

Circulating HER2 Extracellular Domain and Response to Chemotherapy in Metastatic Breast Cancer

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ABSTRACT

Purpose: The present study was designed to demonstrate the association between HER2 and chemotherapy resistance in patients with metastatic breast cancer.

Patients and methods: We performed a prospective assessment of the predictive value of the circulating HER2 extracellular domain (ECD) in patients with metastatic breast carcinoma using paclitaxel and doxorubicin. Serum samples were collected from 45 patients with metastatic breast carcinoma before first-line chemotherapy for metastatic disease and the levels of circulating HER2 ECD were measured using an enzyme immunoassay. Immunohistochemistry with anti-HER2 monoclonal antibody CB11 was used to assess the expression of HER2 in the primary tumors.

Results: When 450 fmol/ml was used as a cutoff, 18 patients (40%) had elevated HER2 ECD levels. Elevated levels of circulating HER2 ECD were associated with the expression of HER2 in the primary tumor tissue and with the metastatic tumor burden (evaluated with the marker CA 15-3; $p = 0.032$ and $p = 0.002$, respectively) but not with variables such as menopausal status, stage at diagnosis, previous adjuvant therapy, or the number of metastatic sites. The levels of circulating HER2 ECD correlated inversely with the response to treatment. The probability of obtaining a complete response to chemotherapy was significantly lower ($p = 0.021$) in patients with elevated HER2 ECD levels (0%; 95% confidence interval, 0-13%) in comparison with patients with non-elevated HER2 (26.9%; 95% confidence interval, 12-45%). In addition, the duration of clinical response was significantly shorter in patients with elevated HER2 ECD, in comparison with the cases with non-elevated HER2 (7.5 versus 11 months; $p = 0.035$).

Conclusion: In conclusion, elevated levels of circulating HER2 ECD in patients with metastatic breast cancer correlate with reduced efficacy of a paclitaxel-doxorubicin chemotherapy combination. We suggest that the poor response rate associated with HER2 expression in advanced breast cancer may not be reversed by aggressive chemotherapy alone.

Key Words: Metastatic breast cancer - Chemotherapy - Circulating HER2.

INTRODUCTION

Some genetic disorders in breast cancer have been associated with a poor prognosis. One of these disorders is the amplification of the HER2 oncogene [31]. The HER2 oncogene (also named *erbB-2* and *HER2/neu*) codifies for the HER2 oncoprotein (also called *p185^{erbB-2}*), which has a structure of growth factor receptor [12]. The HER2 ECD can be found in the circulation [20,37]. Overexpression of the HER2 oncoprotein in primary breast carcinomas [26] and in serum [14] has been related to a higher relapse rate.

The adverse prognostic effect of HER2 overexpression may be related to the resistance to chemotherapy [29]. Two neoadjuvant (preoperative) chemotherapy studies (using mitoxantrone plus methotrexate and CAF/cyclophosphamide-epirubicin-5-fluorouracil, respectively) have found a negative association of HER2 expression with response [19,35]. Furthermore, in metastatic breast carcinoma, two retrospective studies have found an inverse relationship between HER2 expression in primary tumor tissue [36] or serum [13] and the response to single agent mitoxantrone or cyclophosphamide.

The abbreviations used are:

ECD	: Extracellular domain.
CAF	: Cyclophosphamide-Adriamycin-5-fluorouracil.
CMF	: Cyclophosphamide-methotrexate-5-fluorouracil.
CALGB	: Cancer and leukemia group B.
PAF	: L-phenylalanine mustard-5-fluorouracil-doxorubicin.
CI	: Confidence interval.

mid-Novantrone-fluorouracil, respectively. In the adjuvant setting, the predictive value of HER2 is controversial [29]. Four studies using CMF or CMF-like adjuvant chemotherapy (International Breast Cancer Study Group Study V, Intergroup 0011, Guy's/Manchester, and Stockholm Breast Cancer Group) have shown that tumors that overexpress HER2 have shorter disease-free survival times than those with normal amounts of HER2 gene product [1,15,21,33]. No such lack of benefit was noted, however, when conventional doses of adjuvant CAF CALGB 8541 and its ancillary study 8869 [23], PAF (National Surgical Adjuvant Breast and Bowel Project Study B-11) [25], or a single cycle of perioperative CAF (European Organization for Research and Treatment of Cancer study 10854) [8] were used.

