to those shown in Fig. 2, although they do not support as strongly the hypothesis that erbB-2 expression modulates the effect of adding doxorubicin to PF. As before, the benefit from doxorubicin was not statistically significant for patients whose tumors did not overexpress erbB-2; for patients whose tumors showed 100% overexpression, the benefit was statistically significant in terms of DFS, survival, RFS, and DDFS. The numerical values of the estimates of relative risk were also similar in comparing Figs. 2 and 5. However, the results of interaction tests under the continuous model were not statistically significant at the $P = .05$ level for any of the four end points. This result may be due to the fact that the benefit of doxorubicin was just as great among patients whose tumors exhibited focal staining (more than 0% but less than 50% stained cells) as was seen in patients with nearly 100% stained cells, whereas patients with no staining appeared to benefit little, if at all. Since only 26 patients treated with PF and 18 patients treated with PAF exhibited focal staining, it was impossible to determine whether this pattern of interaction is real or simply the result of chance. Although the data appeared to be most consistent with a dichotomous classification of erbB-2 expression, too few patients exhibited focal staining to draw definite conclusions in this regard.

DISCUSSION

In this study, the role of erbB-2 overexpression as a predictor of the clinical response to doxorubicin has been assessed by use of data and materials from NSABP protocol B-11, which evaluated the addition of doxorubicin to PF in lymph node-positive, ER-negative and/or PR-negative patients. It was hypothesized that the addition of doxorubicin to the PF regimen would preferentially benefit those patients in the erbB-2-positive subset. This hypothesis was investigated with respect to four measures of patient outcome: DFS, survival, RFS, and DDFS. Formal statistical tests of the hypothesis were constructed by testing for the equality of the treatment relative risks of erbB-2-negative and erbB-2-positive patients (interaction of treatment with erbB-2 status).

In the protocol B-11 population, erbB-2-positive patients who received PAF had outcomes that were superior to those who received PF. There was little difference in the outcome of erbB-2-negative patients treated with these regimens. Statistical tests for the equality of treatment relative risks of erbB-2-negative and erbB-2-positive patients were significant for DFS ($P = .02$) and DDFS ($P = .02$). Such test results were not significant at the $P = .05$ level for survival ($P = .15$) or RFS ($P = .06$). Since statistical significance was not achieved for all four end points, the results are only weakly confirmatory of the interaction hypothesis. (After adjustment for multiple testing, interaction was significant at $P = .04$.) On the other hand, a general pattern was exhibited by all four end points that was quite consistent with the earlier findings reported in CALGB study 8869/8541. We regard these results as strengthening the proposition that erbB-2 expres-

Outcome	erbB-2 Status	RR: PAF vs PF (0.05 1.00 1.50)	P-Value PAF vs PF	P-Value Interaction
DFS	0% stain		0.49	0.06
	100% stain		0.005	
S	0% stain		0.27	0.31
	100% stain		0.048	
RFS	0% stain		0.23	0.11
	100% stain		0.005	
DDFS	0% stain		0.71	0.09
	100% stain		0.02	

Fig. 5. Risks of patients treated with L-phenylalanine mustard, 5-fluorouracil, and doxorubicin (PAF) relative to those of patients treated with L-phenylalanine mustard and 5-fluorouracil (PF), according to erbB-2 status. Expression of erbB-2 expression is scored in terms of the percentage of positively stained tumor cells. Relative risks of failure (RRs) and *P* values shown on each plot are adjusted (Cox model) for patient's age at surgery, clinical tumor size, pathologic lymph node status, and estrogen receptor expression. Bars indicate 95% confidence intervals. All *P* values are two-sided. DFS = disease-free survival; S = survival; RFS = recurrence-free survival; DDFS = distant disease-free survival.

sion is a marker of preferential response to doxorubicin-containing regimens, especially because the two regimens in the B-11 protocol differed only with respect to the addition of doxorubicin.

