Featured Article

Monitoring of Serum Her-2/*neu* Predicts Response and Progression-Free Survival to Trastuzumab-Based Treatment in Patients with Metastatic Breast Cancer

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Abstract

Purpose: **The present pilot study was performed to elucidate whether early changes in serum Her-2/*neu* extracellular domain (ECD) levels during trastuzumab-based treatment would predict the clinical course of disease in patients with metastatic breast cancer.**

Experimental Design: **Sera from 55 patients with Her-2/*neu*-overexpressing metastatic breast cancer obtained immediately before each weekly administration of trastuzumab were analyzed by a serum Her-2/*neu* ELISA.**

Results: **Whereas response rates were significantly higher in patients with elevated (≥15 ng/ml) ECD levels before initiation of treatment (35% *versus* 7%, $P = 0.045$), progression-free and overall survival did not differ significantly between patients with normal and elevated ECD levels. In patients responding to treatment, ECD levels decreased significantly as early as from day 8 of treatment onwards (all *P* for weekly measurements *versus* baseline <0.001). In contrast, no significant change in ECD levels was observed in patients with progressive disease. Multiple logistic regression analyses identified kinetics of ECD levels as the only factor that allowed for the accurate prediction of response likelihood as early as from day 8 of trastuzumab-based treatment onwards ($P = 0.020$). In addition, determination of serial ECD levels allowed for the prediction of the risk for disease progression within the observed period as early as day 15 of treatment ($P = 0.010$).**

Conclusions: **Serial monitoring of the ECD may represent a valuable tool for early prediction of the probability of response and progression-free survival to trastuzumab-based treatment and is thus likely to contribute to an optimization of treatment and resource allocation.**

Introduction

The human epidermal growth factor (Her-2/*neu*) oncoprotein is composed of an intracellular tyrosine kinase portion, a short transmembrane section, and an extracellular ligand-binding domain (ECD, p105). The latter is frequently cleaved from the cellular surface of Her-2/*neu*-overexpressing cells resulting in ligand-independent receptor activation and downstream signaling (1–5). Overexpression of the Her-2/*neu* protein arises predominantly through amplification of the c-erbB-2/*neu* oncogene and occurs in 25–30% of breast carcinomas. Both Her-2/*neu* overexpression and increased serum levels of the Her-2/*neu* ECD, which can be observed in up to 70% of women with Her-2/*neu*-overexpressing metastatic breast cancer (depending on the intensity of membranous Her-2/*neu* overexpression, tumor mass, and metastatic pattern; Refs. 6, 7) have frequently been associated with increased biological aggressiveness including poorer response rates to alkylating agent-based chemotherapy and endocrine therapy resulting in shorter progression-free and overall survival in this patient cohort (6–9).

Trastuzumab, a monoclonal antibody targeting the Her-2/*neu* oncoprotein has not only demonstrated objective responses when administered as a single agent (6, 10–12), but its combined use with chemotherapy has also prolonged progression-free and overall survival as compared with standard treatment in patients with Her-2/*neu*-overexpressing metastatic breast cancer (9, 13–16). However, the introduction of this treatment modality has caused concern about the availability of appropriate economic resources. With approximately 170,000 cases of breast cancer being diagnosed in the United States and in the European Union per year and major adjuvant treatment trials with trastuzumab underway, the use of trastuzumab is increasingly important for both patients and health economists. To date, further predictive factors for response to trastuzumab-based therapy apart from tumor characteristics of Her-2/*neu* protein overexpression or oncogene amplification, which would allow for the more targeted use of this treatment modality either as single agent or in combination with cytotoxic chemotherapy, are lacking. Therefore, we have attempted to identify such a factor by investigating the predictive potential of the serum Her-2/*neu* ECD. This assumption was based on previous experiments from

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our laboratory that have demonstrated that the Her-2/*neu* ECD competes with full-length p185 in binding to anti-Her-2/*neu* antibodies, thus neutralizing the biological effects of trastuzumab *in vitro* (17, 18). Results from our laboratory also corroborate other clinical studies, which have demonstrated a significantly decreased plasma half-life of trastuzumab in patients with high serum levels of Her-2/*neu* ECD (6, 9), and demonstrate the potential for interference of the Her-2/*neu* ECD with the biological effects of trastuzumab in *vivo*.

By extending these trials, we have now performed the present pilot study using serial measurements of serum Her-2/*neu* ECD to elucidate the predictive value of ECD baseline values and of early changes of serum ECD levels during weekly trastuzumab-based therapy.

We report that early changes in serum ECD levels during treatment with trastuzumab predict both subsequent response and progression-free survival to trastuzumab-based treatment.