This study was designed to report the clinical outcome of 45 patients with metastatic breast cancer treated with a chemotherapy combination of paclitaxel and doxorubicin and analyzed for the expression of HER2 in the serum and primary tumor tissue.

PATIENTS AND METHODS

Forty-five patients with metastatic breast carcinoma were enrolled in the study between August 1997 and August 1999.

Eligibility criteria:

- 1- Histopathologically proven breast carcinoma.
- 2- Measurable metastatic breast carcinoma.
- 3- No previous chemotherapy for advanced disease. Adjuvant anthracyclines was acceptable if the total doxorubicin dose had not exceeded 300 mg/m² and the disease free interval was longer than 18 months.
- 4- Normal cardiac function, with a left ejection fraction of at least 60% measured by echocardiography.
- 5- Adequate hematologic, renal and hepatic function.
- 6- Relatively good performance status (Eastern Cooperative Oncology group index performance status of 2 or better) [19].

A careful history, physical examination, complete blood count, routine chemistry serum

CA 15-3, electrocardiogram, lipid profile, chest X-ray, bone scan, liver ultrasound or C.T., contralateral mammography and left ventricular ejection fraction (LVEF) by echocardiography were performed before starting treatment. To monitor for cardiotoxicity, we evaluated LVEF every three cycles. Blood counts and chemistry were performed at the beginning of each 21-day cycle of treatment. Liver ultrasound or C.T., chest X-rays and bone X-rays were repeated every three cycles (in patients with liver, lung and bone lesions, respectively) to document response to therapy. When possible, paraffin blocks from the primary breast carcinomas were obtained and immunohistochemical analysis was performed.

Treatment schedule:

Outpatient chemotherapy consisted of doxorubicin (50 mg/m², bolus) followed by a 3-hour intravenous infusion of paclitaxel (175 mg/m²), both administered on day 1. Cycles were repeated every 21 days when neutrophil and platelet counts were greater than 1.5 x 10⁹/L and greater than 100 x 10⁹/L, respectively. Treatment was delayed for a maximum of 2 weeks until these counts were reached. Paclitaxel was infused 30 minutes after the administration of a premedication consisting of dexamethazone 40 mg i.v. dexchlorpheniramine 5 mg i.v. and cimetidine 300 mg i.v.

In patients without prior adjuvant anthracycline chemotherapy, the planned duration of treatment was 10 cycles. Treatment was interrupted if the disease progressed or significant toxicities were observed. Doxorubicin was withdrawn after reaching a cumulative dose of 350 mg/m² (i.e. after the seventh cycle). Patients with prior adjuvant anthracycline therapy received 3 cycles of doxorubicin and paclitaxel (reaching a maximal cumulative doxorubicin dose of 450 mg/m²), after which they continued with paclitaxel alone until disease progression or until the 10 cycles were completed.

Efficacy parameters used for this analysis were objective response rate, as defined by WHO criteria [25] and duration of response, defined as the time elapsed from the achievement of an objective response until disease progression.