The doxorubicin-sensitivity interpretation of the B-11 protocol data is supported by studies reporting a clinical association between erbB-2 expression and topoisomerase-IIα expression. In a survey of 230 breast cancer cases analyzed from frozen sections, Jarvinen et al. *(10)* have demonstrated a strong correlation between erbB-2 overexpression and topoisomerase-IIα expression, with a mean topoisomerase-IIα score of 9.0% for erbB-2-negative and 15.8% for erbB-2-positive tumors ($P<.0001$). The topoisomerase-IIα gene has also been reported to be abnormal (either amplified or deleted) in subsets of cases with amplification of the erbB-2 gene (also known as HER-2/neu and ERBB2). Harris and co-workers *(13,14)* reported that three of 25 cases with erbB-2 amplification also had co-amplification of the topoisomerase-IIα gene, whereas another study *(15)* reported more frequent co-amplification (three of six). Topoisomerases II are targets for doxorubicin action *(14)*. Since they are enzymes that alter the topological state of DNA by making staggered double-stranded breaks in DNA and covalently attaching to the 5′ ends of the breaks, another double-stranded DNA molecule can be passed through the break, followed by religation of free ends. Doxorubicin inhibits religation, freezing the so-called cleavable complex *(14)*. Thus, increased levels of topoisomerase-IIα protein are associated with increased sensitivity to doxorubicin *in vitro (14)*. Therefore, it is possible that erbB-2 overexpression is simply a surrogate for topoisomerase-IIα expression.

However, because the study lacked an untreated control arm, the B-11 protocol data can also be interpreted in terms of drug resistance—that tumors overexpressing erbB-2 are more resistant to chemotherapy in general *(3,4,16)*. According to this hypothesis, negligible benefit was seen from the addition of doxorubicin in the erbB-2-negative cohort of protocol B-11 patients because they had received the maximum potential benefit from chemotherapy with the PF regimen. On the other hand, patients with tumors that overexpressed erbB-2 required the more aggressive PAF regimen to overcome the increased level of resistance of their cancers to chemotherapy. Interpretation of the B-11 protocol data in terms of a resistance mechanism is supported by findings from trial V of the International Breast Cancer Study Group (IBCSG) *(2,4)*. In that trial, tumors from 746 axillary lymph node-positive patients treated with either a single cycle of perioperative chemotherapy (PeCT) with cyclophosphamide–methotrexate–5-fluorouracil (CMF) or six cycles of postoperative CMF plus prednisone (CMFp) were assayed for erbB-2. Benefit from CMFp over PeCT was pronounced in the erbB-2-negative cohort (relative risk = 0.57 [95% CI = 0.46–0.72]; $P<.0001$ for 6-year DFS) but less evident in the erbB-2-positive cohort (relative risk = 0.77 [95% CI = 0.51–1.16]; $P = .21$). Although this interaction does not achieve statistical significance, the study has been interpreted to suggest that erbB-2 overexpression may be a marker of resistance to the CMF regimen in particular or to chemotherapy in general. The IBCSG data, however, do not exclude the possibility that erbB-2-positive tumors are selectively sensitive to doxorubicin but are resistant to other regimens including CMF.

The results of CALGB study 8869/8541 have also been explained in terms of a resistance mechanism *(3,16,17)*. In that study, patients were randomly assigned to the CAF regimen at one of three dose levels. Although erbB-2-negative patients had statistically equivalent outcomes at all three dose levels, erbB-2-positive patients displayed a significant dose–response relationship. Importantly, tests for treatment-by-erbB-2 interaction gave significant results for both DFS and survival. The authors noted that, unlike IBCSG trial V, their study regimen included doxorubicin. They speculated that erbB-2 expression may be a marker of relative resistance to chemotherapy but that dose escalation of regimens including anthracyclines may successfully overcome that resistance. On the other hand, they also found it noteworthy that erbB-2-positive patients who were treated at the highest dose level fared somewhat better than erbB-2-negative patients who were treated at the same dose. This observation, if not due to chance alone, is more easily explained by the doxorubicin-sensitivity mechanism than by a resistance mechanism. In addition, since the lowest dose in the CALGB 8869/8541 study contains what is now considered a sub-therapeutic dose of doxorubicin, the similarity of clinical outcomes in the erbB-2-negative cohort can be best explained by lack of sensitivity.

