

# Chemotherapy Response of Breast Cancer Depends on HER-2 Status and Anthracycline Dose Intensity in the Neoadjuvant Setting

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## ABSTRACT

We evaluated the predictive value of a tumor's HER-2 status for chemotherapy response in the neoadjuvant setting and the effect of anthracycline dose intensity on this predictive value. HER-2 status was evaluated by immunochemistry on micro biopsy before neoadjuvant chemotherapy (monoclonal antibody CB-11; Novocastra) in 39 patients (group A) treated with FEC50 (500 mg/m<sup>2</sup> 5-fluorouracil, 50 mg/m<sup>2</sup> epirubicin, and 500 mg/m<sup>2</sup> cyclophosphamide) and 40 patients (group B) treated with FEC100 (500 mg/m<sup>2</sup> 5-fluorouracil, 100 mg/m<sup>2</sup> epirubicin, and 500 mg/m<sup>2</sup> cyclophosphamide). All tumors were stage II or noninflammatory stage III adenocarcinoma. Overall response rate (OR) was evaluated through ultrasound and mammographic measurements. Pathological complete response was evaluated by tumor excision and axillary node resection after six cycles of chemotherapy. Patient and tumor characteristics (age, tumor size, clinical nodal status, SBR grade, hormonal receptor status, and HER-2 expression) were similar in the two groups. In univariate analyses, anthracycline dose was the only factor predictive of response (OR = 61.5% with FEC50; OR = 82.5% with FEC100;  $P = 0.038$ ). When anthracycline dose was correlated with HER-2 status, an OR of 73.9% was demonstrated in HER-2<sup>-</sup> tumors (tumors without HER-2 overexpression), and an OR of 12.5% was demonstrated in HER-2<sup>+</sup> tumors (tumors with HER-2 with overexpression) in group A. In group B, an OR of 69.5% was demonstrated in HER-2<sup>-</sup> tumors, and an OR of 100% was demonstrated in HER-2<sup>+</sup> tumors. There was no difference in OR for HER-2<sup>-</sup> tumors treated with FEC50 or FEC100 ( $P = 0.74$ ). On the other hand, erbB-2<sup>+</sup> tumors treated with

FEC100 had a significantly better OR than HER-2<sup>+</sup> tumors treated with FEC50 ( $P = 0.0003$ ). In a multivariate analysis, the most powerful predictive factor of OR was a conditional variable associating anthracycline dose with HER-2 status. Low-dose anthracycline and HER-2<sup>+</sup> predicted a poor OR, low- or high-dose anthracycline and HER-2<sup>-</sup> predicted an intermediate OR, and high-dose anthracycline and HER-2<sup>+</sup> predicted a high OR. Our results merit additional studies, given the possibility for choosing anthracycline dose according to a tumor's HER-2 status.

## INTRODUCTION

The HER-2 proto-oncogene encodes a transmembrane tyrosine growth factor receptor that is overexpressed in approximately 25–30% of breast cancers (1). In preclinical studies, HER-2 overexpression was found to be associated with a variety of features that make tumor cells more aggressive (2, 3). In clinical studies, amplification or overexpression of HER-2 in tumor cells was associated with poor clinical outcome in multivariate analyses in node-positive patients (4, 5). In node-negative patients, correlation between HER-2 status and prognosis yielded conflicting results (5).

The potential role of HER-2 status for predicting response to chemotherapy in breast cancer was first examined in the adjuvant setting with the CMF<sup>2</sup> regimen. Most of these previous studies reported that overexpression of HER-2 tended to predict resistance to CMF (5). In the IBCSG study, patients were randomized to a single perioperative CMF treatment *versus* a prolonged CMF adjuvant chemotherapy (6). Benefit of prolonged chemotherapy was demonstrated only in patients whose tumors did not overexpress HER-2 (HER-2<sup>-</sup>). In Intergroup Study 0011 (high-risk node-negative patients), CMF adjuvant chemotherapy was compared with observation (7). CMF chemotherapy was not as effective in patients with tumors overexpressing HER (HER-2<sup>+</sup>) as it was in patients with HER-2<sup>-</sup> tumors. However, patients with HER-2<sup>+</sup> tumors receiving CMF had better overall survival than untreated patients with HER-2<sup>+</sup> tumors. The relation between HER-2 status and anthracycline-based regimens in the adjuvant setting was first analyzed in the Cancer and Leukemia Group B 8541 study (8). Adding more patients and additional follow-up, this study suggested that