Patients and Methods

Eligibility. Potentially eligible patients had Her-2/*neu*-overexpressing (immunohistochemistry 2+ or 3+ as determined by the HercepTest; DAKO Diagnostics, Austria) metastatic breast cancer and were scheduled to receive trastuzumab (Herceptin; Roche Pharmaceuticals, Vienna, Austria) ± chemotherapy in accordance with previously published treatment protocols (6, 9, 11, 13, 14, 16, 19, 20) at the discretion of the treating physician. All study participants had bi-dimensionally measurable (with both diameters >1.0 cm and at least one diameter >1.5 cm) disease (excluding previously irradiated lesions) with clearly defined margins and radiologically (computed tomography and/or magnetic resonance imaging and/or ultrasound) documented tumor progression during the preceding 3 weeks. An interval of at least 4 weeks and recovery from all preceding treatment-related toxicities (excluding alopecia) was required between completion of prior therapy and study entry. Data on estrogen and progesterone receptor status of tissue samples from which the original assessment of Her-2/*neu* overexpression had been performed were available from pathology records. Patients who had previously received treatment with monoclonal antibodies, tumor vaccines, or biological-response modifiers were not eligible. Further inclusion criteria were Eastern Cooperative Oncology Group performance status 0–2, age ≥19 years, estimated life expectancy ≥12 weeks. In accordance with our institutional ethical committee guidelines, signed informed consent to participate in the present study was obtained from all patients; before each week, 8 ml of blood were drawn from the same venous access subsequently used for infusion of trastuzumab. Further exclusion criteria were bone metastases as the only site of active disease, second malignancy with the exception of *in situ* cervical cancer, adequately treated basal cell or squamous cell carcinoma of the skin, history of congestive heart failure (unless medically controlled), myocardial infarction within the last 2 years, active infection, altered mental status that would prohibit the understanding and the giving of informed consent, pregnancy, or breast feeding. In case of concomitant treatment with chemotherapy, patients with inadequate hematological function as defined by WBCs $< 2.0 \times 10^9$/liter, absolute neutrophil count $< 1.5 \times 10^9$/liter, platelets $< 50 \times 10^9$/liter, severe hepatic or renal dysfunction (bilirubin > 2.0× or aspartate aminotransferase > 3.0× upper limits of normal, creatinine clearance < 30 ml/min) were not eligible.

Treatments. Trastuzumab (4 mg/kg of body weight i.v. loading dose for 90 min followed by a weekly 2 mg/kg maintenance dose for 30 min, as described previously; Ref. 6) was administered until evidence of disease progression, consent withdrawal, or toxicity prompting cessation of treatment. In addition, patients were allowed to receive chemotherapy according to published protocols (9, 13, 14, 19) immediately after trastuzumab at the discretion of their treating physician. After completion of six to eight treatment cycles, patients achieving an objective response or disease stabilization to trastuzumab plus chemotherapy were offered maintenance therapy with single-agent trastuzumab until disease progression. Cardiac safety was routinely monitored by echocardiography every other month during treatment and if clinically indicated. Treatment was withheld until resolution of symptoms in patients experiencing grade ≥ 2 hematological and/or grade ≥ 3 nonhematological toxicity (with the exception of alopecia) according to National Cancer Institute Common Toxicity Criteria. In addition, patients experiencing grade 4 hematological or grade ≥ 3 nonhematological (with the exception of alopecia) toxicity or a decrease in cardiac-resting ejection fraction of ≥20% of baseline value or to less the lower normal limit went off study. If absolute neutrophil count on the day of scheduled treatment was below 1.5×10^9/liter, chemotherapy was withheld until resolution of neutropenia. Patients with prolonged hematological recovery (>3 weeks) went off study.

Acquisition and Analysis of Serum Probes. Blood was drawn into native tubes immediately before each infusion of trastuzumab and on the date of clinical evaluation of patient response to treatment, centrifuged, and the resulting sera was analyzed using a sequential solid phase sandwich human Her-2/*neu* quantitative ELISA (Her-2/*neu* Microtiter ELISA; Oncogene Science, Cambridge MA) according to the manufacturer's instructions. Microplates were washed using an automated washer (Dias Microplate Washer; Dynex Technologies, Denkendorf, Germany). Absorbance reading was performed using an automated reader (FLUOstar Galaxy; BMG Labtech GmbH, Offenburg, Germany) and calculated using the Fluoscan Galaxy software (versions 4.20–0; BMG Labtech GmbH). Intra- and interassay precision of the assay were <5% coefficient of variation. No cross-reactivity of the assay with epidermal growth factor receptor, trastuzumab, human antimouse antibodies, endogenous substances, vitamins, over-the-counter drugs, or antineoplastic agents has been found (21).

Lesion Measurement and Evaluation of Response to Treatment. Restaging was performed every 8 weeks (or earlier if disease progression was clinically evident). Response evaluation and confirmation was performed in accordance with the Southwest Oncology Group response criteria and end-point definitions (22). In brief, complete response was defined as a complete disappearance of any tumor-related symptoms and all lesions in imaging studies, without appearance of any new lesions lasting for at least 4 weeks. Partial response was defined as >50% decrease under baseline in the sum of products of perpendicular diameters of all measurable lesions and at least

Table 1 Patient characteristics

Patient characteristic	*n*
Gender	
Female	54
Male	1
Chemotherapeutic pretreatment for metastatic disease	
None	29
1 Regimen	15
≥2	11
Grade of Her-2/*neu* overexpression	
Grade 2+	9
Grade 3+	46
Treatment	
Single-agent trastuzumab	8
Trastuzumab & paclitaxel	10
Trastuzumab & docetaxel	9
Trastuzumab & vinorelbine	28
Sites of active disease	
Liver	32
Bone	26
Lymph nodes	25
Lung	23
Skin	9
Other	18
Visceral metastases	43
Soft tissue disease only	12

stabilization of all nonmeasurable lesions lasting for a minimum of 4 weeks. Progressive disease was defined as a >25% increase in the sum of products of all measurable lesions, an unequivocal increase of nonmeasurable disease, or the appearance of new lesions. Disease was classified as being stable if no criteria for classifying responses as complete response, partial response, or progressive disease were met. For subsequent analyses, patients experiencing disease stabilization lasting >6 months were grouped together with patients achieving an objective response to treatment into a "clinical benefit group."