In vitro investigations provide few clues concerning the resistance/sensitivity question or mechanistic explanations of the empirically observed association linking erbB-2 overexpression to the efficacy of doxorubicin. The published data in this regard are quite confusing. In a survey of non-small-cell lung cancer cell lines, Tsai et al. *(18,19)* observed a close correlation between the levels of erbB-2 expression and resistance to doxorubicin. In contrast, Harris and Carmichael *(14)* reported that, among breast cancer cell lines, those with erbB-2 overexpression tended to be sensitive to doxorubicin. In our hands, transfection of the erbB-2 gene into erbB-2-negative MCF-7 breast cancer cells did not result in consistent alterations of doxorubicin sensitivity, with significant variation among individual transfected clones (data not shown). These findings support the concept that erbB-2 expression is a correlative rather than a causative marker for doxorubicin response. While it is possible that erbB-2 is simply a surrogate for topoisomerase-IIα expression, the lack of a reliable assay for topoisomerase-IIα with the use of formalin-fixed, paraffin-embedded sections makes it difficult to address the hypothesis. Molecules other than topoisomerase-IIα are also likely to be abnormally expressed in tumor cells with erbB-2 overexpression. This could be the result of at least two distinct mechanisms: The erbB-2 amplicon (replicating fragment of the chromosome that contains multiple copies of the erbB-2 gene) may physically contain other co-amplified genes such as the topoisomerase-IIα gene; in addition, in cells that overexpress erbB-2, PEA3 (a member of the Ets family of transcriptional activators) is selectively expressed, which could in turn activate other target genes *(20)*.

Whether interpreted in terms of sensitivity or resistance, our findings indicate that the effect of adding doxorubicin to the PF regimen in protocol B-11 was notable in patients whose tumors overexpress erbB-2, suggesting that regimens containing anthracyclines may be of particular benefit in such patients. The implications of these studies in the treatment of erbB-2-negative patients are less clear. While an interpretation of the data in terms of a selective sensitivity mechanism might suggest that

such patients would be better treated with regimens not based on doxorubicin, such an implication does not follow if the findings reported here are due to a generalized resistance to chemotherapy among erbB-2-positive patients. This issue is the focus of an ongoing retrospective study of erbB-2 status and its interaction with treatment efficacy in NSABP protocol B-15, in which lymph node-positive patients less than 50 years of age and patients aged 50–59 years with PR less than10 fmol/mg were randomly assigned to receive either four courses of ''standard'' doxorubicin/cyclophosphamide (AC) (60/600 mg/m^2), six courses of CMF, or four courses of AC followed by three courses of CMF. Findings first reported in 1990 *(21)* indicated that the three regimens were equivalent in terms of outcome. But if the retrospective study were to indicate the existence of qualitative interactions between treatment regimens and patient subsets defined by erbB-2 status, the implications for the management of breast cancer would be significant.

It also will be important to determine whether we can reproduce the results reported here in NSABP protocol B-12 *(11)*. In that study, tamoxifen was added to the same regimens used in protocol B-11 to test the addition of doxorubicin in a population of hormone-responsive patients. In the B-12 protocol, no overall clinical benefit was observed when doxorubicin was added to PFT (PF plus tamoxifen). If indeed erbB-2-positive patients are the only ones who benefit from doxorubicin, the smaller proportion of such patients in this largely receptor-positive cohort could be the reason for the lack of overall difference in outcome. On the other hand, because of the cross-talk between signal transduction pathways of the erbB-2 and ERs, it is not unlikely that the modulation of doxorubicin response by erbB-2 could be different in this cohort of patients. It is also possible that stimulation of erbB-2 expression (up-regulation) by tamoxifen could influence the biologic behavior of tumors within the protocol B-12 cohort.

The current study has some limitations. The size of the B-11 trial was not designed to estimate precisely treatment-by-erbB-2 interactions, since this was not an original aim of the protocol. Even though we were able to include nearly all eligible protocol B-11 patients in this retrospective study, this limitation constrains the strength of the conclusions that can be drawn. Because the majority of retrospective studies designed to test putative therapeutic response markers will suffer from similar limitations in sample size, eventual confirmation of the hypotheses may in some cases require prospective, randomized studies stratified according to the status of the marker in question, with adequate sample size to address the postulated treatment–marker interaction.