HER2 measurements:

Before treatment, a 10-ml blood sample was

drawn and centrifuged at 1000-x g for 5 min. Serum was aliquoted in two parts and stored in polypropylene cryotubes at -20°C. The levels of circulating HER2 were measured using a sandwich enzyme immunoassay, according to the manufacture's instructions (Human neu quantitative ELISA; Calbiochem) [6]. The HER2 ECD values are expressed in fmol/ml. Immunostaining for HER2 in primary carcinomas (Fig. 1) was performed using the anti-c-HER2 antibody CB11 (Biogenex) at a dilution of 1:80 and a streptavidin-biotin detection system (kit LSAB2; DAKO) [37]. Development was performed with diaminobenzidine, using Harris' hematoxylin counterstain. Membrane HER2 staining was quantified in percentages (0-100%). Cases were considered positive when 10% or more of tumor cells had intense membrane staining [37]. HER2 expression was determined in primary tumor tissue of 40 cases.

Statistical methods:

The association of HER2 with clinical parameters was evaluated with the X² test, using the Mantel-Haenszel test to evaluate linear associations. Multivariate analysis of categorical variables was performed using logistic regression. Correlations were performed using the Spearman test. The Mann-Whitney U test was used to test differences between mean values of subgroups. To evaluate the duration of response, the Kaplan-Meier estimation was used and comparisons were made with the log-rank test. For multivariate analysis of time-dependent variables, the Cox proportional hazards regression model was used. Data were entered, checked and analyzed using EPI-INFO (version 6.1) software package [11].

RESULTS

HER2 levels in serum and tumor tissue:

Circulating HER2 ECD levels ranged from 155 to 38.871 fmol/ml (median, 427 fmol/ml). Mean levels were 2085 fmol/ml, with a SE of 810 fmol/ml. When we used 450 fmol/ml as the cutoff level, 18 patients had elevated levels of HER2 (40%) and 27 patients had non-elevated values (60%).

To test the specificity of circulating HER2 ECD in advanced breast cancer, we determined the expression of HER2 in 40 of the primary breast carcinomas. As can be seen in Table (1),

73% of patients expressing HER2 in the primary tumor showed elevated circulating HER2 ECD at relapse, whereas, 66% of the patients with HER2 negative primary tumor were also negative when they developed metastasis ($p = 0.032$).

Correlation of circulating HER2 ECD and clinical parameters:

We performed correlations between circulating HER2 ECD and several clinical parameters. The results are shown in Table (1). Menopausal status, estrogen receptor status, stage at diagnosis (relapsing versus initially metastatic), previous adjuvant chemotherapy, dominant site of metastases and the number of metastatic sites did not correlate with HER2. However, elevated circulating HER2 ECD levels were significantly associated with positivity of the tumor marker CA 15-3 ($p = 0.002$), indicating that the expression of HER2 in the serum is associated with metastatic tumor burden in patients with advanced breast carcinoma.

To study the association between circulating HER2 ECD and tumor burden, we performed a correlation analysis between the logarithmically transformed values of HER2 and CA 15-3. We observed a moderate correlation between HER2 ECD and CA 15-3, which was statistically significant ($r = 0.5$; $p < 0.001$). In addition, patients with elevated circulating HER2 had higher CA 15-3 values compared with patients with non-elevated HER2 (23.5 versus 34.7 units/ml; $p = 0.011$). This confirms the association between the levels of circulating HER2 ECD and the extent of metastatic breast cancer.

We assessed the relative importance of the clinical variables and tissue HER2 on the elevation of circulating HER2 ECD. A logistic regression analysis showed that only tissue HER2 ($p = 0.016$) and CA 15-3 ($p = 0.019$) were independent variables associated with elevated serum HER2 ECD.

Correlation of circulating HER2 ECD and treatment efficacy:

Treatment efficacy was evaluable in 44 patients with circulating HER2 measurement. One patient was not evaluable for response (developed a hypersensitivity adverse event after the second treatment). Table (2) shows that 0% of the patients with elevated HER2 had a complete

response (95% CI, 0-13%), whereas 26.9% of patients with non-elevated HER2 had a complete clinical response (95% CI, 12-45%). In addition, almost twice the proportion of patients with elevated HER2 did not respond to therapy when compared with the patients with non-elevated HER2 (38.9 versus 22.2%; 95% CI, 19-59% and 10-41%, respectively). Therefore, there was a statistically significant inverse relationship between the levels of circulating HER2 and the clinical response ($p = 0.021$). The efficacy of treatment was evaluated using the duration of response. The overall median duration of response was 10 months (95% CI, 7-12 months). When we compared the duration of response in the patients with elevated or non-elevated levels of circulating HER2 ECD, we found that there were statistically significant differences (7.5 versus 11 months; $p = 0.035$). Fig. (2) illustrates that elevated circulating HER2 ECD level are associated with a shorter response duration. Cox analysis of the response duration using HER2 ECD as a continuous variable showed that HER2 ECD had a borderline statistical significance ($p = 0.06$).

Because previous treatment with adjuvant anthracyclines might have an impact on the interaction of HER2 with the chemotherapy that we used in the metastatic setting (paclitaxel and doxorubicin), analysis of treatment efficacy was performed, removing the 16 patients with past anthracycline exposure. Again the duration of response was shorter in the HER2 ECD-positive patients than in the HER2 ECD-negative patients (6.4 versus 14.9 months; $p = 0.07$).

Correlation of treatment efficacy with tissue HER2 and CA 15-3:

Table (2) shows that there was a trend for tissue HER2 expression to correlate inversely with the response to treatment. However, the association was not significant. On the other hand, CA 15-3 levels in patients with advanced breast carcinoma showed a significant inverse correlation with the response to chemotherapy.

Multivariate analysis of treatment efficacy:

To assess the relative importance of circulating HER2 on treatment efficacy in comparison with other variables, multivariate analyses was done (Table 3). A logistic regression analysis was performed first, using objective response (response versus no response) as the dependent

variable. The circulating HER2 level was the only variable that showed a significant association in the multivariate analysis ($p = 0.03$). Second, a Cox regression analysis of the duration of response was performed. This showed that of the variables entered in the model, only circulating HER2 ECD retained statistical significance in the multivariate analysis ($p = 0.04$). Therefore, circulating HER2 ECD is a significant predictor of treatment efficacy that is independent of other clinical variables.

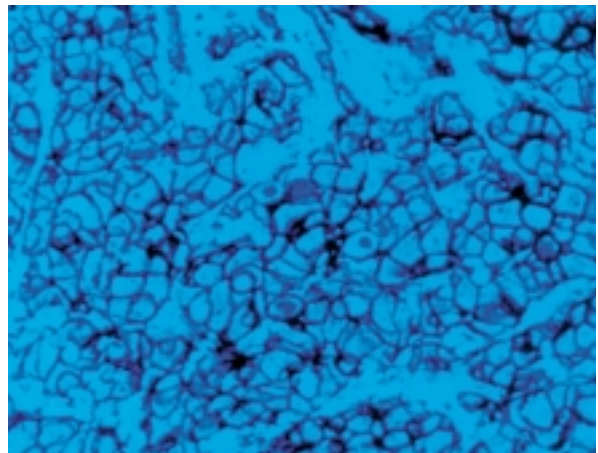


Fig. (1): HER2 expression in tumor tissue was significantly associated with its level in serum.

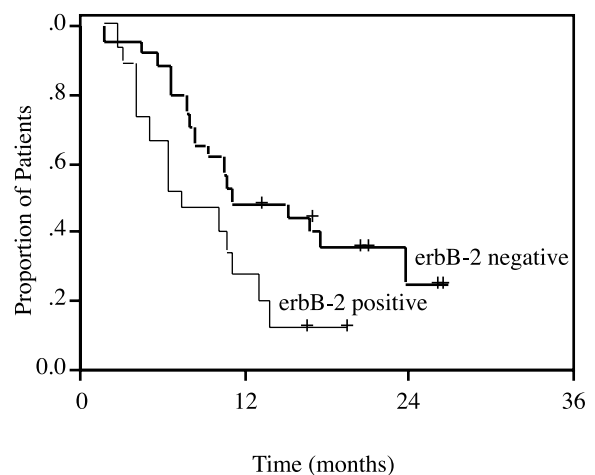


Fig. (2): Response duration stratified by HER2 ECD status. The Kaplan-Meier plot shows that patients with elevated HER2 ECD (—) had shorter duration of response than patients with non-elevated HER2 (---). The difference was significant using the log-rank test ($p = 0.035$).