Statistical Analysis. Frequencies of patients' characteristics were compared by Fisher's exact test. Wilcoxon-signed ranks test was used for comparisons between ECD measurements at baseline and throughout treatment within and between the groups of patients experiencing a response, clinical benefit, or disease progression to trastuzumab.

In patients with elevated serum Her-2/*neu* levels before initiation of treatment, multivariate analyses were used to determine whether changes in concentrations of serum ECD could predict response, clinical benefit, progression-free survival, and overall survival from trastuzumab-based treatment. Grade of Her-2/*neu* overexpression (2+ *versus* 3+), patient age, recurrence-free interval (duration from initial diagnosis to appearance of metastatic disease), anthracycline pretreatment (for early or metastatic breast cancer), number of prior chemotherapeutic regimens for metastatic disease, estrogen receptor and progesterone receptor status, performance status, sites of active disease (visceral *versus* soft tissue only), number of organs involved by metastatic disease, type of treatment (single-agent trastuzumab *versus* combination with chemotherapy) were entered as potential confounders. Multiple logistic regression analyses were used to determine whether any of these variates could predict response or clinical benefit; multiple Cox regression models were used to identify the properties of the predictors mentioned above on progression-free and overall survival. Confounders without significant influences were removed by the backward selection method based on the Wald statistic. The related risk, the odds ratio, and 95% confidence interval (CI) were calculated with the proportional hazard method with respect to logistic regression and Cox regression, respectively. Survival curves (progression-free and overall survival) were compared with the log-rank test. For all analyses, a $P < 5\%$ was considered statistically significant. SPSS statistical software system (SPSS Inc., Chicago, IL, version 10.0) was used for all calculations.

Results

Study Population. Between April 2000 and July 2001 a total of 55 patients (median age, 53.0; range 27.6 to 81.0 years) with Her-2/*neu*-overexpressing metastatic breast cancer were enrolled. An overview of patients' characteristics and corresponding baseline ECD values is depicted in Tables 1 and 2. During the study period, a total of 885 infusions of trastuzumab were performed. Sera for Her-2/*neu* measurements were obtained before each infusion. All patients were evaluable for response. Overall, six complete and nine partial responses (objective-response rate 27%)—all of which occurred in patients with grade 3+ Her-2/*neu*-overexpressing tumors—were observed (*P* for response according to the grade of Her-2/*neu* overexpression, 0.052). Disease stabilization lasting >6 months was achieved in 13 (24%) patients (including 2 of 9 patients with grade 2+ Her-2/*neu*-overexpressing tumors), whereas 27 (49%) patients (including 7 of 9 patients with grade 2+ overexpressing tumors) progressed despite treatment (*P* for clinical benefit according to the grade of Her-2/*neu* overexpression, 0.078). Objective responses were observed in 1 of 8 (13%) patients receiving single-agent trastuzumab and 14 of 47 (30%) patients additionally receiving chemotherapy ($P = 0.040$); clinical benefit was achieved in 3 of 8 (38%) patients treated with trastuzumab only and 25 of 47 (53%) patients additionally receiving chemotherapy ($P = 0.469$).

The present analysis covers a median observation period of 26.4 (range 20.7 to 39.5) months. Treatment was discontinued in 49 patients because of progressive disease ($n = 46$), hematological ($n = 1$), or cardiac ($n = 1$) toxicity or consent withdrawal ($n = 1$). Median progression-free survival calculated from the survival function was 4.9 (range 0.7–32.6) months. As of June 2003, 30 (55%) deaths were observed, all of which were attributed to disease progression. Median overall survival cal-

Table 2 Correlation of clinical characteristics with baseline serum Her-2/*neu* levels

	Median (range) baseline ECD[a] (ng/ml)	*P*
Grade 2+ Her-2/*neu* overexpression	12.1 (9.3–19.1)	
Grade 3+ Her-2/*neu* overexpression	53.4 (5.2–6076.2)	0.002
Visceral involvement	61.4 (9.2–6076.2)	
Soft tissue disease only	11.9 (5.2–49.7)	<0.001

[a] ECD, extracellular domain.

culated from the survival function was 19.9 (range 1.7–38.5) months.

