Serum Levels of HER2 ECD Can Determine the Response Rate to Low Dose Oral Cyclophosphamide and Methotrexate in Patients with Advanced Stage Breast Carcinoma

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Abstract. *Background: The proto-oncogene HER2/neu encodes for a transmembrane receptor protein whose overexpression in breast cancer may be associated with poor prognosis. Its extracellular domain (HER2 ECD) can be shed into the circulation. The purpose of this study was to evaluate the predictive value of HER2 ECD in patients with advanced breast cancer. Patients and Methods: HER2 ECD was determined in 39 patients with advanced breast cancer, treated with oral cyclophosphamide and methotrexate (CM) at low doses. HER2 ECD levels were determined with the Bayer Immuno 1 HER2/neu assay before and after 2 months of chemotherapy, when all the patients were re-evaluated. Results: Based on the response to chemotherapy, the patients were divided into two groups: progressive disease (PD, 14 patients) and patients with clinical benefit (CB; i.e. patients with stable or responsive disease, 25 patients). The patients with PD had a mean baseline value of HER2 ECD of 38.3±69.2 ng/ml, while the group of CB showed lower levels (9.2±2.3 ng/ml, p<0.05). After 2 months, the mean value of HER2 ECD in the PD group increased to 71.6±146 ng/ml, while in the other group the levels did not change (9.7±2.4 ng/ml). At follow-up, significant differences were noted in both the time to progression and overall survival, with patients with increased levels of HER2 ECD at baseline (≥15 ng/ml) showing a worse clinical outcome.*

Conclusion: Increased pretreatment levels of HER2 ECD identify a subset of patients with more aggressive tumors and less response to CM chemotherapy. During therapy, increasing levels of HER2 ECD are associated with disease progression.

Amplification and/or overexpression of the HER2/*neu* oncogene is detectable in approximately 30% of primary breast tumors (1-3). This oncogene encodes for a transmembrane growth factor receptor with tyrosine kinase activity, playing a role in cell differentiation, adhesion and motility. The association between HER2/*neu* amplification and poor prognosis was first indicated by Slamon *et al.* (1). Since then, a large number of studies have evaluated the prognostic and predictive role of HER2/*neu* overexpression. It has been reported to be associated with poorer prognosis in patients with node-positive disease, while conflicting results are reported for patients with node-negative tumors (4, 5). Furthermore, tissue HER2/*neu* overexpression has been correlated with a relative resistance to the CMF regimen, a dose-response relationship to anthracycline therapy and a relative resistance to tamoxifen (6). Due to proteolytic cleavage, the extracellular domain of the HER2/*neu* receptor (HER2 ECD) may be shed into the blood. Increased circulating levels of this protein have been reported in 8-16% of women with primary breast cancer and in women with metastatic breast cancer (7), with a steady increase from non-metastatic disease to regional and to distant metastases. In the metastatic setting, most studies reported a negative prognostic value of high HER2 ECD levels (8-11), which may be related to their role in predicting the absence of response to chemotherapy or to hormonal manipulation (10, 12).

In 1997, we started a clinical trial to evaluate the clinical efficacy and tolerance of low-dose oral cyclophosphamide and methotrexate (CM) in patients with advanced breast cancer (13). Serial serum samples were collected at baseline and at monthly intervals during treatment. The purpose of this study was to determine the relationship between response rate to

treatment and HER2 ECD levels and to evaluate whether HER2 ECD correlates with the clinical course of the disease.

Patients and Methods

Patients. From July 1997 to May 2000 a total of 64 patients were enrolled in the study. Eligibility criteria included clinical stage IV breast cancer, histologically confirmed and measurable metastases, maintained bone marrow reserve, adequate renal and hepatic function, age <80 years and at least one previous line of chemotherapy for metastatic disease.

Baseline evaluation included clinical examination, chest X-ray, liver ultrasound or CT scan, bone nuclear scan, ECG and routine biochemical and hematological tests. The patients were treated with oral methotrexate (2.5 mg twice a day on days 1 and 2 every week) and cyclophosphamide (50 mg a day). Pretreatment and post-treatment blood samples were available for 39 of the 64 patients enrolled in the clinical trial. The response to treatment was evaluated after 2 months according to the WHO criteria. In case of disease progression or of toxicity the therapy was stopped, while in case of stable disease or response (partial or complete) the treatment was continued until disease progression.