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<sup>2</sup> The abbreviations used are: CMF, cyclophosphamide, methotrexate, and 5-fluorouracil; OR, overall response rate; pCR, pathological complete response; CAF, cyclophosphamide, Adriamycin, and 5-fluorouracil; FEC50, 500 mg/m<sup>2</sup> 5-fluorouracil, 50 mg/m<sup>2</sup> epirubicin, and 500 mg/m<sup>2</sup> cyclophosphamide; FEC100, 500 mg/m<sup>2</sup> 5-fluorouracil, 100 mg/m<sup>2</sup> epirubicin, and 500 mg/m<sup>2</sup> cyclophosphamide; CR, complete response; PR, partial response; PbR, probability of response; IHC, immunohistochemistry; FISH, fluorescence *in situ* hybridization.

Table 1 Patient characteristics

	Group A (FEC50)	Group B (FEC100)	<i>p</i>
No. of patients	39	40	
Median age (yrs)	46 (26–65)	49 (31–66)	0.37
Median tumor size (mm)	40 (25–80)	40 (25–90)	0.43
Clinically positive axillary nodes	23 (59%)	19 (47%)	0.37
SBR grade III	14 (35%)	12 (30%)	0.33
Hormonal receptor negativity	12 (31%)	12 (29%)	1
HER-2 determination	31 (79%)	33 (82.5%)	0.78
HER-2 overexpression	8 (25.8%)	10 (30%)	0.78

patients with HER-2+ tumors benefit from dose-intensive CAF (9). Retrospective analysis of two large randomized studies (NSABP B-11 and Southwest Oncology Group 8814) indicated that the addition of anthracyclines to adjuvant treatment improved clinical outcome in patients with HER-2+ tumors (10, 11).

We focused on a neoadjuvant setting to evaluate the predictive value of a tumor's HER-2 status on chemotherapy response and the effect of anthracycline dose intensity on this predictive value. Before 1996, FEC50 was the standard neoadjuvant regimen in our institution. This regimen was modified to FEC100 after 1996, when the results of the French Adjuvant Study Group were taken into consideration (12). This study in the adjuvant setting compared FEC50 and FEC100 in 565 patients with more than three involved axillary nodes or less than three nodes and an additional pejorative prognostic factor. Relapse-free survival and overall survival were statistically increased in patients treated with FEC100 with a median follow-up of 5 years. In the present study, we analyzed HER-2 expression in 39 breast tumors treated in the neoadjuvant setting before 1996 and 40 breast tumors treated after 1996. We correlated radiological and pathological responses to chemotherapy with HER-2 expression and epirubicin dose intensity.

## PATIENTS AND METHODS

**Patient and Tumor Characteristics.** Patient and tumor characteristics are listed in Table 1. Characteristics of patients in group A and group B were similar. No patient received tamoxifen as part of her neoadjuvant treatment. Pathological diagnosis, hormonal receptor and HER-2 status identification, and tumor histological grading were performed on a microbiopsy of each breast tumor before chemotherapy. All tumors were adenocarcinomas.

Immunohistochemical analysis of HER-2 status was performed using monoclonal antibody CB-11 (Novocastra). Three- $\mu$ m sections from formalin-fixed, paraffin-embedded tissues were cut and mounted on positively charged slides. Tissue sections were deparaffinized and rehydrated in graded alcohol. The slides were subjected to heat-induced epitope retrieval by immersing them in 0.01 M boiling citrate buffer (pH 6) in a pressure cooker for 3 min, followed by a 20-min cooling-off period. Slides were then incubated overnight with the monoclonal antibody directed against HER-2 protein (diluted 1:25) at 4°C. Antibody was localized using the Histostain Plus detection system (Zymed Inc.) according to the manufacturer's instructions. Diaminobenzidine (Sigma Chemical Co.) was used to

visualize antibody binding. Tumors were considered to overexpress HER-2 if more than 10% of invasive tumor cells showed definite membrane staining resulting in a so-called fishnet appearance. Negative (normal breast tissue) and positive (strongly positive carcinoma) control slides were included with each assay.

