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Monitoring of Serum Her-2/*neu* Predicts Histopathological Response to Neoadjuvant Trastuzumab-based Therapy for Breast Cancer

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Abstract. *Background: This prospective pilot study was performed to elucidate whether early changes in serum levels of the Her-2/neu extracellular domain (ECD) reflect histopathological response to trastuzumab-based neoadjuvant therapy in patients with Her-2/neu-overexpressing breast cancer. Patients and Methods: ECD levels were measured throughout neoadjuvant trastuzumab-based treatment in 16 patients using a Her-2/neu Microtiter ELISA. Results: In 9 (56%) patients with Her-2/neu shedding tumors (ECD >15ng/ml), ECD values (in % of baseline) of non-responders vs. responders were 117% vs. 55% on day 8 (p=0.014), 157% vs. 58% on day 22 (p=0.061) and 114% vs. 46% at restaging (p=0.049). Conclusion: Serial monitoring of serum Her-2/neu ECD levels may represent a valuable tool to predict pathological response to trastuzumab-based neoadjuvant therapy in patients with Her-2/neu-overexpressing tumors.*

Neoadjuvant chemotherapy can increase surgical resectability and breast conserving surgery rates in patients with breast cancer (1-5). Assuming that response of the primary tumor mirrors the effects on occult micrometastases, most of these studies have demonstrated that histopathological response to induction therapy represents the most valuable predictor of disease-free and overall survival, whereas clinical response correlates poorly with histopathological response or long-term clinical outcome (1-10). Thus, the identification of parameters indicative of pathological response remains an issue of great interest to reduce treatment-related morbidity and optimize resource allocation. Aimed at an increase in response rates, a series of neoadjuvant trials are currently evaluating agents that have been shown to be highly active in patients with metastatic disease, including trastuzumab (11-13).

We and others have previously demonstrated that the Her-2/*neu* extracellular domain (ECD) is proteolytically cleaved from the cellular surface of Her-2/*neu*-overexpressing tumors and can be detected at increased levels in the sera of patients with Her-2/*neu*-overexpressing breast cancer (14-17). Based upon previous reports from our laboratory demonstrating that early changes in serum Her-2/*neu* levels predict clinical response to trastuzumab-based treatment in patients with metastatic breast cancer (18), the present pilot study was performed to evaluate the potential of ECD levels to predict histopathological response to trastuzumab-based neoadjuvant treatment.

Patients and Methods

Study population. Patients had immunohistochemically (HercepTest®, DAKO Diagnostics, Austria) confirmed grade 3+ Her-2/*neu*-overexpressing AJCC (19) stage IIA to IIIB breast cancer and received weekly neoadjuvant treatment with trastuzumab (Herceptin®, Roche Pharmaceuticals, Vienna, Austria), in combination with epirubicin and docetaxel (13), or with vinorelbine (20) in two multiinstitutional phase II studies. Histological diagnosis was performed by core needle biopsy and staging included thorough history and clinical examination, mammography and breast ultrasound, bone scan and thoracic and abdominal CT scan, respectively. In accordance with our institutional ethical committee guidelines, signed informed consent to participate in the present study was obtained from all patients before blood was drawn from the same venous access afterwards

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Table I. *Patient characteristics and clinical disease stage in relation to serum Her-2/neu ECD levels observed before neoadjuvant trastuzumab-based therapy.*

		Serum Her-2/neu ECD levels	
		<15ng/ml	>15ng/ml
T	T2	4	4
	T3	1	4
	T4	2	1
N	N0	3	4
	N1	4	5
Stage	IIA	1	2
	IIB	3	3
	IIIA	1	3
	IIIB	2	1
Grading	G2 (moderately-differentiated)	4	3
	G3 (poorly-differentiated)	3	6
Inflammatory	yes	3	2
breast cancer	no	4	7
Estrogen receptor	positive	4	5
	negative	3	4
Progesterone	positive	0	0
receptor	negative	7	9
Histopathological	complete response	0	1
response	*in situ* carcinoma only	0	1
	regressive changes	2	6
	no change	5	1

used for infusion of trastuzumab. Major exclusion criteria for this trial were previous therapy for breast cancer, previous antibody-based therapy for any (non)malignant condition, autoimmune disease, second malignancy, active infection and altered mental status not allowing the giving of informed consent. A total of two 6-week cycles were administered unless there was evidence of disease progression according to the Southwest Oncology Group standard response criteria (21), consent withdrawal or toxicity prompting cessation of treatment. Surgery (segmental resection with axillary lymph node dissection or modified radical mastectomy as surgically indicated) was scheduled within 4 weeks after completion of the neoadjuvant therapy.

Sixteen patients (median age: 56.1 years, range 27.6 to 77.4) with Her-2/*neu*-overexpressing breast cancer were enrolled. An overview of the patients' characteristics, serum ECD levels and frequencies of response is depicted in Table I. After a median (range) observation period of 24.9 (16.4-33.0) months, 1 patient (not achieving a pathological response) had progressed to metastatic disease and all patients were alive. Median progression-free and overall survival have not been reached.