Baseline Serum Her-2/*neu* ECD Levels. The median (range) baseline serum ECD levels was 45.1 (range 5.2–6076.2) ng/ml. As defined by the upper normal limit of 15 ng/ml (7, 23–25), a total of 40 (73%) patients presented with increased serum levels suggesting tumoral shedding of the Her-2/*neu* ECD into the bloodstream. As shown in Table 2, patients with grade 3+ Her-2/*neu* tumoral overexpression had significantly higher baseline ECD values (median 53.4, range 5.2–6076.2 ng/ml) than those with grade 2+ overexpression (median 12.1, range 9.3–19.1 ng/ml, $P = 0.002$). Patients with liver and/or lung metastases (median 61.4, range 9.2–6076.2 ng/ml) had significantly higher ECD levels at baseline, as compared with patients without visceral involvement (median 11.9, range 5.2–49.7 ng/ml, $P < 0.001$).

Impact of Baseline ECD Levels on Response, Progression-Free, and Overall Survival. Response rates in patients with elevated (≥15 ng/ml) baseline serum ECD levels were significantly higher (35%, 14 of 40) as compared with the response rate observed in patients with normal (<15 ng/ml) baseline ECD values before initiation of treatment (7%, 1 of 15; $P = 0.045$). Likewise, responding patients had significantly higher absolute baseline ECD values (median 107.0, range 11.9–230.0 ng/ml) as compared with patients with progressive disease (median 17.4, range 5.2–6076.2 ng/ml, $P = 0.028$). The rates of clinical benefit were similar between patients with elevated (22 of 40, 55%) and patients with normal ECD levels at baseline (6 of 15, 40%; $P = 0.375$). Likewise, absolute baseline ECD levels of patients experiencing a clinical benefit (median 53.2, range 6.1–245.4 ng/ml) did not differ significantly from those observed in patients with progressive disease (median 17.4, range 5.2–6076.2 ng/ml, $P = 0.195$).

Median (range) progression-free survival was 5.7 (0.7–28.4) months in patients with elevated baseline ECD values and 4.5 (0.9–32.6) months in those with normal ECD levels ($P = 0.813$). Median (range) overall survival in patients with elevated baseline ECD levels was 17.4 (2.1–32.9) months, which did not differ significantly from the overall survival observed in patients with baseline ECD values <15 ng/ml (median 27.0, range 7.2–38.5 months, $P = 0.410$). In multivariate Cox regression analyses, there was a trend toward longer progression-free ($P = 0.099$) and overall survival ($P = 0.060$) in patients with higher absolute baseline ECD values and toward longer overall survival in patients with grade 3+ overexpressing tumors ($P = 0.066$).

Kinetics of Serum Her-2/*neu* ECD Concentrations During Trastuzumab-Based Treatment. Kinetics of serum ECD levels (expressed as % of baseline values) in the course of treatment are depicted grouped according to the observed responses in Fig. 1. A significant decrease of ECD levels was observed in patients experiencing an objective response or clinical benefit from trastuzumab-based therapy as early as from day 8 of treatment onwards (all *P*s for weekly measurements *versus* baseline were <0.001 in both the total collective and in patients with baseline values ≥15 ng/ml, respectively). In contrast, no significant change in ECD levels was observed in patients with progressive disease (all *P*s > 0.05).

For instance, on day 15 of treatment, patients progressing despite therapy had a mean (±SD) decrease in serum Her-2/*neu* ECD levels of 11.5 ± 31.4%. Patients experiencing a clinical benefit had a mean decrease of 41.5 ± 20.9%, and patients responding to treatment had a mean decrease of 49.5 ± 20.8%. The magnitude of all these changes was much larger than the coefficient of variation of the assay.

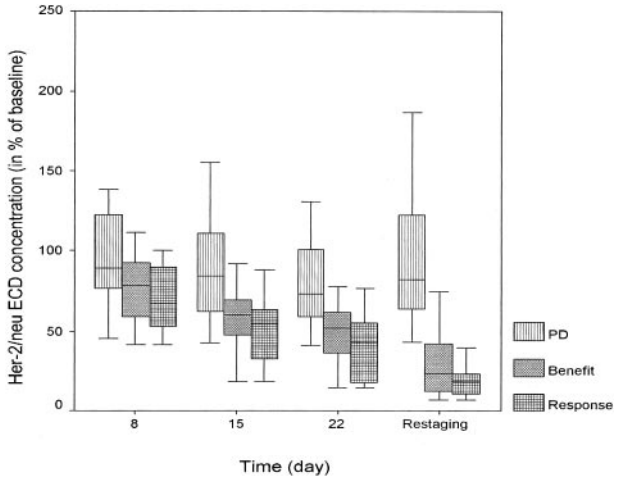

Fig. 1 Dynamics of serum Her-2/*neu* extracellular domain (ECD) during trastuzumab-based treatment. Serial serum Her-2/*neu* levels in patients with elevated (≥15 ng/ml) baseline serum Her-2/*neu* ECD concentrations are depicted in accordance to the clinical course of disease (*response*, complete or partial response; *benefit*, response, or stable disease >6 months; *PD*, progressive disease). Measurements are expressed as % of the baseline value.