**Tumor Response Evaluation.** Assessment of tumor response was undertaken by bidimensional ultrasonic and mammographic measurements before chemotherapy and after six cycles of chemotherapy before surgery. Radiological response was recorded according to the UICC criteria: (a) CR, a disappearance of the primary tumor; (b) PR, a tumor reduction of  $\geq 50\%$ ; (c) stable disease, a tumor reduction of  $< 50\%$  or an increase in tumor size of  $< 25\%$ ; and (d) progressive disease, an increase in tumor size of  $\geq 25\%$ .

pCR was evaluated by tumor excision and axillary node resection after six cycles of chemotherapy. The OR was given as the sum of CR and PR. Pathological response was graded as being complete (pCR) if no residual invasive tumor was found in the tumorectomy or mastectomy specimen or in the axillary lymph nodes. Specimens with only noninvasive tumor cells were included in the pCR category.

**Statistical Analysis.** Categorical data were compared using Fisher's exact test.  $P < 0.05$  was considered to indicate statistical significance, and all resulting  $P$ s were two-tailed.

To estimate the simultaneous effects of prognostic factors on response rate to neoadjuvant chemotherapy, we performed a multivariate analysis using stepwise logistic regression in which the assumption of multivariate normality need not to be verified. In logistic regression, the PbR is assumed to follow the logistic model:  $\text{PbR} = \exp(\mu)/(1 + \exp(\mu))$  where  $\mu$  is a linear function of one or more independent variables expressed as  $\mu = b_0 + b_1x_1 + \dots + b_nx_n$ .

To define the model, stepwise selection was used. Variables were retained in the model if the associated two-tailed  $P$ s were 0.10 or less.

The statistical analysis was performed using BMDP statistical software (Biomedical Computer Programs, Los Angeles, CA).

## RESULTS

**HER-2 Determination.** Paraffin blocks of breast tumor before chemotherapy were available in 31 of 39 tumors in group A (79.5%) and in 33 of 40 tumors in group B (82.5%; Table 1). Overexpression of HER-2 was discovered in 8 patients in group A (25.8%) and 10 patients in group B (30.3%).

Table 2 Response rate according to patient characteristics

	OR	P
Chemotherapy		
FEC50	24/39 (61.5%)	0.038
FEC100	33/40 (82.5%)	
Age (yrs)		
<50	34/47 (72%)	0.96
>50	23/32 (71.9%)	
Tumor size (mm)		
<50	38/51 (74.5%)	0.52
>50	19/28 (67.9%)	
Clinical nodal status		
Present	29/37 (78.4%)	0.24
Absent	28/42 (66.7%)	
SBR grade		
I	6/11 (54.5%)	0.16*
II	31/42 (73.8%)	
III	20/26 (76.9%)	
Estrogen receptors		
Absent	23/29 (79.3%)	0.27
Present	34/50 (68.0%)	
Progesterone receptors		
Absent	25/33 (75.8%)	0.54
Present	32/46 (69.6%)	
HER-2 status		
Negative	33/46 (71.7%)	0.40
Positive	11/18 (61.1%)	

\* : SBR I compared to SBR II and SBR III.

**Univariate Analysis of Response Rate.** In group A, 24 patients had an overall response with 5 CRs and 4 pCRs (Table 2). In group B, 33 patients had an overall response with 8 CRs and 7 pCRs. Anthracycline dose was the only predictive factor of response, with a significantly better OR in patients treated with FEC100 (OR = 82.5%) than in patients treated with FEC50 (OR = 61.5%;  $P = 0.038$ ). Neither CR (20% versus 12.8%,  $P = 0.55$ ) nor pCR (17.5% versus 10.5%,  $P = 0.52$ ) was significantly different in patients treated with FEC100 compared with patients treated with FEC50.

**Univariate Analysis of Response Rate after Conditioning on Anthracycline Dose.** Among the HER-2<sup>-</sup> tumors, groups A and B were similar with respect to OR and pCR (Table 3). ORs were 73.9% (17 of 23 patients) and 69.6% (16 of 23 patients) respectively in patients treated with FEC50 and FEC100; pCR rates were 13.0% (3 of 23 patients) in each group. On the other hand, a significant increase in the OR and a trend toward better pCR rate were demonstrated when the regimen was changed from FEC50 to FEC100 in patients with HER-2<sup>+</sup> tumors. In these patients, OR was 12.5% (1 of 8 patients) with FEC50 and 100% (10 of 10 patients) with FEC100 ( $P = 0.0003$ ), and the pCR rate was 0% with FEC50 and 30% (3 of 10 patients) with FEC 100 ( $P = 0.22$ ).