Table (1): Correlation of circulating HER2 ECD and the expression of HER2 in the corresponding primary tumors and clinical variables.

	Circulating HER2 ^a		<i>p</i>
	Nonelevated, n (%)	Elevated, n (%)	
<i>Tissue HER2:</i>			
Negative	19 (66)	10 (34)	0.032
Positive ^b	3 (27)	8 (73)	
<i>Menopausal status:</i>			
Pre	10 (66.7)	5 (33.3)	NS ^c
Post	17 (56.7)	13 (43.3)	
<i>Estrogen receptor status:</i>			
Negative	8 (53.3)	7 (46.7)	NS
Positive	19 (63.3)	11 (36.7)	
<i>Type of advanced disease:</i>			
Relapse	18 (60)	12 (40)	NS
Initially metastatic	9 (60)	6 (40)	
<i>Adjuvant chemotherapy:</i>			
None	12 (52.2)	11 (47.8)	NS
Without anthracyclines	8 (80)	2 (20)	
With anthracyclines	7 (58.3)	5 (41.7)	
<i>Number of metastatic sites:</i>			
1	15 (60)	10 (40)	NS
≥ 2	12 (60)	8 (40)	
<i>Dominant site of metastases:</i>			
Soft tissue	7 (53.8)	6 (46.2)	NS
Bone	5 (83.3)	1 (16.7)	
Visceral	15 (57.7)	11 (42.3)	
<i>CA 15-3 levels:</i>			
Negative	19 (86.4)	3 (13.6)	0.002
Positive	8 (34.8)	15 (65.2)	

^a Elevated circulating HER2, ≥ 450 fmol/ml.

^b Positive tumor HER2, ≥ 10% cells with intense membrane staining.

^c NS, not significant.

Table (2): Correlation of the response to chemotherapy with circulating HER2 ECD levels, tissue HER2 expression and the levels of CA 15-3.

Objective response	Circulating HER2		Tissue HER2		CA 15-3 levels	
	Nonelevated	Elevated	Negative	Positive	Negative	Positive
CR	7 (26.9%)	0 (0%)	5 (18%)	1 (9%)	6 (27.2%)	1 (4.3%)
PR	13 (50%)	11 (61.1%)	18 (64%)	6 (55%)	9 (40.9%)	15 (65.2%)
NR	6 (22.2%)	7 (38.9%)	5 (18%)	4 (36%)	7 (31.8%)	6 (26.1%)
<i>p</i>	0.021		0.219		0.043	

Results given as n (%).

Table (3): Multivariate analysis of response rate and duration of response.

	Response rate ^a			Duration of response		
	Univariate	Multivariate		Univariate	Multivariate	
	P	p	Relative risk ^b	P	p	Relative risk ^b
Menopausal status (pre vs. post)	0.39	0.15	1.4 (0.3-5.9)	0.74	0.81	1.0 (0.3-3.1)
Type of advanced disease (relapse vs. initially metastatic)	0.79	0.57	0.5 (0.1-3.5)	0.83	0.95	1.2 (0.4-3.4)
Adjuvant chemotherapy (None vs. non-anthracycline vs. anthracycline)	0.91	0.88	0.4 (0.1-1.3)	0.85	0.64	1.2 (0.6-2.3)
Number of metastatic sites (1 vs. ≥ 2)	0.32	0.35	0.9 (0.2-3.2)	0.53	0.37	1.5 (0.6-3.5)
CA 15-3 levels (< 30 vs. ≥ 30 units/ml)	0.36	0.83	0.5 (0.1-2.3)	0.03	0.21	2.0 (0.7-5.6)
Circulating HER2 ECD (< 450 vs. ≥ 450 fmol/ml)	0.01	0.03	3.0 (0.6-13.3)	0.03	0.04	2.2 (1.0-4.9)

^a Response vs. no response. ^b Relative risk (95% CI).

