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Serum HER-2/neu as a Prediction and Monitoring Parameter in a Phase II Study with Weekly Paclitaxel in Metastatic Breast Cancer

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Abstract. *Elevated levels of the extracellular domain of HER-2/neu in serum (sHER-2/neu) have been shown to be of prognostic importance. In this phase II study with weekly paclitaxel in metastatic breast cancer, we investigated the predictive quality of this serum oncoprotein by correlating the outcome of therapy to sHER-2/neu levels. Paclitaxel (90 mg/m^2 weekly x6, q9w) was administered to 35 patients with complete outcome assessment and biochemical follow-up. sHER-2/neu was measured using standardized enzyme-linked immunoassays. We found that 62.9% (22/35) of the patients had elevated levels (≥15 ng/ml) of sHER-2/neu. The overall response rate (RR) to weekly paclitaxel was 40.0% (14/35). There was no difference in RR between sHER-2/neu-positive patients (40.9%) and sHER-2/neu-negative patients (38.5%; p=0.4). The progression-free interval was longer for sHER-2/neu-negative patients (53.2 weeks) in comparison to sHER-2/neu-positive patients (31.2 weeks; p=0.098). Responses were significantly more durable in sHER-2/neu-negative patients (65.2 weeks) than in the sHER-2/neu-positive subgroup (25.7 weeks; p=0.042). Introducing hypothetical cut-offs into the sHER-2/neu-positive subset, we found that in patients with a sHER-2/neu level of greater than 22 ng/ml, the progression-free survival decreased significantly with increasing sHER-2/neu levels (p≤0.022). Considering the high impact of progression-free survival and duration of response as outcome parameters, the sHER-2/neu status is a predictive indicator for benefit from paclitaxel chemotherapy.*

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The activation of cellular oncogenes plays an important role in the development of human cancer. One important member of the oncogene family encodes the growth factor receptor known as HER-2/neu (human epidermal growth factor receptor) or c-erbB-2 (1, 2). This transmembrane growth factor receptor is found on cells of epithelial origin and exerts its effect on cell growth through autophosphorylation. The full-length protein has a molecular weight of 185 kDa (p185) and is composed of three domains: the internal tyrosine kinase portion, a short transmembrane section and the extracellular ligand-binding domain that is heavily glycosolated (referred to as the extracellular domain or ECD). The C-terminal, cytoplasmic end of the molecule is responsible for the tyrosine kinase activity and initiation of signal transduction. A hydrophobic transmembrane portion connects the internal tyrosine kinase region and the ECD.

The HER-2/neu protein is a normal epithelial protein that is present on many organs such as lungs, colon, prostate and breast and therefore is not specific to any tissue (3). After proteolytic processes mediated by metalloproteases, the ECD portion is shed into the circulation (4). This shed ECD has been shown to be present in the sera of healthy women and to be elevated above the normal range in women with breast cancer and, in particular, women with metastatic breast cancer. While the membrane-bound protein is over-expressed in about 30% of primary breast cancers, the shed ECD has been reported to be elevated in the sera of as many as 50% of women with metastatic breast cancer (5-8).

The question of whether HER-2/neu-overexpressing breast cancer is sensitive or resistant to the cytotoxic drug, paclitaxel, is still unanswered (9,10). The pre-clinical and clinical investigations on this topic are difficult to interpret due to confounding factors like heterogeneous patient populations (locally advanced breast cancer *vs.* stage IV disease), influences of other therapies (radiation; combined analyses on paclitaxel and docetaxel) and small

patient numbers. Another important aspect is the different methods of HER-2/neu evaluation employed by the studies: immunohistochemistry (IHC), fluorescence *in situ* hybridisation (FISH) or enzyme-linked immunosorbent assays (ELISA) of serum HER-2/neu (sHER-2/neu). The choice of method *per se* can determine a study result as shown by Harris *et al.* who found no correlation of overall survival, after high-dose chemotherapy to the HER-2/neu status by immunohistochemistry, but showed a worse outcome for those patients with an elevated sHER-2/neu level (11).

In this phase II study with weekly fractionated, dose-intensified paclitaxel (schedule: 90 mg/m^2 weekly x 6, q9w) for metastatic breast cancer patients, we correlated the clinical outcome parameters to the baseline, and the longitudinal levels of sHER-2/neu to: (1) determine its value as a predictive marker for paclitaxel chemotherapy, and (2) to test sHER-2/neu for its utility for monitoring. The strategy of single-agent paclitaxel in this dose-intensified, weekly schedule seemed promising in addressing these questions.

Materials and Methods

Study design. Paclitaxel was applied as a 1-hour infusion after premedication with dexamethasone (16 mg), clemastine (2 mg) and ranitidine (50 mg) on days 1, 8, 15, 22, 29 and 36 with a 3-week interval before the start of the next cycle on day 57. Patients were enrolled if they fulfilled the following inclusion and exclusion criteria: 1) *Inclusion criteria:* histological diagnosis of stage IV breast adenocarcinoma; 1 or 2 previous chemotherapy regimens for metastatic disease one of which had to be anthracycline-based (1st-line therapy with weekly paclitaxel was possible in the case of contraindications against anthracyclines); minimum Karnofsky performance status at study entry >70%; sufficient bone marrow reserve with the leucocyte count, platelet count and hemoglobin ≥3,500/μl, ≥100,000/μl and ≥10 g/dl, respectively; adequate liver, renal and blood-clotting function; calcium within normal ranges; age >18 years; life expectancy >12 weeks; childbearing potential terminated or use of contraceptive methods. 2) *Exclusion criteria:* serious concomitant disease (especially cardiac arrhythmias); peripheral neuropathy of any reason; severe allergic disposition or known allergy against Cremophor EL as component of the paclitaxel medication; previous or current CNS metastases; second primary malignancy (except from carcinoma *in situ* of the cervix or adequately treated basal cell carcinoma of the skin).

The study was approved by the local ethics committee and was subject to the principles set out in the World Medical Association Declaration of Helsinki. All patients provided signed informed consent.