A second limitation of the study is that the materials used were not ideal, being derived from either archived, unstained sections or sections prestained with H&E. This choice was necessitated by the availability of materials and the need to include as many patients as possible in the analysis. Our investigations indicated a very high (98%) level of agreement between results obtained from unstained slides and H&E-stained slides and justified the pooling of results obtained by the two methods. A greater concern was the comparison of results obtained from freshly cut sections with those derived from archived precut, unstained slides. These comparisons indicated that use of unstained slides was highly specific (i.e., never scored as positive those tumors that were negative on the basis of freshly cut sections) but not completely sensitive (i.e., a 12% false-negative rate was noted among tumors that were positive on the basis of freshly cut sections). This loss of sensitivity should have the effect of slightly attenuating the measurement of differences that might exist in the benefit of doxorubicin for erbB-2-negative and erbB-2-positive patients. Therefore, its effect on our analysis is conservative, in the sense that it will bias against the rejection of the hypothesis of equivalent treatment effects in the two cohorts.

The need to use unstained and H&E-stained slides as sources of material influenced our decision to base primary analyses on a dichotomized (negative/positive) scoring of erbB-2 expression rather than on the basis of the percentage of stained cells as was done in CALGB study 8869/8541 *(3)*. The percentage of cells that stained positively appeared—in our hands—to have more to do with fixation anomalies, leading to variable losses of epitope and focal patterns of staining, than to any intrinsic heterogeneity of the cells themselves. Thus, we believed that quantitation of erbB-2 expression in terms of the percentage of cells that stained was not advisable under the given conditions. Even if ideal materials were available, use of the percentage of cells that stained to quantify erbB-2 expression may be questionable, because in snap-frozen sections expression is generally present in either none or almost all cells, although the intensity of staining may reflect the quantity of the erbB-2 protein on the cell *(22)*. This would suggest that quantitative scoring of expression should be done, if at all, on the basis of intensity. Unfortunately, given the materials available for the B-11 retrospective study, quantitation in terms of intensity was not feasible.

Finally, it should be noted that the dose intensity of the doxorubicin delivered in the PAF arm of protocol B-11 (30 mg/m^2 every 3 weeks) was lower than is currently considered appropriate, and the comparator arm (PF) is no longer considered standard therapy. It is true that these considerations obscure the implications of the findings to current practice. But it is also true that, under the hypothesis of either a doxorubicin-sensitivity mechanism or a generalized resistance mechanism, these limitations should have a conservative impact on the analyses presented here: Just as is the case for the reduced sensitivity of staining due to the use of non-ideal materials, these limitations would bias against the rejection of the hypothesis of equivalent treatment effects in the two cohorts.

REFERENCES

(1) Hayes DF, Bast RC, Desch CE, Fritsche H Jr, Kemeny NE, Jessup JM, et al. Tumor marker utility grading system: a framework to evaluate clinical utility of tumor markers. J Natl Cancer Inst 1996;88:1456–66.

(2) Gusterson BA. The changing role of pathologist in the prediction of tumor behavior and response to treatment. In: Dickson RB, Lippman ME, editors. Drug and hormonal resistance in breast cancer. New York: Ellis Horwood; 1995. p. 39–53.

(3) Muss HB, Thor AD, Berry DA, Kute T, Liu ET, Koerner F, et al. c-erbB-2 expression and response to adjuvant therapy in women with node-positive early breast cancer [published erratum appears in N Engl J Med 1994;331: 211]. N Engl J Med 1994;330:1260–6.

(4) Gusterson BA, Gelber RD, Goldhirsch A, Price KN, Save-Soderborgh J, Anbazhagan R, et al. Prognostic importance of c-erbB-2 expression in breast cancer. International (Ludwig) Breast Cancer Study Group. J Clin Oncol 1992;10:1049–56.

(5) Allred DC, Clark GM, Tandon AK, Molina R, Tormey DC, Osborne CK, et al. HER-2/neu in node-negative breast cancer: prognostic significance of

overexpression influenced by the presence of *in situ* carcinoma. J Clin Oncol 1992;10:599–605.

(6) Arteaga CL, Winnier AR, Poirier MC, Lopez-Larraza DM, Shawver KL, Hurd SD, et al. p185c-erbB-2 signalling enhances cisplatin-induced cytotoxicity in human breast carcinoma cells: association between an oncogenic receptor tyrosine kinase and drug-induced DNA repair. Cancer Res 1994; 54:3758–65.

(7) Baselga J, Seidman AD, Rosen PP, Norton L. HER2 overexpression and paclitaxel sensitivity in breast cancer: therapeutic implications. Oncology (Huntingt) 1997;11(3 Suppl 2):43–8.