When comparing the response in group A between HER-2<sup>-</sup> and HER-2<sup>+</sup> tumors, a significantly better OR (73.9% versus 12.5%,  $P = 0.0041$ ) was demonstrated for HER-2<sup>-</sup> tumors. In group B, there was just a trend toward better OR in HER-2<sup>+</sup> tumors (OR = 100%) compared with HER-2<sup>-</sup> tumors (OR = 69.5%,  $P = 0.073$ ).

**Multivariate Analysis of Response Rate.** To express that HER-2 overexpression could predict poor response rate (12.5%) or excellent response rate (100%) according to the

Table 3 Response rate according to epirubicin dose and HER-2 status

HER-2 status	No. of patients	OR	P
Group A—FEC50			
HER-2 <sup>-</sup>	23	17/23 (73.9%)	0.004
HER-2 <sup>+</sup>	8	1/8 (12.5%)	
Group B—FEC100			
HER-2 <sup>-</sup>	23	16/23 (69.5%)	0.07
HER-2 <sup>+</sup>	10	10/10 (100%)	

anthracycline dose, we generated a conditional variable HER-2/anthracycline dose. HER-2/anthracycline dose was coded as 1 when patients with HER-2<sup>+</sup> tumors were treated with FEC50, as 2 when patients with HER-2<sup>-</sup> tumors were treated with FEC50 or FEC100, and as 3 when patients with HER-2<sup>+</sup> tumors were treated with FEC100.

We used a stepwise logistic regression in which response rate to neoadjuvant chemotherapy was the dependent variable, and age, tumor size, clinical nodal status, anthracycline dose, HER-2 status, SBR grade, estrogen receptor and progesterone receptor status, and HER-2/anthracycline dose were the potential independent variables.

HER-2/anthracycline dose was the first variable to enter the model with a significant  $P$  (b coefficient = 3.408,  $P < 0.0001$ ). SBR grade was the second factor to enter the model (SBR I was coded as 0, and SBR II or III was coded as 1; b coefficient = 1.525,  $P = 0.048$ ). No other variables entered the model. Thus, the PbR can be expressed by the logistic model  $PbR = \exp(\mu)/(1 + \exp(\mu))$  where  $\mu = -7.021 + (3.408 \times \text{HER-2/anthracycline dose}) + (1.525 \times \text{SBR})$ . Such a model adequately fits the data with a goodness of fit  $\chi^2$  equal to 0.360 ( $P = 0.98$ ).

## DISCUSSION

The present retrospective study was the first to be carried out in the neoadjuvant setting to study the relationship between HER-2 status and anthracycline dose intensity. In 39 patients treated with FEC50 and 40 patients treated with FEC100, anthracycline dose was the only predictive factor of response with a significantly better OR in patients treated with FEC100 (OR = 82.5%) than in patients treated with FEC50 (OR = 61.5%;  $P = 0.038$ ) in univariate analyses. These two regimens were equally effective in patients with HER-2<sup>-</sup> tumors. On the contrary, FEC50 had less efficacy in HER-2<sup>+</sup> tumors. Treatment with FEC100 resulted in a trend toward better OR in patients with HER-2<sup>+</sup> tumors compared with patients with HER-2<sup>-</sup> tumors. In a multivariate analysis, the most powerful prognostic factor of response was a conditional variable combining HER-2 with the anthracycline dose defined as follows: (a) HER-2<sup>+</sup> tumor and a low dose of anthracyclines predicted a poor response rate; (b) HER-2<sup>-</sup> tumor and a low or high dose of anthracyclines predicted an intermediate response rate; and (c) HER-2<sup>+</sup> tumor and a high dose of anthracyclines predicted a high response rate. Such a model should be validated in a prospective study.

The association of HER-2 and topoisomerase II expression supports the hypothesis that HER-2 overexpression increases sensitivity to epirubicin because topoisomerase II is the molecular target of epirubicin (13).