Study end-points. For disease description, metastatic organ manifestations were counted with subgroups being formed for patients with only 1 organ involved, 2 organs or >2 organs affected. The dominant site of the disease was defined as being located either in the visceral organs (*i.e.* liver, lung), soft tissue (*i.e.* skin, lymph nodes) or bone. Outcome was defined according to internationally accepted criteria (12). For response evaluation, bidimensionally measurable (product of longest diameter and greatest perpendicular diameter) and evaluable (*e.g.* lymphatic pulmonary metastases) lesions were considered. Complete remission (CR) was documented in the case of disappearance of all known disease with confirmation at ≥4 weeks. For a partial remission (PR), the tumor load had to decrease by >50% from baseline with confirmation after 4 weeks. Any appearance of new lesions or a >25% increase of the size of one or more lesions led to the diagnosis of progressive disease. Disease was found to be stable (SD) if neither PR nor PD criteria were met. SD in evaluable lesions reduced a CR in measurable lesions to on overall PR while a SD in non-measurable lesions did not influence the staging of a PR. Progression-free survival in weeks was defined from the start of chemotherapy to the first diagnosis of disease progression. The duration of response was defined as the time from the first evidence of partial remission to the date PD was first noted.

The study was designed with the overall response rate as primary end-point. Secondary end-points were progression-free survival and duration of response. In addition to the main trial, there were 3 other study objectives: to assess the predictive value of, and monitoring by, sHER-2/neu; to assess the quality of life using standardized questionnaires; and to assess neurotoxicity by electrophysiological methods. As not all patients could take part in every aspect of this study, the final patient numbers differ between the main trial and the additional study objectives. As the serum collection started several months after initiation of the study and as some patients did not receive at least one complete cycle of fractionated paclitaxel, only 35 patients qualified for the sHER/neu analysis.

sHER-2/neu measurements. Three to six serum samples were taken per patient and cycle to measure the sHER-2/neu level. Both the Oncogene® Science Human HER-2/neu assay (Oncogene Science/Bayer Corporation, 80 Rogers Street, Cambridge, MA 02142-1168, USA) and the former Chiron® Diagnostics enzyme immunoassay were used in this study.

The Oncogene Science assay is a sandwich enzyme immunoassay. Monoclonal anti-HER-2/neu antibodies, immobilized on the interior surface of microplate wells, specifically bind to the ECD of HER-2/neu. The serum sample (20 μl) is mixed with 980 μl of sample diluent. One hundred μl of this serum dilution is then added to the microplate wells. After an incubation time of 3 hours at 37˚C and 3 washes, 100 μl of mouse monoclonal antibody is added to each well. This step is followed by another hour of incubation at 37˚C and 3 washes. During this incubation, the detector reagents react with the immobilized ECD HER2-/neu antigen. The amount of detector antibody-antigen-complex is measured after reaction with a streptavidin-horseradish peroxidase conjugate (100 μl) which is added to each well. The streptavidin-horseradish peroxidase conjugate catalyzes the conversion of the chromogenic substrate o-phenylenediamine into a colored product. This chromogenic substrate (100 μl) is added to the microplate wells after 30 minutes of incubation at room temperature and a final 3-cycle wash. The plate is then incubated for 45 minutes at room temperature, followed by stopping the reaction with 100 μl of stop solution (2.5 N H_2SO_4). The colored reaction product is quantified by spectrophotometry at 490 nm. To establish the calibration curve, 6 different standards of recombinant HER-2/neu protein fragment p105 are run with each assay. The standard curve is constructed by plotting the average absorbance value for the HER-2/neu standards

versus the known HER-2/neu standard concentration (ng/ml). The assay has a lower detection limit of 3.4 ng/ml and an upper linear dilution limit of 36 ng/ml. The cut-off level for sHER-2/neu positivity was previously established as 15 ng/ml by comparing a collective of healthy women with a collective of patients with metastatic breast cancer (13).

The Chiron Diagnostics assay (which is no longer commercially available) for the quantitative measurement of human HER-2/neu protein fragment in serum was also a monoclonal antibody-based immunoenzymetric assay. Serum samples (50 µl) were added to streptavidin-coated tubes containing 200 µl of sample diluent. This serum dilution was mixed and combined with 200 µl of combined monoclonal antibody conjugate solution (horseradish peroxidase-labeled and isothiocyanate (FITC)-labeled anti-HER-2/neu monoclonal antibodies). After 2 hours of incubation, 200 µl of biotinylated antibody reactive with FITC were added, followed by another 2 hours of incubation. The resulting immune complexes were bound to the coated tubes. After aspiration and 3 washes, colour development was initiated by adding tetramethylbenzidine and hydrogen peroxide. The colorimetric reaction was stopped after 25 minutes by the addition of 1 ml of 1 M phosphoric acid. Colour density was measured spectrophotometrically at 450 nm. A calibration curve was generated with 3 reference calibrators. As with the Oncogene assay, a sHER-2/neu level of 15 U/ml was previously established.

Retrieval of paraffin-embedded tumor blocks for the determination of HER-2/neu in tissue by immunohistochemistry and fluorescence *in situ* hybridisation for correlation of HER-2/neu tissue and serum results was only partly successful. The number of tissue results (<20) was not sufficient to be integrated into this analysis, but will be part of a separate paper on concordance of HER-2/neu results by different methods to be published elsewhere.

Statistics. The estimated patient number for the phase II study with weekly fractionated paclitaxel was 50 women, including a drop-out rate of 20%. The statistical calculations were performed using SPSS for Windows 95 release 7.5. For the statistical comparison of the response rates, the Mantel-Haenszel-test of linear by linear association was applied. The comparison of progression-free survival and duration of response between sHER-2/neu-positive and sHER-2/neu-negative patients was done using the log-rank test. As error of first kind (significance level), 0.05 was chosen for all analyses. Cox regression including the parameters of the primary breast tumor could not numerically be accomplished because of the moderate number of patients and partly unknown variables.