Informed consent was obtained from all patients and the study was approved by the Ethical Committee of our Institution.

HER2 ECD evaluation. Serum HER2 ECD levels were determined at baseline and after two months of therapy in all the patients. For a subset of 16 patients with responsive or stable disease, the determinations were performed also during the following months of therapy. Blood samples were obtained by venipuncture and allowed to clot at room temperature. The sera were collected after centrifugation at 1080 x g for 10 min and immediately frozen at -30°C until the test was performed. The samples were analyzed with the Bayer Immuno 1 automated HER-2/neu assay (Bayer Corporation, Tarrytown, NY, USA), according to the manufacturer's instructions (14). The intra-assay and inter-assay coefficients of variation were 2% and 2.2%, respectively. A cut-off of 15 ng/ml was considered as a threshold to discriminate between normal and pathological values, as previously reported (15, 16).

HER2/neu immunohistochemistry. HER2/*neu* overexpression was immuno-cytochemically investigated on formalin-fixed paraffin-embedded sections of the primary tumors of 20 patients, using the HercepTest kit (Dako, Glostrup, Denmark) according to the manufacturer's instructions. The results were evaluated taking into account the staining intensity, the percentage of immunoreactive neoplastic cells and the completeness of membrane staining. Cases showing more than 10% neoplastic cells with moderate to intense staining of the entire cell membrane were regarded as HER2/*neu* overexpressing.

Statistical analysis. Data are represented as mean±SD of each group. For statistical evaluation, the Student's *t*-test for quantitative results and the Fisher's exact test for qualitative results were used. Time to progression (TTP) was defined as the time between the date of enrolment and the date of documented disease progression, while overall survival (OS) was defined as the time between the date of enrolment and the date of death. The Kaplan-Meier method was used to estimate survival distributions. The log-rank test was used to compare the curves. Statistical significance was taken as *p*<0.05.

Table I. *Patient characteristics.*

		No	%
Age (years)	Mean	56 ± 11	
	Range	36 – 81	
No. of metastatic sites			
1		10	25.6
2		15	38.6
≥ 3		14	35.8
Site of metastasis			
Bone		20	51.3
Node		16	41.0
Liver		14	35.8
Skin		14	35.8
Lung		12	30.8
Pelvic effusion		5	12.8
Chest wall		1	2.5
Brain		1	2.5
Breast		1	2.5
Subcutaneous		1	2.5
Spleen		1	2.5
No. of prior chemotherapy regimens for metastatic disease			
1		9	23.2
2		14	35.8
≥ 3		16	41.0
Prior hormonal therapy		25	64.1
Prior radiotherapy		1	2.5

Results

The main clinico-pathological characteristics of the 39 patients under study are shown in Table I. According to the response after 8 weeks of treatment, the patients were divided into two groups: progressive disease (PD), 14 patients and clinical benefit (CB), *i.e.* patients with stable disease or partial response, 25 patients. The mean baseline HER2 ECD concentrations were below the cut-off level for all the patients with CB (9.2±2.2 ng/ml). Higher levels were found in patients with progressive disease (38.3±69.2 ng/ml, *p*<0.05). After two months, the levels of HER2 ECD had not changed in the patients of the CB group (9.7±2.4 ng/ml); in the patients with progressive disease the levels had increased although not significantly (71.6±146 ng/ml, *p*=0.1309), and 4 patients showed a conversion from negative to positive HER2 ECD levels while under therapy. All the 8 patients with increased HER2 ECD levels at baseline or after 2 months of chemotherapy had progressive disease, whereas 25 of the 31 patients with low levels had stable or responsive disease (*p*<0.001).