Prediction of Response Probability by Determination of Serum Kinetics of the ECD. Patients with elevated baseline ECD levels ($n = 40$) were entered into multiple logistic regression analyses to calculate the odds ratio of response and clinical benefit predicted by early changes of ECD levels during trastuzumab-based treatment. In these analyses, changes in ECD concentrations in relation to baseline were the only predicting variables for the occurrence of response or clinical benefit with higher relative ECD concentrations predicting lower probabilities. Kinetics of serum ECD levels allowed for an accurate prediction of response probability to trastuzumab-based therapy as early as by day 8 after treatment (and onwards; all *P*s < 0.05). The odds ratio and 95% CI for response corresponding to a 1% decrease in ECD levels observed on day 8 of treatment was 1.043 (1.007–1.080, $P = 0.020$); *i.e.*, each 1% decrease of ECD values (from baseline values) observed on day 8 increased the probability of response by 4.3% (0.7–8.0%). The odds ratio (95% CI) for response corresponding to each 10% change of ECD levels in the course of treatment is shown in Table 3.

The prediction of probability of clinical benefit based on changes in ECD serum levels from baseline was possible from day 8 of treatment onwards (all *P*s < 0.05; Table 3). Measuring early ECD changes the probability of response or clinical benefit could be predicted not only in the total cohort but also in patients with grade 3+ Her-2/*neu*-overexpressing tumors or patients receiving trastuzumab plus chemotherapy, the latter representing the largest patient subgroups (Table 3).

In addition, cut-point analyses for prediction of response

Table 3 The odds ratios (OR) and 95% confidence intervals (95% CI) of response and clinical benefit (response or disease stabilisation >6 months) in dependence on early changes of Her-2/*neu* extracellular domain (ECD) during treatment with trastuzumab

The OR of response and clinical benefit for each 10% decrease of Her-2/*neu* ECD from baseline concentrations in the course of treatment are depicted for the total collective, patients with grade 3+ Her-2/*neu*-overexpressing tumors and patients receiving trastuzumab plus chemotherapy. Lower relative Her-2/*neu* ECD concentrations predict higher odds of response and benefit: i.e., in the total collective the OR of response doubles (1.989) in patients with an observed 10% decrease (from baseline) of ECD values on day 15, whereas the OR of response in patients with a 50% decrease on day 15 is 31.130 (1.989^5). In analogy, the OR of response for a 50% increase of ECD values observed on day 15 is 0.032 [$(1/1.989)^5$].

	OR (95% CI) for response for each 10% decrease of ECD from baseline	*P*	OR (95% CI) for clinical benefit for each 10% decrease of ECD from baseline	*P*
All patients				
Day 8	1.519 (1.068–2.159)	0.020	1.365 (1.021–1.825)	0.036
Day 15	1.989 (1.139–3.471)	0.016	1.632 (1.131–2.357)	0.009
Day 22	2.052 (1.192–3.533)	0.010	1.649 (1.142–2.381)	0.008
Patients with grade 3+ Her-2/*neu* overexpressing tumors				
Day 8	1.475 (1.044–2.085)	0.027	1.359 (1.013–1.824)	0.041
Day 15	1.906 (1.114–3.261)	0.019	1.684 (1.133–2.502)	0.010
Day 22	2.199 (1.193–4.052)	0.012	1.721 (1.144–2.589)	0.009
Patients receiving trastuzumab and concomitant chemotherapy				
Day 8	1.622 (1.074–2.450)	0.021	1.531 (1.058–2.215)	0.024
Day 15	2.041 (1.123–3.711)	0.019	1.662 (1.094–2.525)	0.017
Day 22	2.0277 (1.130–3.640)	0.018	1.602 (1.085–2.365)	0.018

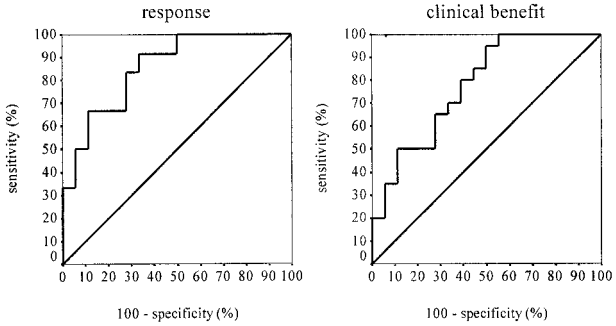

Fig. 2 Receiver-operating characteristics curves for response and clinical benefit in dependence on the relative Her-2/*neu* extracellular domain (ECD) concentration observed on day 15 of treatment in patients with elevated ECD levels at baseline. Cut-point analyses revealed a cutoff at 66.1 and 71.3% of the baseline ECD concentration for prediction of response and clinical benefit, respectively.

and clinical benefit based on ECD levels (in % of baseline levels) observed on day 15 were performed (see receiver-operating characteristics curves in Fig. 2). For prediction of response, cut-point analysis revealed a cutoff at 66.1% of the baseline ECD concentration. Sensitivity and specificity were 0.833 and 0.722, respectively. The area below the curve was 0.856 (95% CI, 0.725–0.988; $P = 0.001$). Objective responses were observed in 10 of 15 (67%) patients whose ECD levels had dropped below 66.1% of baseline levels, whereas only 2 of 15 (13%) patients with ECD levels above 66.1% of baseline levels on day 15 responded.