Results

Patients. A serum sample set together with the clinical follow-up was available from 35 patients treated with at least one cycle of dose-intensified weekly fractionated paclitaxel. The detailed demographic characteristics of these 35 patients are listed in Table I for the total population as well as for the sHER-2/neu-positive and the sHER-2/neu-negative subgroups. The mean age of the patients at the time of the primary diagnosis and at stage IV disease is representative of breast cancer patients in general. Most women (91%) were postmenopausal at the time of study entry. The features of the primary tumor (histology, pathological staging, grading) and therapeutic interventions were comparable between the sHER-2/neu-positive and -negative subsets. For some of the patients, the data on the TNM classification of the primary tumor are missing, since standard of care procedures did not necessarily require that this information be provided at the time of their first diagnosis. Of note, nearly all patients (97%) had had an anthracycline-based chemotherapy before therapy with weekly paclitaxel, either as an adjuvant treatment or for stage IV disease. For 60% of the patients, paclitaxel was the second-line chemotherapy for metastatic disease and 34% had already failed 2 lines of palliative chemotherapy. In addition, more than half of the patients had also been given a palliative hormonal therapy. In general, the patients had highly advanced breast cancer with visceral involvement as the dominant site of the disease. A difference in the distribution of the demographic parameters between sHER-2/neu-positive and -negative patients was demonstrated for the steroid hormone receptor status. While the percentage of estrogen receptor (ER) or progesterone receptor (PR)-positive patients is similar between the sHER-2/neu-positive and the sHER-2/neu-negative patients (36% *vs.* 31%), there were more patients with complete steroid hormone receptor negativity in the sHER-2/neu-positive group. This is consistent with other studies which have demonstrated that HER-2/neu overexpression is associated with ER negativity (14,15). The steroid receptor status was unknown in nearly half of the patients for the same reasons as previously mentioned above.

sHER-2/neu assays. In this biochemical study, 2 different enzyme-linked immunosorbent assays (ELISA) were used for the measurement of sHER-2/neu. At the beginning of the phase II study, the Chiron Diagnostics assay was commercially available and frequently used for experimental studies with relevant results for clinical practice (16,17). However, while the study was ongoing, the assay was removed from the market and, thus, we converted to the Oncogene Science assay for a parallel investigation. It is important to note that although the 2 assays are different in several aspects (use of wavelengths for quantitation by spectrophotometry, units reported for final results), they are linearly comparable with excellent significance as shown in Figure 1. In this linear regression, we compared 260 longitudinal Oncogene and Chiron sHER-2/neu results for 73 patients with metastatic breast cancer, including all patients treated with weekly paclitaxel. The correlation factor of r=0.9553 (confidence interval: 0.9409-0.9659; $p<0.0001$) indicates the excellent standardisation of sHER-2/neu testing, in general, which is important in the discussion of reliability of HER-2/neu methods (18). The percent coefficient of variation (%CV) of the sHER-2/neu results for both assays was < 5%. From this point onward,

Table I. *Demographic data of 35 patients under weekly fractionated paclitaxel.*

Parameter			Total		sHER-2/neu-positive		sHER-2/neu-negative	
			n=35	(%)	n=22	(%)	n=13	(%)
Age of the patient at the time of first diagnosis		range	31-63		34-63		31-56	
		mean	48		49		48	
Age of the patient at the time of diagnosis of stage IV disease		range	35-66		35-66		35-62	
		mean	51		51		52	
Age of the patient at the time of study with weekly paclitaxel		range	36-67		36-67		36-65	
		mean	54		53		54	
Surgical procedure	modified radical mastectomy		24	(69%)	15	(68%)	9	(69%)
	breast conserving		10	(29%)	7	(32%)	3	(23%)
	none		1	(3%)	0	(0%)	1	(8%)
Histology	invasive ductal		20	(57%)	13	(59%)	7	(54%)
	inflammatory		2	(6%)	1	(5%)	1	(8%)
	medullary		0	(0%)	0	(0%)	0	(0%)
	other		13	(37%)	8	(36%)	5	(38%)
Pathological staging of primary disease	T	1-2	25	(71%)	16	(73%)	9	(69%)
		3-4	5	(14%)	3	(14%)	2	(15%)
		x	5	(14%)	3	(14%)	2	(15%)
	N	0	8	(23%)	5	(23%)	3	(23%)
		1-2	20	(57%)	14	(64%)	6	(46%)
		x	7	(20%)	3	(14%)	4	(31%)
	M	0	31	(89%)	19	(86%)	12	(92%)
		1	4	(11%)	3	(14%)	1	(8%)
Grading	G	1	2	(6%)	2	(9%)	0	(0%)
		>1	21	(60%)	13	(59%)	8	(62%)
		x	12	(34%)	7	(32%)	5	(38%)
Receptor status	ER or PR positive		12	(34%)	8	(36%)	4	(31%)
	ER and PR negative		6	(17%)	5	(23%)	1	(8%)
	unknown		17	(49%)	9	(41%)	8	(62%)
Menopausal status	premenopausal		3	(9%)	2	(9%)	1	(8%)
	postmenopausal		32	(91%)	20	(91%)	12	(92%)
Metastases at the time of study entry	number of involved organs	1	9	(26%)	5	(23%)	4	(31%)
		2	11	(31%)	8	(36%)	3	(23%)
		>2	15	(43%)	9	(41%)	6	(46%)
	dominant site	visceral	29	(83%)	19	(86%)	10	(77%)
		soft tissue	6	(17%)	3	(14%)	3	(23%)
		bone	0	(0%)	0	(0%)	0	(0%)
Therapy for malignancy								
Chemotherapy	weekly paclitaxel as 1st-line		2	(6%)	0	(0%)	2	(15%)
	weekly paclitaxel as 2nd-line		21	(60%)	15	(68%)	6	(46%)
	weekly paclitaxel as 3rd-line		12	(34%)	7	(32%)	5	(38%)
	previous adjuvant chemotherapy		18	(51%)	13	(59%)	5	(38%)
	previous anthracycline-based therapy		34	(97%)	22	(100%)	12	(92%)
Hormonal therapy	adjuvant		8	(23%)	3	(14%)	5	(38%)
	palliative		21	(60%)	11	(50%)	10	(77%)
Radiation	adjuvant		19	(54%)	12	(55%)	7	(54%)
	palliative		15	(43%)	11	(50%)	4	(31%)

we will preferably refer to the sHER-2/neu results as measured by the Oncogene Science assay because currently this is one of the only sHER-2/neu tests with U.S. FDA approval for monitoring of metastatic breast cancer.