DISCUSSION

HER2 oncogene expression has been related to an unfavorable prognosis in breast cancer [26,31]. Several retrospective studies have suggested that HER2 expression is associated with a reduced efficacy of adjuvant chemotherapy [29]. The present study has confirmed this point in a different patient population by finding that the level of HER2 ECD in the prospectively collected sera of patients with metastatic breast cancer correlates inversely with the response to chemotherapy. In our study, we observed marked differences in the probability of response between patients with elevated circulating HER2 ECD and those with non-elevated HER2. In addition, the quality of the response was affected by HER2 ECD level because the duration of response was significantly shorter in patients with elevated circulating HER2 ECD than in patients with non-elevated HER2 ECD (7.5 versus 11 months).

In the present study, we categorized the levels of circulating HER2 ECD using 450 fmol/ml as a cutoff. The proportion of patients with elevated HER2 in our study was identical to the positivity rate that has been reported by other authors in patients with metastatic breast cancer using the same or similar ELISAs (43, 45 and 34%, respectively) [16,22,38].

Our results showing a relationship between HER2 ECD expression and resistance to chemotherapy which are consistent with several reports in the adjuvant and neoadjuvant settings. Retrospective analysis of adjuvant chemotherapy in node-negative patients, such as the Inter-

national Breast Cancer Study Group study V [15] and the Intergroup study 0011 [1] and in node-positive patients, such as the Guy's/Manchester [21], have shown that adjuvant CMF chemotherapy has reduced efficacy in the subset of HER2-positive patients. A study by Fehm et al. [14] in node-positive breast carcinoma found that HER2 ECD-positive patients had a worse outcome than HER2-negative patients when treated with either adjuvant CMF or cyclophosphamide-Novantrone-fluorouracil. Two recent prospective studies evaluated the efficacy of preoperative chemotherapy in relation to HER2 status [19,35]. In the study by Makris et al. [19], the response rate was significantly lower in HER2-positive than in HER2-negative cases (57 versus 93%; $p = 0.007$) and in the report by Vargas-Roig et al. [35], 89% of HER2-positive patients developed distant metastases, whereas only 38% of the HER2-negative cases did ($p = 0.006$). The results of the present study were further supported by a study of Colomer et al. [9] that was carried out in 53 patients with metastatic breast cancer treated with a biweekly combination of paclitaxel and gemcitabine. In the latter study, the response rate to paclitaxel-gemcitabine was 85% in HER2-positive patients and 40% in the HER2-negative cases ($p = 0.003$) while the duration of response was 6 and 10.5 months, respectively ($p = 0.06$) [9].

Other studies, in contrast, have not found an association between HER2 expression and resistance to chemotherapy. The CALGB study 8869 [23] evaluated three doses of adjuvant CAF chemotherapy (standard, low, or very low) in 396 cases with node-positive breast cancer. They found that standard CAF was more active

than the lower doses and that this was especially true in the HER2-positive cases. The National Surgical Adjuvant Breast and Bowel Project study B-11 [26] showed that PAF adjuvant treatment in ER-negative, node-positive patients was superior to L-phenylalanine mustard-5-fluorouracil. An evaluation of HER2 expression in this latter study [26] observed that the benefit of PAF is restricted to HER2-positive patients. Because some of the drugs in the CMF and L-phenylalanine mustard-5-fluorouracil regimens are identical to those in CAF or PAF, it has been hypothesized that doxorubicin, the drug that is not common in the comparative regimens, may be more active in HER2-positive cases. A recent update of CALGB study 8541-8869, although validating the results in the 396 cases of the first report, has not been able to replicate the results in an additional cohort of 595 patients [30]. Two published retrospective studies, one in primary breast cancer [28] and another in advanced breast cancer [24], have not found a correlation between treatment response to CAF and HER2 tumor tissue expression. The retrospective design of all of these studies and the limitations of immunohistochemical analysis in archival samples [7,28] may explain some of the discrepancies observed and make the point for prospectively designed trials when evaluating biological endpoints.