For prediction of clinical benefit, cut-point analysis calculated a cutoff at 71.3% of the baseline ECD concentration (sensitivity 0.800, specificity 0.611). The area below the curve was 0.778 (95% CI, 0.630–0.925; $P = 0.003$). Thus, 16 of 23 (70%) patients with ECD levels below 71.3% of baseline levels on day 15 experienced a clinical benefit from trastuzumab-based treatment. In contrast, only 4 of 15 (27%) patients with ECD levels above 71.3% of pretreatment serum levels on day 15 experienced a clinical benefit.

Prediction of Progression-Free and Overall Survival by Serum Kinetics of the Her-2/*neu* ECD. Multiple Cox regression analyses in patients with elevated baseline ECD levels revealed that early changes in ECD serum levels after initiation of trastuzumab-based treatment allowed for the prediction of progression-free survival. In these analyses, determination of

Table 4 The related risk (RR) and 95% confidence interval (95% CI) for disease progression depending on early changes of the serum Her-2/*neu* extracellular domain (ECD)

The RR is the factor by which the risk of disease progression within the observed period (median, 26.4; range 20.7–39.5 months) is multiplied, if ECD values (in % baseline) of treatment rise by 10% (depicted for the total collective, patients with grade 3+ Her-2/*neu*-overexpressing tumors and patients receiving trastuzumab plus chemotherapy). Lower relative serum Her-2/*neu* ECD values raise the probability of progression-free survival. For instance, in the total collective the RR for progression is 1.231 times higher in patients with an observed 10% increase (from baseline) of ECD values on day 22 and 2.827 times higher (1.231^5) in patients with a 50% increase of ECD values over baseline observed on day 22, respectively. In contrast, the RR for progression with a 50% decrease of ECD values on day 22 is 0.354 ($1/1.231^5$).

	RR (95% CI) for progression for a 10% increase of ECD values	
	RR (95% CI)	*P*
All patients		
Day 8	1.033 (0.879–1.214)	0.691
Day 15	1.194 (1.043–1.367)	0.010
Day 22	1.231 (1.057–1.434)	0.008
Patients with grade 3+ Her-2/*neu* overexpressing tumors		
Day 8	1.037 (0.882–1.219)	0.662
Day 15	1.219 (1.057–1.405)	0.006
Day 22	1.240 (1.056–1.457)	0.009
Patients receiving trastuzumab and concomitant chemotherapy		
Day 8	1.038 (0.870–1.239)	0.678
Day 15	1.193 (1.013–1.405)	0.034
Day 22	1.230 (1.044–1.450)	0.013

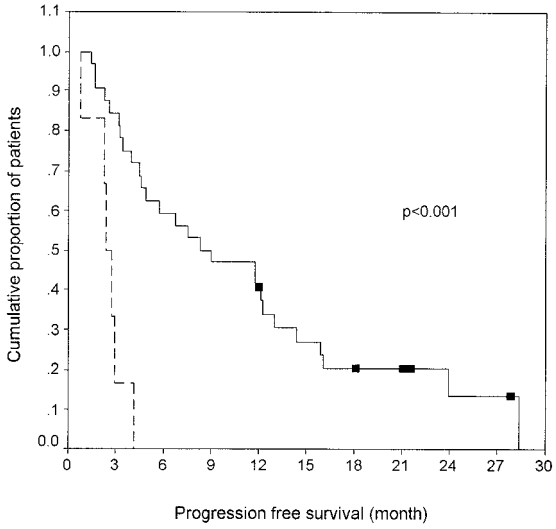

Fig. 3 Kaplan-Meier functions of progression free survival in patients with elevated pretreatment serum Her-2/*neu* extracellular domain (ECD) levels comparing patients whose ECD levels had decreased (—, $n = 32$) with patients whose ECD levels had increased (– – –, $n = 6$) from baseline on day 15 of trastuzumab-based treatment (■, censored cases, log-rank $P < 0.001$).

the risk for disease progression within the observation period (median 26.4, range 20.7–39.5 months) was possible as early as from day 15 of trastuzumab-based treatment onwards; the related risk for progression within the observed period corresponding to a 1% increase of ECD values observed on day 15 of treatment was 1.018 (95% CI, 1.004–1.032; $P = 0.010$); *i.e.*, each 1% increase of ECD values (from baseline values) observed on day 15 increased the risk for disease progression by 1.8% (95% CI, 0.4–3.2%). The probability values for progression-free survival corresponding to early changes in ECD values could not only be calculated within the total cohort but also in patients with grade 3+ Her-2/*neu*-overexpressing tumors and in patients receiving trastuzumab plus chemotherapy. None of the other patient characteristics analyzed was found to influence progression-free survival significantly. The related risk and 95% CI for disease progression corresponding to each 10% change in ECD levels from baseline values observed in the course of treatment are shown in Table 4. Survival curves comparing progression-free survival in patients whose ECD levels had increased on day 15 of treatment to those whose ECD levels had decreased are depicted in Fig. 3.

No significant prediction of the probabilities of overall survival could be made by any of the factors analyzed, probably because the majority of patients (43 of 55, 75%) had received further cytotoxic therapy after progression on trastuzumab-based treatment.