sHER-2/neu and outcome of paclitaxel therapy. In this biochemical study, we correlated the sHER-2/neu results with treatment outcome and tested for the monitoring validity of sHER-2/neu. The first serum specimen was collected at the beginning of paclitaxel treatment. These baseline levels ranged from 5-2373 ng/ml with a mean and median value of 142 ng/ml and 18 ng/ml, respectively. Twenty-two patients (62.9%) showed an elevated level of sHER-2/neu ≥15 ng/ml. A total of 14/35 patients (40%) responded to weekly paclitaxel by complete or partial remission (Table II). Taking into consideration the patients

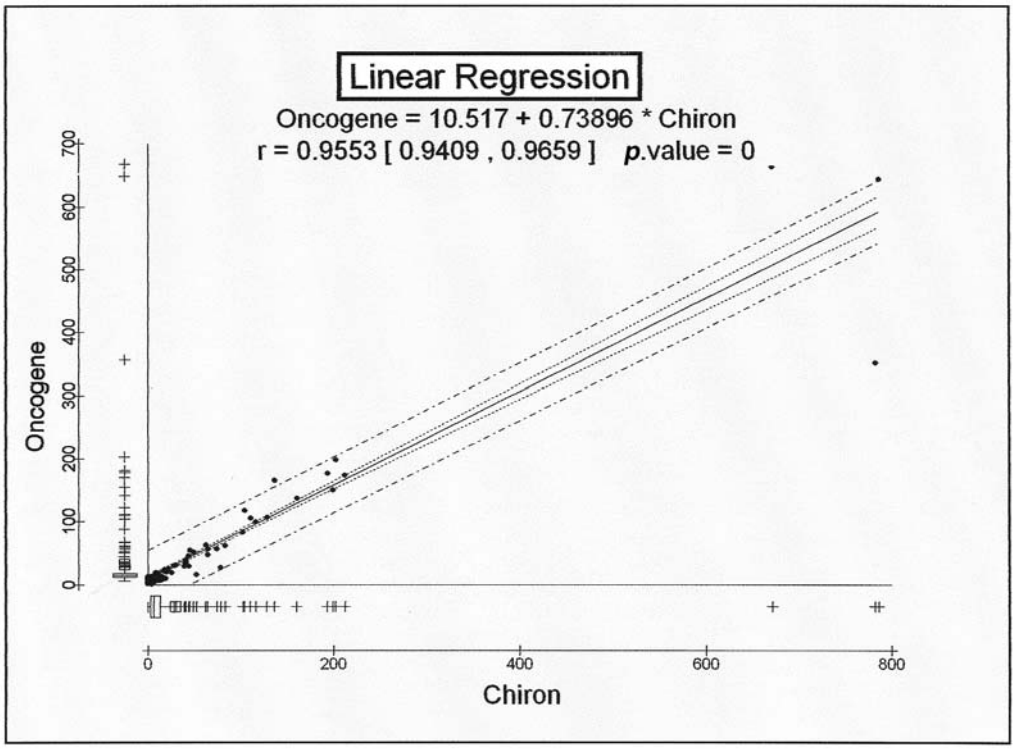

Figure 1. *Correlation of the measurement of the sHER-2/neu concentration with the Oncogene Science assay and the Chiron assay.*

with stable disease (34.3%) and the advanced line of chemotherapy for metastatic disease, weekly paclitaxel must be considered as a very effective treatment modality in this patient setting. The response rate among sHER-2/neu-positive patients and sHER-2/neu-negative patients did not show any statistically significant difference ($p=0.4$).

In terms of the progression-free survival, there was a non-statistically significant trend toward an improved outcome for the sHER-2/neu-negative patients (mean progression-free interval: 53.1 weeks) as compared to the sHER-2/neu-positive patients (mean progression-free interval: 31.2 weeks; $p=0.098$). The corresponding Kaplan-Meier curves are shown in Figure 2. The correlation of the baseline sHER-2/neu level with the duration of response showed a statistically significant difference ($p=0.042$). In this case, the sHER-2/neu-negative patients demonstrated a duration of response that was 254% better (65.2 weeks) than the duration of response for the sHER-2/neu-positive subgroup (25.7 weeks) (Figure 3).

As a next step, we analysed the data for further biological subgroups. When the patients with a sHER-2/neu level ≥ 20 / 22 / 25 / 30 ng/ml were compared with those with a sHER-2/neu level < 20 / 22 / 25 / 30 ng/ml, we found a statistically significantly shorter ($p=0.022$) progression-free survival for those with a sHER-2/neu level of 22 ng/ml or greater with decreasing p-values (Table III). Thus, we could

Table II. *Correlation of sHER-2/neu levels with best response, progression-free survival and duration of response.*

	Best response (n=35)			
sHER-2/neu	Number of patients	Outcome	Number of patients	%
Total	35	CR or PR or SD:	26	74.3%
		CR or PR:	14	40.0%
		PD:	9	25.7%
Positive	22	CR or PR or SD:	17	77.3%
		CR or PR:	9	40.9%
		PD:	5	22.7%
Negative	13	CR or PR or SD:	9	69.2%
		CR or PR:	5	38.5%
		PD:	4	30.8%

	Progression-free survival (n=26)		Duration of response (n=14)	
sHER-2/neu	Number of Patients	Progression-free survival (weeks)	Number of Patients	Duration of response (weeks)
Total	26	38.8	14	39.8
Positive	17	31.2	9	25.7
Negative	9	53.1*	5	65.2*
		*$p=0.098$		*$p=0.042$