Acquisition and analysis of serum probes. Blood was drawn into native tubes immediately before initiation of trastuzumab-based treatment, on day 8, day 22 and on the date of response evaluation, centrifuged and the resulting sera analyzed using a Sequential Solid Phase Sandwich Human Her-2/*neu* Quantitative ELISA (Her-2/*neu* Microtiter ELISA, Oncogene Science/Bayer Immunodiagnostics, Cambridge, MA, USA). Microplates were washed using an automated washer (Dias Microplate Washer, Dynex Technologies, Denkendorf, Germany). The absorbance reading was performed using an automated reader (FLUOstar Galaxy, BMG Labtechnologies, Offenburg, Germany) and calculated using the Fluoscan Galaxy software (vers. 4.20-0, BMG Labtechnologies, Offenburg, Germany). The intra- and inter-assay precision were <5% CV.

Evaluation of histopathological response to treatment. Response assessment was performed on surgical specimens in accordance with previous neoadjuvant trials (5, 22, 23). In brief, the criteria of pathological responses were: pathological grade 1: disappearance of all tumor on macroscopic and microscopic assessment; grade 2: presence of *in situ* carcinoma of the breast without invasive tumor and no tumor found in the lymph nodes; grade 3: presence of invasive carcinoma with marked stromal alteration, such as sclerosis or fibrosis and no macroscopic disease evident in the surgical specimen; grade 4: no or few modifications of the tumoral appearance. Specimens exhibiting grade 1 to 3 changes were classified as pathological responses, whereas grade 4 samples were considered as failures.

Statistical analysis. Frequencies of patients' characteristics were compared using Fisher's exact test while proportional ECD values (percentage of baseline values) between patients with or without pathological response to trastuzumab-based therapy were compared by the Mann-Whitney's *U*-test using SPSS statistical software system (SPSS Inc., Chicago, IL, USA version 10.0).

Results

Influence of clinical and tumor characteristics and baseline ECD values on pathological response. When initial clinical tumor characteristics including clinical tumor and lymph node status, clinical stage, presence or absence of inflammatory histology, histopathological grading and hormone receptor status were analysed, no significant differences in pathological response rates to subsequent trastuzumab-based treatment were found, mostly because the study size was small (all p-values >0.05, descriptive data not shown).

As defined by the upper normal limit of 15 ng/ml (24-27), 9/16 (56%) patients presented with increased serum levels suggesting tumoral shedding of the Her-2/*neu* ECD into the bloodstream. Overall, the mean (range) serum ECD level was 31.83 (5.27-165.36) ng/ml. No significant differences in pathological response rates were found between patients with normal and those with elevated baseline Her-2/*neu* levels (all p-values >0.05, Table I). None of the patients with normal baseline ECD levels had elevated ECD values at any time point during treatment.

Monitoring of serum Her-2/neu ECD concentrations during neoadjuvant trastuzumab. In patients with elevated ECD levels before initiation of treatment, a significant increase in ECD levels was observed in those without histopathological

Table II. *Comparison of Her-2/neu ECD levels between the patients with and without histopathological response to neoadjuvant trastuzumab in patients with elevated (>15ng/ml) baseline ECD levels.*

	Median (range) Her-2/neu ECD values (in % of baseline)		
	No response	Histopathological response	*P*-value*
Day 8	117.3 (106.7-151.2)	55.1 (19.2-106.8)	0.014
Day 22	156.6 (90.7-222.5)	57.5 (22.4-132.1)	0.061
Restaging	113.7 (54.9-186.8)	45.9 (8.1-98.9)	0.049

* Mann-Whitney test

evidence of response to treatment (all *p*-values for measurements on day 8, day 22 and restaging *vs*. baseline <0.05), whereas in those responding to treatment a significant decrease in ECD levels was found (all *p*-values for measurements on day 8, day 22 and restaging *vs*. baseline <0.05). As shown in Table II, patients achieving a histopathological response to treatment had lower ECD levels (relative to baseline) at all time points throughout treatment as compared to those not responding to treatment.

Discussion

A series of studies have attempted to identify predictive factors for the efficacy of neoadjuvant chemotherapy including tumor stage, histopathological grade, DNA ploidy, proliferation markers, hormone receptor status and tumoral Her-2/*neu* expression, but results have been discordant due to differences in patient characteristics, treatment protocols or assessment of these parameters. Aside from the intensity of immunohistochemical Her-2/*neu* overexpression and the presence of amplification of the Her-2/*neu* oncogene, factors predictive for response to trastuzumab are so far lacking. Since patients with Her-2/*neu*-overexpressing tumors, and particularly those with inflammatory breast cancer (the latter representing almost one-third of our study cohort), are at highest risk of relapse and death, the evaluation of innovative treatment modalities represents a valuable task. However, treatment with trastuzumab is resource intensive and potential toxicity must be considered in patients treated with curative intent. Thus, the identification of patients with Her-2/*neu*-overexpressing breast cancer most likely to benefit from this treatment modality has to be emphasized.

In this pilot study we demonstrated that, in patients with elevated pretreatment serum Her-2/*neu* ECD levels, the dynamics of ECD levels in the course of treatment mirror the histopathological response of the tumor. Thus, early changes in serum levels of this oncoprotein may have the potential of predicting histopathological response to treatment. However, our pilot study was underpowered to determine whether monitoring of serum Her-2/*neu* ECD levels can also reliably identify patients achieving a complete pathological response, which represents the strongest indicator of long-term disease control.

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