Discussion

The weekly use of trastuzumab as either a single agent or in combination with cytotoxic chemotherapy has intensified the need for patients to undergo frequent therapeutic interventions as well as increased economic burden. Therefore, it seems a desirable goal to develop predictive indicators for response to treatment aside from tumoral Her-2/*neu* overexpression and chemotherapeutic pretreatment.

In the present study, we are able to confirm previous reports demonstrating that serum Her-2/*neu* ECD does not interfere with the biological effects of trastuzumab-based treatment in the majority of patients (6, 9). Our results indicate that patients with elevated serum ECD levels are more likely to respond to trastuzumab-based treatment. Furthermore, we report that in patients with elevated baseline ECD levels, changes of ECD levels throughout trastuzumab-based treatment do not only parallel (6, 9, 20) but even precede the clinical course of disease, thus allowing for a significant prediction of response, clinical benefit, and progression-free survival as early as after 1 and 2 weeks of treatment, respectively.

Within this context, it is interesting to note that serum ECD levels have not only been reported to correlate with the intensity of membranous Her-2/*neu* overexpression (6) and tumor burden (7) but that proteolytic cleavage of the ECD represents a ligand-independent activation mechanism leading to Her-2/*neu* phosphorylation and active Her-2/*neu* signaling (5). In our study, the higher-response rates observed in patients with elevated ECD levels may thus represent the increased sensitivity of tumors with more intense Her-2/*neu* expression and signaling toward trastuzumab-based treatment. Likewise, the rapid and profound decrease of ECD levels observed in patients responding to treatment may also represent inhibition of ECD release and inhibition of Her-2/*neu* activation in trastuzumab-sensitive tumors (5), then solely parallel the declining tumor mass.

We conclude, therefore, that serial measurements of the Her-2/*neu* ECD could have the potential to predict the clinical outcome of patients with Her-2/*neu*-overexpressing metastatic breast cancer treated with trastuzumab-based therapy. Thus, monitoring of serum Her-2/*neu* ECD levels should be incorporated in future, larger trials. The use of this predictive potential could not only focus and tailor treatment individually but also adapt to economic needs.

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References

1. Coussens, L., Yang-Feng, T. L., Liao, Y. C., Chen, E., Gray, A., McGrath, J., Seeburg, P. H., Libermann, T. A., Schlessinger, J., Francke, U., *et al.* Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with *neu* oncogene. Science (Wash. DC), *230:* 1132–1139, 1985.

2. Brandt-Rauf, P. W., Pincus, M. R., and Carney, W. P. The c-erbB-2 protein in oncogenesis: molecular structure to molecular epidemiology. Crit. Rev. Oncog., *5:* 313–329, 1994.

3. Breuer, B., DeVivo, I., Luo, J. C., Smith, S., Pincus, M. R., Tatum, A. H., Daucher, J., Minick, C. R., Miller, D. G., Nowak, E. J., *et al.* erbB-2 and myc oncoproteins in sera and tumors of breast cancer patients. Cancer Epidemiol. Biomark. Prev., *3:* 63–66, 1994.

4. Zabrecky, J. R., Lam, T., McKenzie, S. J., and Carney, W. The extracellular domain of p185/*neu* is released from the surface of human

breast carcinoma cells, SK-BR-3. J. Biol. Chem., *266:* 1716–1720, 1991.

5. Molina, M. A., Codony-Servat, J., Albanell, J., Rojo, F., Arribas, J., and Baselga, J. Trastuzumab (herceptin), a humanized anti-Her2 receptor monoclonal antibody, inhibits basal and activated Her2 ectodomain cleavage in breast cancer cells. Cancer Res., *61:* 4744–4749, 2001.

6. Cobleigh, M. A., Vogel, C. L., Tripathy, D., Robert, N. J., Scholl, S., Fehrenbacher, L., Wolter, J. M., Paton, V., Shak, S., Lieberman, G., and Slamon, D. J. Multinational study of the efficacy and safety of humanized anti-HER2 monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J. Clin. Oncol., *17:* 2639–2648, 1999.

7. Lipton, A., Ali, S. M., Leitzel, K., Demers, L., Chinchilli, V., Engle, L., Harvey, H. A., Brady, C., Nalin, C. M., Dugan, M., Carney, W., and Allard, J. Elevated serum Her-2/*neu* level predicts decreased response to hormone therapy in metastatic breast cancer. J. Clin. Oncol., *20:* 1467–1472, 2002.

8. Yamauchi, H., Stearns, V., and Hayes, D. F. When is a tumor marker ready for prime time? A case study of c-erbB-2 as a predictive factor in breast cancer. J. Clin. Oncol., *19:* 2334–2356, 2001.

9. Pegram, M. D., Lipton, A., Hayes, D. F., Weber, B. L., Baselga, J. M., Tripathy, D., Baly, D., Baughman, S. A., Twaddell, T., Glaspy, J. A., and Slamon, D. J. Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/*neu* monoclonal antibody plus cisplatin in patients with HER2/*neu*-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J. Clin. Oncol., *16:* 2659–2671, 1998.

10. Pegram, M., and Slamon, D. Biological rationale for HER2/*neu* (c-erbB2) as a target for monoclonal antibody therapy. Semin. Oncol., *27:* 13–19, 2000.