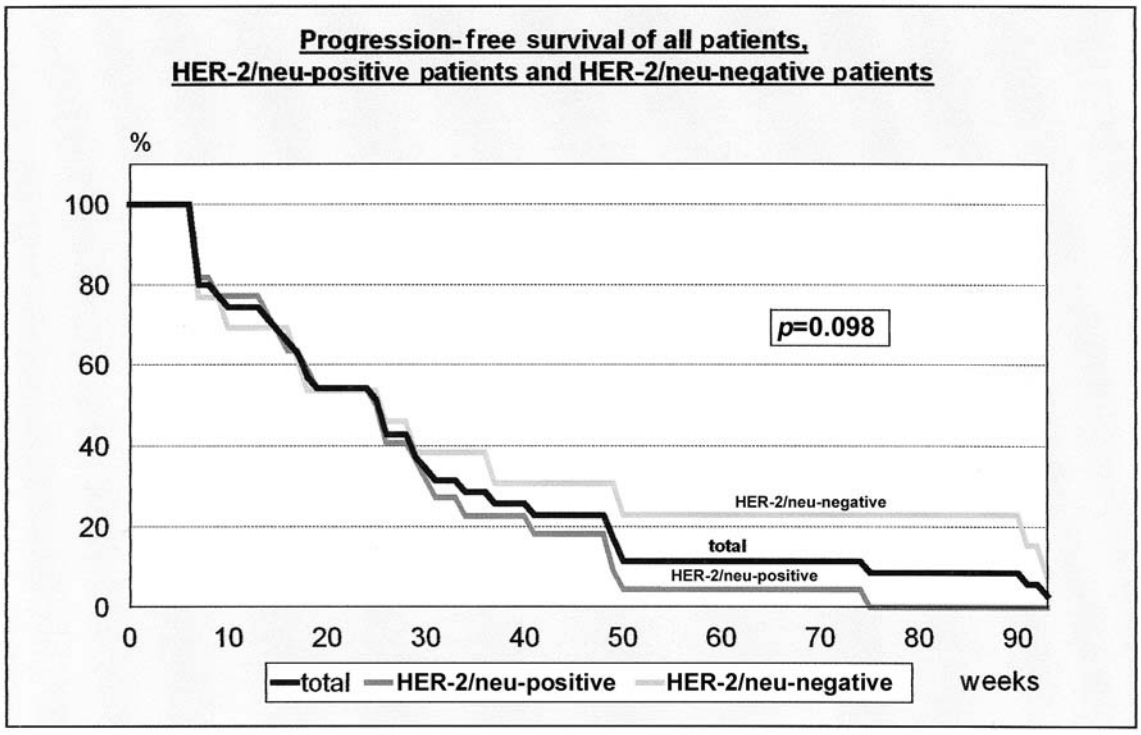

Figure 2. *Kaplan-Meier curve for the progression-free survival of all patients irrespective of the sHER-2/neu level (n=35) and for sHER-2/neu-positive patients (n=22) as compared to patients with normal sHER-2/neu level (n=13).*

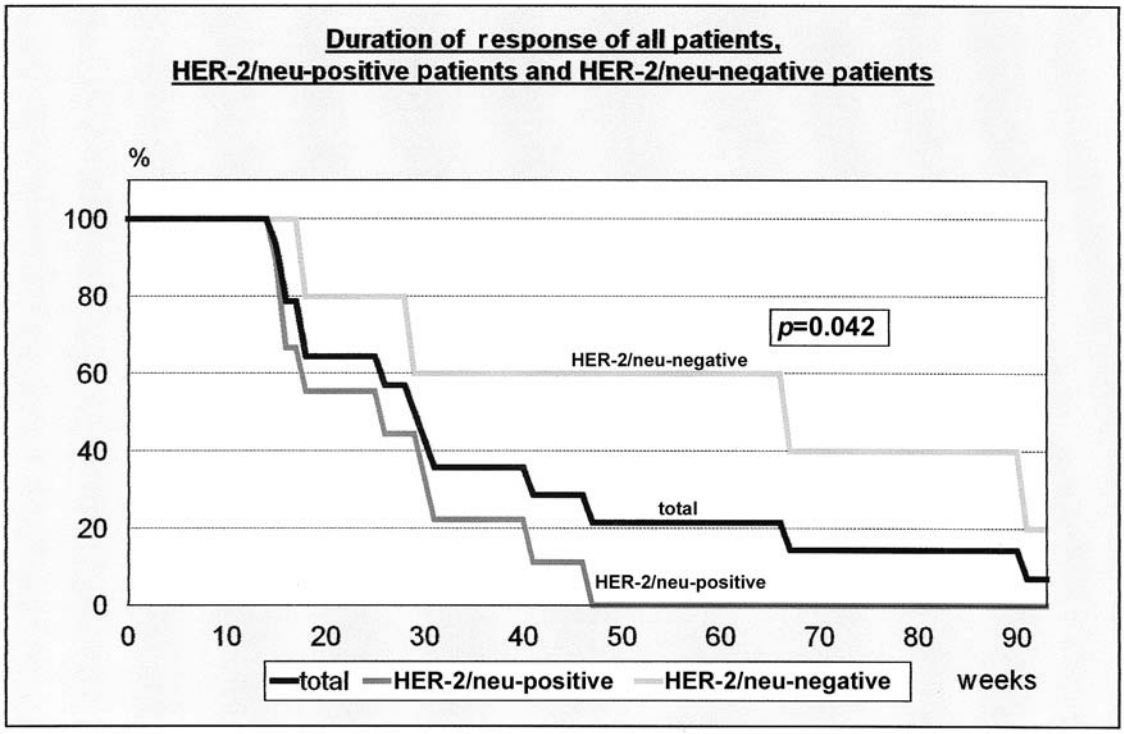

Figure 3. *Kaplan-Meier curve for the duration of response for all patients irrespective of the sHER-2/neu level (n=14) and for sHER-2/neu-positive patients (n=9) as compared to patients with normal sHER-2/neu level (n=5).*

Table III. *Definition of biological subgroups of sHER-2/neu positivity beyond the cut-off of normal of 15 ng/ml. The progression-free survival increases highly significantly even with small increases of sHER-2/neu.*

Definition of biological subgroups of sHER-2/neu positivity beyond the cut-off of normal of 15 ng/ml

Variation of the sHER-2/neu cut-off (ng/ml)	sHER-2/neu status total:	Number of patients n=26	Progression-free survival (weeks) 38.8	
15	positive:	17	31.2	$p=0.098$
	negative:	9	**53.1**	
20	positive:	12	21.1	$p=0.065$
	negative:	14	**72.1**	
22	positive:	11	16.8	$p=0.022$
	negative:	15	**80.3**	
25	positive:	10	13.9	$p=0.0089$
	negative:	16	**85.7**	
30	positive:	9	11.6	$p=0.0039$
	negative:	17	**90.1**	

identify a quantitative, negative correlation between outcome and sHER2/neu status. HER-2/neu-positive patients are heterogeneous and can be further subdivided using a strictly quantitative HER-2/neu serum assay.

sHER-2/neu and monitoring of disease. Finally, we correlated the clinical outcome with the longitudinal change of the sHER-2/neu concentration. In Figures 4A and 4B, the courses of the sHER-2/neu levels of two patients measured by the Oncogene Science assay and the Chiron assay are shown. Figure 4A shows the course of a patient responding to therapy by a partial remission as best response. During therapy, this patient's sHER-2/neu concentration had fallen below the upper limit of normal of 15 ng/ml. At the time of verified disease progression, the sHER-2/neu levels increased again. Figure 4B shows the sHER-2/neu changes of a patient failing weekly paclitaxel therapy by primary progression. The sHER-2/neu concentrations increased at the time of verified disease progression. As in the overall linear regression, the sHER-2/neu levels as measured by both ELISA assays provided the same information.