In our study, we used a combination of doxorubicin and paclitaxel, two drugs that have been suggested to be more active in HER2-positive breast cancer [5,23,25,39]. HER2-associated chemoresistance has been reported to be independent of the multidrug resistance gene *mdr-1* [27,39] and recent animal experiments using cells transfected with HER2 have suggested that the lack of response to chemotherapy of HER2-positive tumors is related to the rapid proliferation of the tumor cells that survive the chemotherapy and not to an intrinsic resistance to chemotherapy [27]. In agreement with these observations, a recent investigation in which the apoptotic index was measured in primary breast carcinomas before and 24h after doxorubicin-containing chemotherapy showed that HER2-positive tumors have a markedly reduced apoptotic response to chemotherapy [2]. Therefore, the expression of HER2 may not indicate a pleiotropic resistance to chemotherapy, but rather it represents a cellular growth advantage that allows the regrowth of tumor cells after treatment. This is consistent

with the shorter duration of responses that we observed in the patients with elevated circulating HER2 ECD.

In the present study, we observed a significant association between circulating HER2 ECD levels in the serum with the expression of HER2 in the primary tumor, although the assay reagents used for the detection of HER2 in the serum and in the tumor were not the same. Our results agree with the concordance indices for HER2 of 70-80% that have been reported with variations in the diagnostic HER2 antibodies [5,7]. In our series, circulating HER2 ECD levels also correlated with an indicator of tumor burden, the marker CA 15-3 [10], reflecting that HER2 ECD serum levels depend on the amount of metastatic tumor cells. This is in agreement with the results of Krainer et al. [17], who found a very similar correlation coefficient between serum HER2 ECD and CA 15-3 in patients with metastatic breast cancer. The detection of elevated HER2 ECD levels in the absence of HER2 overexpression in the primary tumor tissue reflects the different timeframes in the collection of samples. Whereas HER2 was determined at the time of primary surgery, HER2 ECD was determined much later in the disease course, when distant metastases were present. It has been suggested that HER2 amplification may be involved in the progression of breast cancer from a hormone-dependent to a hormone-independent phenotype. A study of multiple biopsies of patients with advanced breast cancer undergoing hormonal treatment showed that HER2 amplification appeared (9-31 months after the initiation of therapy) in 6 of 34 HER2-negative patients [18].

Our data suggest that circulating HER2 ECD levels may be a better indicator of resistance to chemotherapy than the expression of HER2 in the primary tumor. However, the small number of patients in our study does not allow a definitive conclusion. An advantage for the use of serum samples over archival tissue in patients with advanced breast carcinoma is that, in general, serum samples may be obtained more easily after relapse. Therefore, the measurement of circulating HER2 in the serum may be more reliable and reproducible than the measurement of HER2 in archival paraffin blocks. Our study cannot distinguish whether HER2 ECD predicts for resistance to paclitaxel or to doxorubicin because we used the drugs in combination. The

Eastern Cooperative Oncology Group study 1193 measured circulating HER2 in patients with advanced breast cancer who received paclitaxel, doxorubicin, or the combination paclitaxel-doxorubicin. The results of this study will provide important information on this issue when published.

With the results obtained in our study, a recommendation for selecting conventional chemotherapy regimens in advanced breast cancer based on HER2 status can not be justified. In contrast, we suggest that the poor response rate that is associated with HER2 expression might be reversed with anti-HER2-specific therapies, an approach that has been effective in the laboratory [3,4] and in randomized clinical trials [32] with monoclonal antibodies.

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