The difference in serum HER2 ECD concentration (8 weeks post-chemotherapy *vs.* baseline) was significantly associated with clinical outcome (disease progression *vs.* stable and responsive disease, *p*<0.05) (Figure 1, A and B).
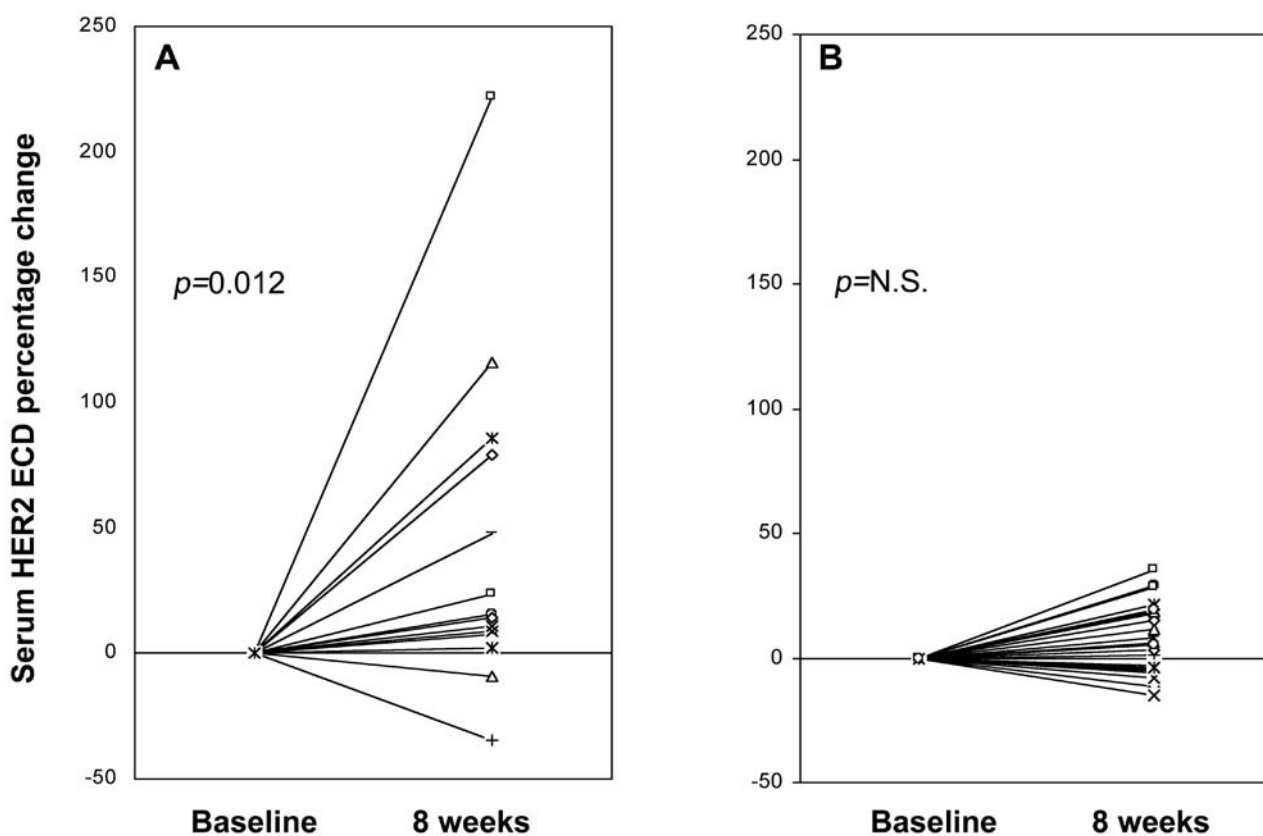