In the overall analysis on the monitoring quality of sHER-2/neu, we determined the mean change of the sHER-2/neu concentration for patients with disease regression by partial or complete remission as compared to patients with stable disease and compared to patients with primary progression. All sHER-2/neu-positive patients were entered, except for one patient who biased the calculation due to an extremely high baseline level > 2000 ng/ml. The mean decrease of the sHER-2/neu level was 19.6 ng/ml for responders, while the sHER-2/neu level increased by 17.3 ng/ml in patients with progressive disease (Table IV). This different course was statistically not significant ($p=0.15$) between the two groups. Interestingly, in the subgroup of patients with stable disease, the sHER-2/neu level increased by more than 200 ng/ml. Most probably this indicates an earlier biochemical progression, in comparison to morphological progression as determined by standard restaging procedures like clinical examination and imaging techniques. Furthermore, in 66.6% of the patients with an increased sHER-2/neu baseline level and objective response to therapy, sHER-2/neu fell into the normal range (Figure 5A). However, a decrease of the sHER-2/neu level to normal could rarely be demonstrated in non-responders (Figure 5B). Patients with negative sHER-2/neu level at baseline did not show any relevant change in their sHER-2/neu level. In 19 patients, sHER-2/neu results were available from previous chemotherapies with a median interval of 31 weeks before initiation of weekly paclitaxel therapy, *i.e.* from a time with markedly lower tumor load. While the mean sHER-2/neu level of these patients was 22.2 ng/ml at this early chemotherapy, the mean concentration increased approximately 10-fold by the time of weekly paclitaxel.

Table IV. *Change of the sHER-2/neu concentration under therapy with weekly, dose-intensified paclitaxel.*

Longitudinal course of the sHER-2/neu concentration (n=33)

	Number of patients		Mean change of the serum HER-2/neu concentration	
	HER-2/neu-positive	HER-2/neu-negative	HER-2/neu-positive	HER-2/neu-negative
CR+PR	9	4	-19.6	-0.8
SD	8	3	213.5	1.0
PD - reduced*	4	4	17.0	0.5

*In this group we did not take into consideration one patient with an extremely high baseline value of sHER-2/neu.

Discussion

In this analysis on the value of sHER-2/neu as a predictive marker for outcome of weekly fractionated paclitaxel chemotherapy, we found a statistically significant shorter duration of response for patients with elevated sHER-2/neu levels at the initiation of therapy. Using the established cut-off of normal of 15 ng/ml for sHER-2/neu, we also found a trend for a decreased progression-free survival for this patient subgroup. To our knowledge, this is the first analysis on sHER-2/neu showing that the higher