11. Vogel, C. L., Cobleigh, M. A., Tripathy, D., Gutheil, J. C., Harris, L. N., Fehrenbacher, L., Slamon, D. J., Murphy, M., Novotny, W. F., Burchmore, M., Shak, S., Stewart, S. J., and Press, M. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J. Clin. Oncol., *20:* 719–726, 2002.

12. Baselga, J., Tripathy, D., Mendelsohn, J., Baughman, S., Benz, C. C., Dantis, L., Sklarin, N. T., Seidman, A. D., Hudis, C. A., Moore, J., Rosen, P. P., Twaddell, T., Henderson, I. C., and Norton, L. Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/*neu*-overexpressing metastatic breast cancer. J. Clin. Oncol., *14:* 737–744, 1996.

13. Slamon, D. J., Leyland-Jones, B., Shak, S., Fuchs, H., Paton, V., Bajamonde, A., Fleming, T., Eiermann, W., Wolter, J., Pegram, M., Baselga, J., and Norton, L. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N. Engl. J. Med., *344:* 783–792, 2001.

14. Burstein, H. J., Kuter, I., Campos, S. M., Gelman, R. S., Tribou, L., Parker, L. M., Manola, J., Younger, J., Matulonis, U., Bunnell, C. A., Partridge, A. H., Richardson, P. G., Clarke, K., Shulman, L. N., and Winer, E. P. Clinical activity of trastuzumab and vinorelbine in women with HER2-overexpressing metastatic breast cancer. J. Clin. Oncol., *19:* 2722–2730, 2001.

15. Burris, H. A. III. Docetaxel (Taxotere) plus trastuzumab (Herceptin) in breast cancer. Semin. Oncol., *28:* 38–44, 2001.

16. Miller, K. D., Sisk, J., Ansari, R., Gize, G., Nattam, S., Pennington, K., Monaco, F., and Sledge, G. W., Jr. Gemcitabine, paclitaxel, and trastuzumab in metastatic breast cancer. Oncology (Huntingt.), *15:* 38–40, 2001.

17. Brodowicz, T., Wiltschke, C., Budinsky, A. C., Krainer, M., Steger, G. G., and Zielinski, C. C. Soluble HER-2/*neu* neutralizes biologic effects of anti-HER-2/*neu* antibody on breast cancer cells *in vitro*. Int. J. Cancer, *73:* 875–879, 1997.

18. Krainer, M., Brodowicz, T., Zeillinger, R., Wiltschke, C., Scholten, C., Seifert, M., Kubista, E., and Zielinski, C. C. Tissue expression and serum levels of HER-2/*neu* in patients with breast cancer. Oncology, *54:* 475–481, 1997.

19. Seidman, A. D., Fornier, M. N., Esteva, F. J., Tan, L., Kaptain, S., Bach, A., Panageas, K. S., Arroyo, C., Valero, V., Currie, V., Gilewski, T., Theodoulou, M., Moynahan, M. E., Moasser, M., Sklarin, N., Dickler, M., D'Andrea, G., Cristofanilli, M., Rivera, E., Hortobagyi, G. N., Norton, L., and Hudis, C. A. Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification. J. Clin. Oncol., *19:* 2587–2595, 2001.

20. Esteva, F. J., Valero, V., Booser, D., Guerra, L. T., Murray, J. L., Pusztai, L., Cristofanilli, M., Arun, B., Esmaeli, B., Fritsche, H. A., Sneige, N., Smith, T. L., and Hortobagyi, G. N. Phase II study of weekly docetaxel and trastuzumab for patients with HER-2-overexpressing metastatic breast cancer. J. Clin. Oncol., *20:* 1800–1808, 2002.

21. Payne, R. C., Allard, J. W., Anderson-Mauser, L., Humphreys, J. D., Tenney, D. Y., and Morris, D. L. Automated assay for HER-2/*neu* in serum. Clin. Chem., *46:* 175–182, 2000.

22. Green, S., and Weiss, G. R. Southwest Oncology Group standard response criteria, endpoint definitions and toxicity criteria. Investig. New Drugs, *10:* 239–253, 1992.

23. Cook, G. B., Neaman, I. E., Goldblatt, J. L., Cambetas, D. R., Hussain, M., Luftner, D., Yeung, K. K., Chan, D. W., Schwartz, M. K., and Allard, W. J. Clinical utility of serum HER-2/*neu* testing on the Bayer Immuno 1 automated system in breast cancer. Anticancer Res., *21:* 1465–1470, 2001.

24. Molina, R., Jo, J., Filella, X., Zanon, G., Pahisa, J., Munoz, M., Farrus, B., Latre, M. L., Gimenez, N., Hage, M., Estape, J., and Ballesta, A. M. C-erbB-2 oncoprotein in the sera and tissue of patients with breast cancer. Utility in prognosis. Anticancer Res., *16:* 2295–2300, 1996.

25. Molina, R., Jo, J., Filella, X., Zanon, G., Pahisa, J., Munoz, M., Farrus, B., Latre, M. L., Escriche, C., Estape, J., and Ballesta, A. M. c-erbB-2 oncoprotein, CEA, and CA 15.3 in patients with breast cancer: prognostic value. Breast Cancer Res. Treat., *51:* 109–119, 1998.