(8) Leitzel K, Teramoto Y, Konrad K, Chinchilli VM, Volas G, Grossberg H, et al. Elevated serum c-erbB-2 antigen levels and decreased response to hormone therapy of breast cancer. J Clin Oncol 1995;13:1129–35.

(9) Wood WC, Budman DR, Korzun AH, Cooper MR, Younger J, Hart RD, et al. Dose and dose intensity of adjuvant chemotherapy for stage II, node-positive breast carcinoma [published erratum appears in N Engl J Med 1994;331:139]. N Engl J Med 1994;330:1253–9.

(10) Jarvinen TA, Kononen J, Pelto-Huikko M, Isola J. Expression of topoisomerase IIalpha is associated with rapid cell proliferation, aneuploidy, and c-erbB2 overexpression in breast cancer. Am J Pathol 1996;148:2073–82.

(11) Fisher B, Redmond C, Wickerham DL, Bowman D, Schipper H, Wolmark N, et al. Doxorubicin-containing regimens for the treatment of stage II breast cancer: the National Surgical Adjuvant Breast and Bowel Project experience. J Clin Oncol 1989;7:572–82.

(12) Paik S, King CR, Simpson S, Lippman ME. Quantification of erbB-2/neu levels in tissue. Methods Enzymol 1991:198:290–300.

(13) Smith K, Houlbrook S, Greenall M, Carmichael J, Harris AL. Topoisomerase II alpha co-amplification with erbB2 in human primary breast cancer and breast cancer cell lines: relationship to m-AMSA and mitoxantrone sensitivity. Oncogene 1993;8:933–8.

(14) Harris AL, Carmichael J. Topoisomerase inhibitors and multiple drug resistance mechanisms in human breast cancer. In: Dickson RB, Lippman ME, editors. Drug and hormonal resistance in breast cancer. New York: Ellis Horwood; 1995. p. 303–22.

(15) Keith WN, Douglas F, Wishart GC, McCallum HM, Geroge WD, Kaye SB, et al. Co-amplification of erbB2, topoisomerase II alpha and retinoic acid receptor alpha genes in breast cancer and allelic loss at topoisomerase I on chromosome 20. Eur J Cancer 1993;10:1469–75.

(16) Tripathy D, Henderson IC. Clinical resistance in breast cancer. In: Dickson RB, Lippman ME, editors. Drug and hormonal resistance in breast cancer. New York: Ellis Horwood; 1995. p. 3–20.

(17) Berry DA, Thor A, Cirrincione C, Edgerton S, Muss H, Marks J, et al. Scientific inference and predictions; multiplicities and convincing stories: a case study in breast cancer therapy. In: Bernardo JM, Berger JO, Dawid AP, Smith AF, editors. Bayesian statistics, vol 5. Oxford (U.K.): Oxford University Press; 1996. p. 45–67.

(18) Tsai CM, Chang KT, Wu LH, Chen JY, Gazdar AF, Mitsudomi T, et al. Correlations between intrinsic chemoresistance and HER-2/neu gene expression, p53 gene mutations, and cell proliferation characteristics in non-small cell lung cancer cell lines. Cancer Res 1996;56:206–9.

(19) Tsai CM, Yu D, Chang KT, Wu LH, Perng RP, Ibrahim NK, et al. Enhanced chemoresistance by elevation of p185neu levels in HER-2/neu-transfected human lung cancer cells. J Natl Cancer Inst 1995;87:682–4.

(20) Benz CC, O'Hagan RC, Richter B, Scott GK, Chang CH, Xiong X, et al. HER2/Neu and the Ets transcription activator PEA3 are coordinately upregulated in human breast cancer. Oncogene 1997;15:1513–25.

(21) Fisher B, Brown AM, Dimitrov NV, Poisson R, Redmond C, Margolese RG, et al. Two months of doxorubicin–cyclophosphamide with and without interval reinduction therapy compared with 6 months of cyclophosphamide, methotrexate, and fluorouracil in positive-node breast cancer patients with tamoxifen-nonresponsive tumors: results from the National Surgical Adjuvant Breast and Bowel Project B-15. J Clin Oncol 1990;8:1483–96.

(22) Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, et al. Studies of the HER-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989;244:707–12.

NOTES

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