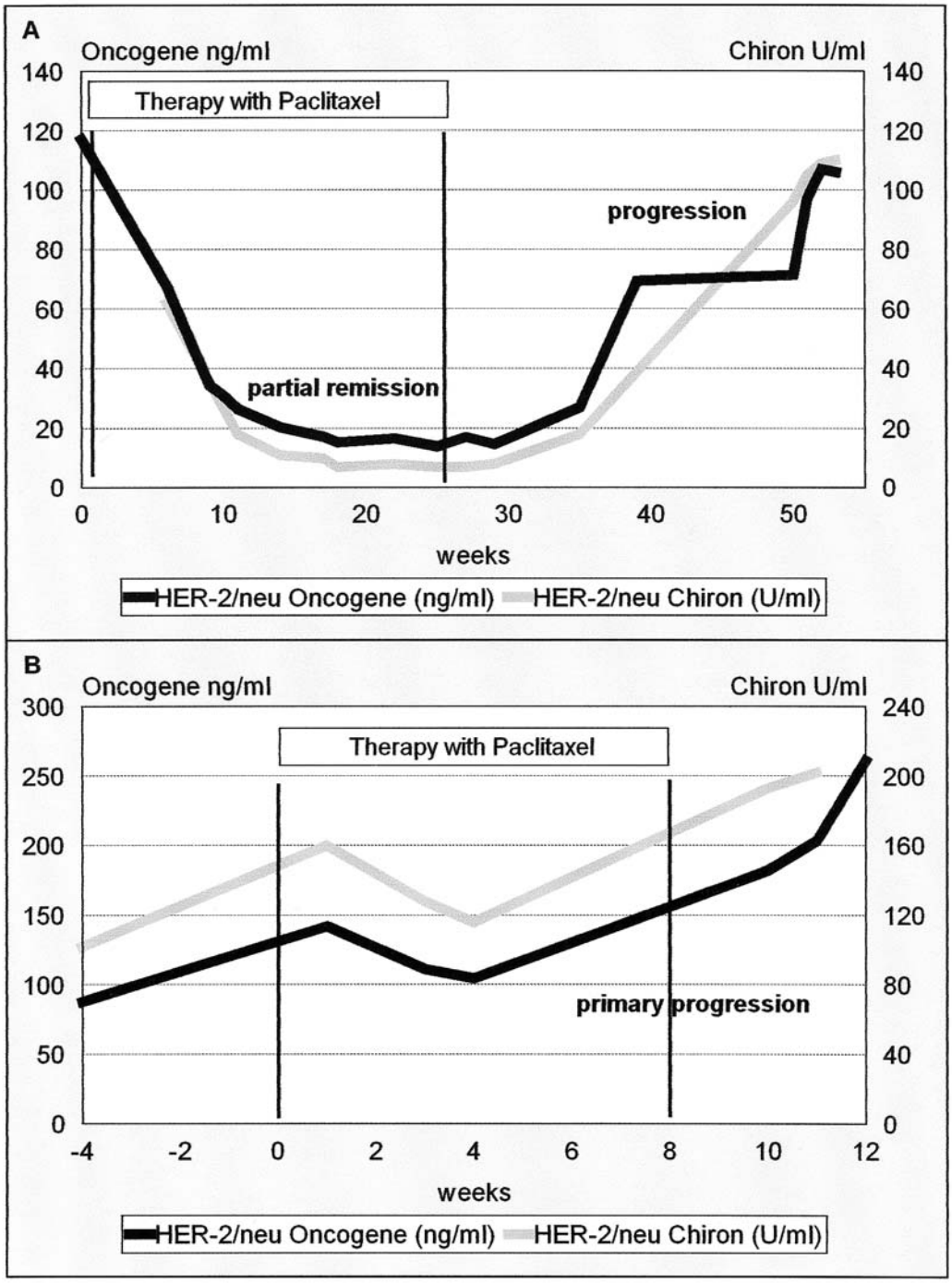

Figure 4. *Longitudinal course of sHER-2/neu. A: Patient with partial remission and later progressive disease. B: Patient with primary disease progression.*

the sHER-2/neu level is, the worse the progression-free interval becomes. We found the first statistically significant *p*-value at a sHER-2/neu level of 22 ng/ml indicating that there is a further biological heterogeneity beyond the cut-off of normal which could not be detected by semi-quantitative tissue assays for HER-2/neu. It must be emphasized that the difference between the cut-off of normal of 15 ng/ml and this first biological cut-off of 22 ng/ml is biologically not relevant. Our results indicate not only that patients with an elevated sHER-2/neu level do worse, but also that the higher this sHER-2/neu level is the worse the outcome.

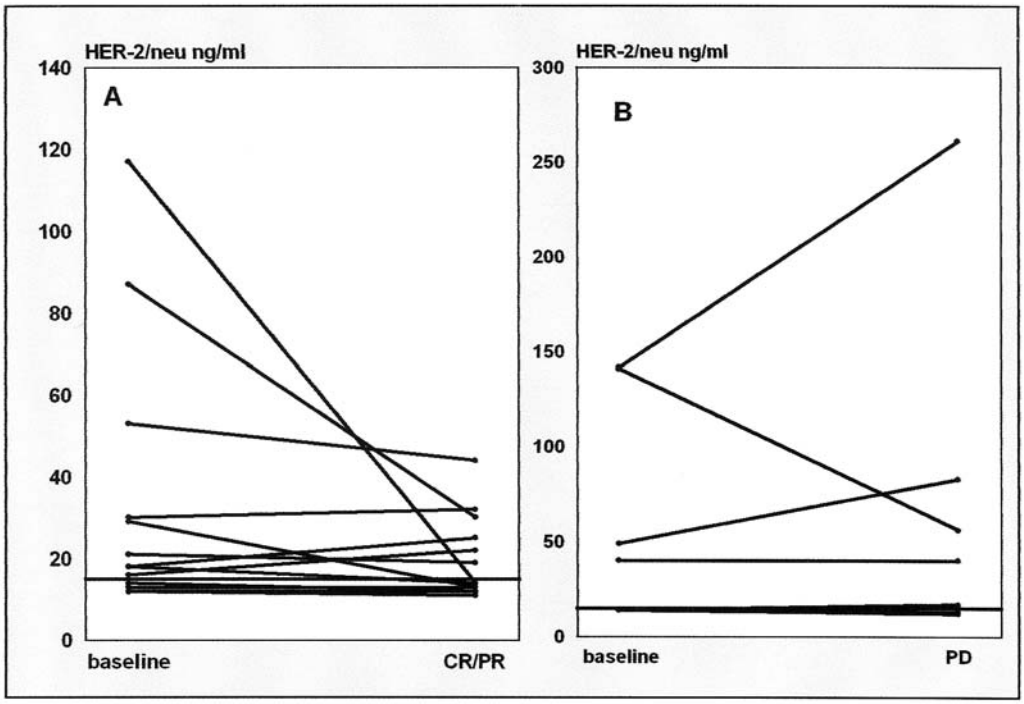

Figure 5. *Graphic display of the courses of the sHER-2/neu levels. A: Responding patients. B: Patients with primary progression. (One patient could not be shown because of her extremely high sHER-2/neu level with >2000 ng/ml).*

We did not find any difference in response rate between the sHER-2/neu-positive and the sHER-2/neu-negative subgroups. In a similar analysis, Colomer *et al.* found a correlation between high sHER-2/neu levels and low probability of achieving a response to the combination of doxorubicin and paclitaxel for first-line therapy in 58 metastatic breast cancer patients (18). While the progression-free survival results were identical between Colomer *et al.'s* and our analyses, the lack of correlation for response to therapy may be related to the different size of the patient population and, even more so, to the lineage of therapy. In general, responses are more difficult to induce the later the line of therapy is given which, thus, reduces the number of responses in our analysis.

The results from our study fit with data from other investigators. With the former Chiron assay, no association between sHER-2/neu status and response to paclitaxel chemotherapy was shown, while the circulating sHER-2/neu antigen strongly predicted for poorer overall survival in metastatic breast cancer with 30.2 months for the sHER-2/neu-negative patients as compared to 17.7 months for the sHER-2/neu-positive patients (19). Thus, HER-2/neu overexpression seems to confer sensitivity to taxanes in metastatic breast cancer as reflected by a good objective response, in spite of a persistent positive correlation of HER-2/neu positivity with poor prognostic features and more precise outcome measures (9,20). These results fit with preclinical research showing a more rapid regrowth of HER-2/neu-overexpressing xenografts than control xenografts following initial response to chemotherapy. The findings suggest that a high rate of tumor cell proliferation rather than intrinsic drug resistance may be responsible for the adverse outcome associated with HER-2/neu overexpression (21). The combination of paclitaxel plus an anthracycline might even help to overcome the unfavorable situation of HER-2/neu positivity in the metastatic setting, leading to a better response rate and progression-free survival for HER-2/neu-positive patients as compared to HER-2/neu-negative patients (22).