HER-2 is routinely determined using IHC or FISH. IHC detects the protein product of the HER-2 gene on the cell membrane (1) whereas FISH detects the number of HER-2 copies/cell (14). In the present study, HER-2 status was tested by IHC using the CB-11 antibody (Novocastra). HER-2 determinations by FISH and by IHC with four different antibodies (Herceptest, P-AB 1, TAB 250, and CB-11) were compared in a clinical trial involving weekly paclitaxel and trastuzumab (15). In this study, the IHC method using the CB-11 antibody gave results that were very similar to those from the FISH method, although it is often claimed that the FISH method is more precise than IHC in evaluating overexpression of HER-2.

The results of the present study strengthen the hypothesis that HER-2+ tumors are sensitive to anthracycline dose intensity, a hypothesis that has already been put forth in three large studies in adjuvant setting.

The Cancer and Leukemia Group B 8541 study examined the cyclophosphamide, doxorubicin, 5-fluorouracil regimen with dose intensification of all three drugs (low dose, 300, 30, and 300 mg/m<sup>2</sup>; moderate dose, 400, 40, and 400 mg/m<sup>2</sup>; and high dose, 600, 60, 600 mg/m<sup>2</sup>; Ref. 16). The moderate and high-dose CAF treatments were superior to the low-dose CAF treatment, and there was a nonsignificant trend toward the highest dose for disease-free survival and overall survival. Immunohistochemical analysis of HER-2 was performed on blocks of 1013 breast cancers randomized in this study (8). In patients with HER-2+ tumors, the highest dose arm performed significantly better than either the moderate or the low-dose arm. On the contrary, patients with HER-2- tumors did not benefit from dose-intensive CAF. Because all three drugs were dose-intensified in this study, it is not possible to conclude that the effect of dose intensification on survival in patients with HER-2+ tumors is due only to doxorubicin.

Three other retrospective analyses of HER-2 status by IHC were performed in studies in which doxorubicin was the only variable. The NSABP B-11 study treated 638 breast tumors (10). This protocol tested the effect of adding doxorubicin to a regimen of l-phenylalanine and 5-fluorouracil (17). After a mean follow-up of 13.5 years, this addition significantly improved disease-free survival in patients with HER-2+ tumors, but not in patients with HER-2- tumors. A second retrospective evaluation of HER-2 status was carried out in the Southwest Oncology Group 8814 protocol, which randomized postmenopausal patients with node-positive hormone receptor-positive tumors between tamoxifen and tamoxifen + CAF regimens (11). Patients with HER-2- tumors did not gain any benefit from the addition of CAF. Patients with HER-2+ tumors had a very poor prognosis in the tamoxifen arm, but had a prognosis similar to that of patients with HER-2- tumors in the tamoxifen + CAF arm. Similar results were reported after retrospective analysis of HER-2 status in a randomized trial comparing CMF versus CAF in the adjuvant setting (18).

Other studies failed to demonstrate any predictive value of HER-2 status for response to anthracycline-based chemotherapy (19–21). These studies had fewer patients and therefore had reduced statistical power.

Anthracycline dose intensity raises several major issues. The reintroduction of anthracyclines in the metastatic setting depends on the cumulative anthracycline dose already used in a

neoadjuvant or an adjuvant setting. The estimated risk of congestive failure is 0.1% for a cumulative epirubicin dose of 300 mg/m<sup>2</sup>, 1% for a cumulative epirubicin dose of 600 mg/m<sup>2</sup>, and 5.7% for a cumulative epirubicin dose of 900 mg/m<sup>2</sup> (22). However, an increase of anthracycline dose intensity seems to be beneficial to patients with HER-2+ tumors. Use of dexrazoxane, which protects against anthracycline-caused myocardial damage, should allow us to increase the anthracycline dose in this subgroup of patients (23). However, congestive heart failure is not the only limiting factor for dose increase. Topoisomerase II inhibitors like anthracyclines have leukemogenic potential that might be enhanced by higher dose intensity (24).

These results imply that anthracycline dose intensity can be selected according to a tumor's HER-2 status. There is currently not enough data to conclude that patients with HER-2- tumors gain no benefit from increased anthracycline dose intensity, but HER-2 status should be taken into consideration if anthracyclines are to be included in the chemotherapy regimen.

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