Figure 1. *Percentage change of HER2 ECD levels after 8 weeks of treatment. In patients with PD (A) a significant increase was observed (p=0.012), while (B) no change was found in patients with CB.*
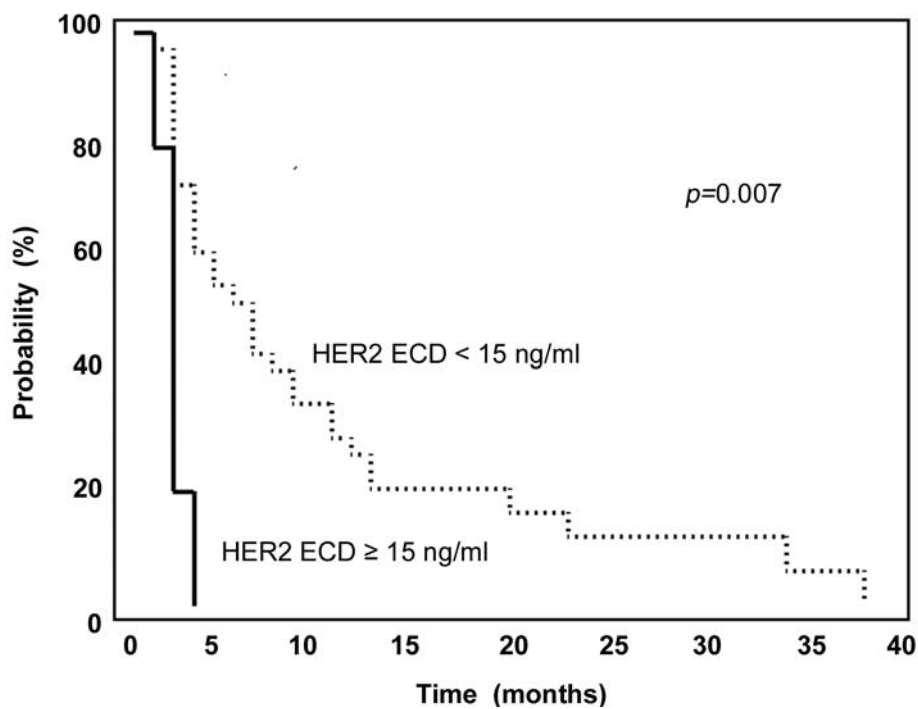

Figure 2. *Kaplan-Meier curve for the prediction of disease progression in patients with HER2 ECD levels < or ≥ 15 ng/ml at baseline. Patients with levels ≥ 15 ng/ml showed a significantly shorter TTP than patients with low levels (p=0.007).*
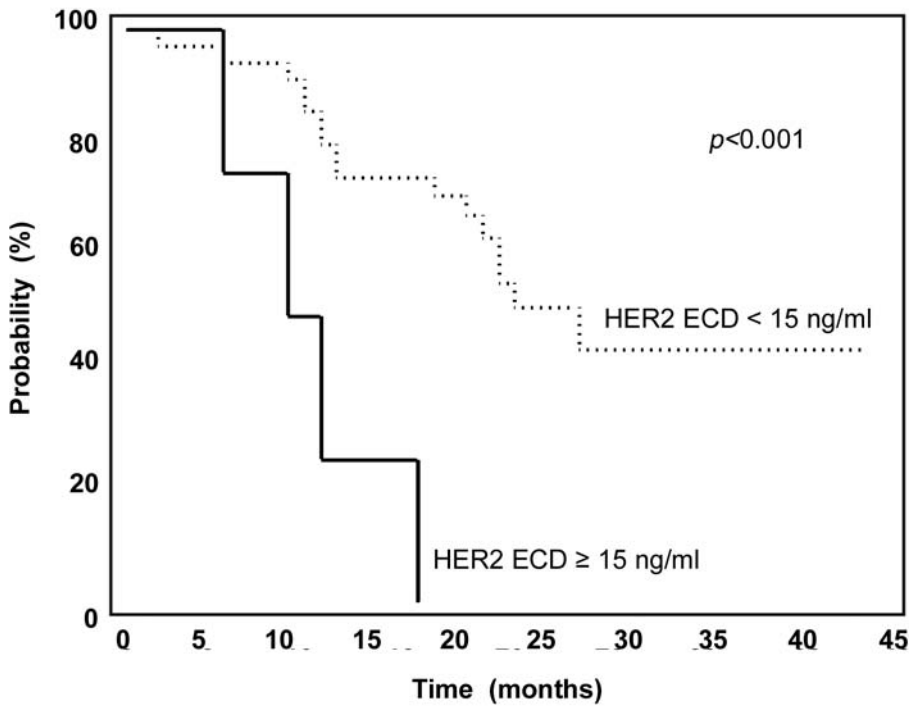
Figure 3. *Kaplan-Meier survival curve for the prediction of disease-related death in patients with HER2 ECD levels < or ≥ 15 ng/ml at baseline. Patients with levels ≥ 15 ng/ml showed a significantly shorter OS than patients with low levels (p<0.001).*
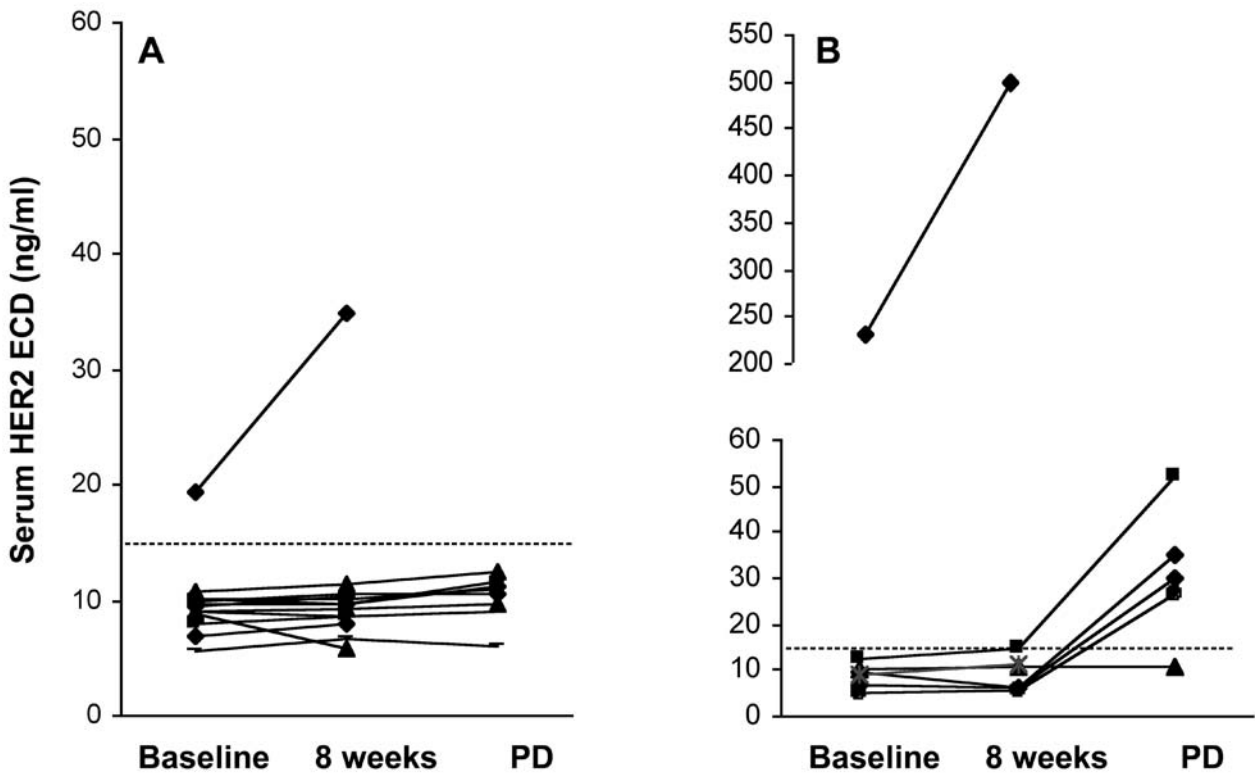

Figure 4. *Serum HER2 ECD levels at progression in patients with CB, according to tissue HER2 status: (A) patients without tissue HER2 overexpression, (B) patients with tissue HER2 overexpression.*
------- cut-off value 15 ng/ml.

Pretreatment number and site of metastases did not show any correlation with baseline levels of HER2 ECD, except for increased levels of HER2 ECD in the presence of bone metastasis ($p<0.05$).

We determined the significance of baseline HER2 ECD levels in predicting TTP and OS. We considered serum HER2 ECD as a dichotomous variable (*i.e.*, <15 ng/ml and ≥15 ng/ml). As shown in Figures 2 and 3, patients with levels ≥15 ng/ml had a shorter TTP and OS. The mean TTP and OS were 8±9 and 16±7 months in the patients with baseline HER2 ECD levels below the cut-off and 2±1 and 11±6 months in the patients with HER2 ECD levels above the cut-off ($p=0.007$ and $p<0.001$, respectively). All the patients with increased levels of HER2 ECD died within 18 months, while at that time 68 % of HER2 ECD-negative patients were still alive.

In the subset of 20 patients with available data on HER2/*neu* tissue expression, there were 13 patients without HER2/*neu* overexpression and 7 patients with overexpressing tumors. Five (71%) of these latter patients also had increased HER2 ECD levels at baseline or after 8 weeks of therapy; conversely, only 1 (8%) of the 13 patients without HER2/*neu* overexpression showed increased HER2 ECD levels at baseline or at progression. ($p=0.007$) (Figure 4).

Discussion

The results of this study indicate that increased levels of HER2 ECD in the sera of patients with advanced breast cancer identify a subset of patients with lower response rate to low dose CM chemotherapy.

Previous studies have addressed the question of whether increased levels of circulating HER2 ECD in patients with advanced breast cancer may have a role in predicting the response to various treatments. Failure to respond to endocrine treatment of metastatic ER/PR-positive breast cancer is observed in 30-50% of patients. As tumor evolution is a complex process, different autocrine or paracrine pathways may be activated that facilitate cancer growth. Wright *et al.* (17) first reported that coexpression of HER2 in ER-positive patients was associated with a reduced response rate to first-line hormone therapy of metastatic breast cancer, possibly favored by mechanisms of hormone resistance induction by HER2 overexpression. Furthermore, several studies (18-21) indicate a significantly lower response rate to endocrine therapy in women with increased levels of circulating HER2 ECD too. While HER2 overexpressing tumors show a relative resistance to chemotherapy with alkylating agents, the role of HER2 ECD in this setting is less well established (22). Increased HER2 ECD levels are not correlated with the response to anthracyclines (10), while the correlation with taxanes has not yet been elucidated, possibly because these drugs are very often administered in association

with other chemotherapics (12, 23). Finally, few publications have analyzed the role of HER2 ECD in patients treated with trastuzumab. The data published to date, though insufficient to draw definite conclusions, indicate that increased pretreatment HER2 ECD concentrations are predictive of response to Herceptin (24, 25), and that serial changes in HER2 ECD levels parallel the clinical course of the disease (24, 26, 27).

In the present study all the patients with increased pretreatment levels of HER2 ECD showed progressive disease after 8 weeks of therapy, while only 25% of HER2 ECD-negative patients failed to respond to the treatment. The proportion of positive patients at the entry of the study was quite low (10.2%), in comparison with previous studies reporting values up to 63% (7). This finding might be ascribed to the fact that the majority of our patients (76.9%) had been heavily pretreated, thus possibly affecting HER2 ECD levels. Moreover, this discrepancy may be due to differences in the assays and in the cut-off levels chosen by the different authors (8-10, 18, 20).

The patients with increased HER2 ECD levels showed a trend to a shorter OS (11±6 months *vs*. 16±7 months for patients with low levels), in keeping with previous reports documenting that patients with increased HER2 ECD levels have shorter TTP and/or OS (7). Moreover, all the patients with increased baseline HER2 ECD levels died within 18 months, while at that time 68% of the negative patients were still alive.

During the follow-up, a conversion from normal to increased HER2 ECD levels was further detected in 4 patients at the time of progression. No patient showed a decrease of HER2 ECD levels while progressing.

Increased HER2 ECD levels were significantly more prevalent in the subset of patients with HER2/*neu*-overexpressing tumors (71% *vs* 8%). Moreover, at least in some patients, the increase of HER2 ECD levels anticipated by months the occurrence of disease progression as documented by other diagnostic modalities. This is in agreement with previous studies (28, 29), showing that increased HER2 ECD levels indicate disease progression, and that the sensitivity of this assay is significantly higher if it is used to monitor patients with HER2/*neu*-overexpressing tumors.

In conclusion this study further confirmed the potential clinical utility of HER2 ECD in monitoring patients with advanced breast cancer, being associated with response to chemotherapy and disease progression.

Acknowledgements

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