The percentage of sHER-2/neu-positive patients (60%) in this analysis is relatively high. There are several possible explanations for this observation. First of all, the patient number in this phase II study may result in selection bias. More importantly, it is very common for patients with advanced breast cancer to over-express HER-2/neu in serum (7,8). The reason for the discrepancy between the tissue HER-2/neu status of primary breast cancer patients with approximately 30% over-expressing HER-2/neu and the increased number of patients with sHER-2/neu positivity at the time of metastatic disease remains to be elucidated. One possible explanation is the heterogeneity of the primary tumor and the possibility that only selected populations of

cancer cells are those that are able to produce metastatic lesions. For example, positivity for HER-2/neu on bone marrow micrometastases was detected in 60% of primary breast cancer patients independent of the overexpression of HER-2/neu on the primary tumor cells (23). In other solid tumors such as gastric and bladder cancers, HER-2/neu expression levels have been shown to increase over the course of the disease. Specifically, the HER-2/neu expression level of the primary tumor was shown to be less than the HER-2/neu expression level of regional lymph nodes, which was shown to be less than the HER-2/neu expression level of distant metastases (24,25). A possible explanation for these phenomena could be the high potential for locomotion and extravasation of cytokeratin/HER-2 double-positive clustered circulating breast cancer cells. Blood-borne epithelium-derived HER-2/neu-positive clustered cells could be the precursor cells responsible for the formation of distant metastases (26,27).

Clinically relevant discordance of the HER-2/neu status has been shown with other methods, too. Edgerton *et al.* described discordance between primary breast carcinomas and their metachronic metastases in nearly 20% of cases, measured by immunohistochemistry and confirmed by FISH (28). It should be noted that, in a very recent analysis, the discordance of biological markers in primary breast cancers as compared to metastatic ipsilateral axillary lymph nodes approached 30%. In that particular study not one biological marker out of a panel of markers including HER-2/neu, *p53, bcl-2,* topoisomerase II-α and heat shock proteins 27 and 70 had 100% concordant results (29). The discordance between HER-2/neu results for immunohistochemistry, FISH and serum methods and between primary tumor and metastases was also seen for the patients in this phase II trial and will be reported elsewhere. To summarize, the percentage of advanced breast cancer patients with elevated HER-2/neu levels in serum is generally higher than HER-2/neu expression in tissue at the time of first diagnosis of the disease. The biological role of this feature is currently under investigation in clinical trials, among others focusing on the selection of stage IV breast cancer patients for trastuzumab (Herceptin®) antibody therapy.

The utilization of sHER-2/neu determinations for monitoring has been reproduced in clinical trials with and without integration of trastuzumab into the treatment schedule. Pegram *et al.* showed, in a trastuzumab phase II trial, that patients with stable or responsive disease had a significant decrease in sHER-2/neu after therapy with cisplatinum plus trastuzumab (30). They concluded that the decrease of the sHER-2/neu concentration was not sufficient to discriminate between patients with stable disease and those with objective clinical responses, because some patients with stable disease also showed decreasing sHER-2/neu levels. However, this is not astonishing as trastuzumab *per se* has been shown to diminish the shedding process of the extracellular domaine of HER-2/neu by inhibiting metallomatrix proteases which are responsible for this proteolytic process (4,31). In our analysis, the mean sHER-2/neu level increased in patients with stable disease, which could be an indicator of early biochemical progression while the proof of morphological progession by standard imaging techniques shows a certain lag time. In another trial with trastuzumab as single agent treatment for metastatic breast cancer patients with progression after chemotherapy, no correlation was demonstrated between sHER-2/neu and response status (32). A possible explanation for this result could be that they used a non-standardized assay for the measurements of sHER-2/neu for which insufficient data on the performance were presented, no cut-off of normal was established and nearly 40% of the sHER-2/neu results were below the detectable level.

The activity of weekly fractionated paclitaxel monotherapy in this heavily pretreated patient population with an overall response rate of 40% and a progression-free interval of nearly 39 weeks is strikingly good but not surprising. In previous trials, similar results were presented with a response rate of 53% and a duration of response of 7.5 months in a less pretreated patient population with n=30 (33). In our analysis, the responses were even more durable with a mean time of nearly 10 months. In a trial with a high recruitment goal of 200 patients, a preliminary analysis on the first 130 patients showed a response rate of 24% in a patient cohort with multiple lines of pretreatment including high-dose chemotherapy (34).

The high systemic activity of weekly, dose-intensified paclitaxel was the basis for combination with other weekly schedules of cytotoxic (gemcitabine, vinorelbine) or antibody therapy. The combination of weekly paclitaxel plus trastuzumab showed response rates of 41-46% for HER-2/neu-negative patients (depending on the antibody used for tissue immunophenotype determination) and an overall response of 67-81% for the HER-2/neu-positive subgroup (35,36). These promising results led to the CALGB 9840 programme for HER-2/neu-negative patients comparing weekly paclitaxel with/without trastuzumab to paclitaxel q3w plus weekly trastuzumab and compared to a control arm of paclitaxel q3w. In the HER-2/neu-positive subgroup, weekly trastuzumab was combined with either weekly paclitaxel or paclitaxel q3w. Irrespective of the results from this project, upcoming trials should evaluate combinations of weekly paclitaxel plus an anthracycline (plus trastuzumab for HER-2/neu-positive patients) as this combination has shown high, possibly synergistic, activity in the pre-clinical and clinical setting (37,38).

Conclusion

In this analysis of sHER-2/neu under weekly fractionated paclitaxel chemotherapy, we found a statistically significant shorter progression-free survival and duration of response for patients with slightly elevated sHER-2/neu baseline levels ≥ 22 ng/ml. Furthermore, the higher the sHER-2/neu levels became, the worse the progression-free interval was. We did not find any difference in response rate between the sHER-2/neu-positive and the sHER-2/neu-negative subgroups, possibly reflecting some sensitivity of HER-2/neu-positive breast cancers to paclitaxel therapy. As HER-2/neu-positive breast cancers are associated with sensitivity to anthracycline-based types of chemotherapy, the combination of these 2 drugs for the treatment of advanced breast cancer should be the focus of interest in prospective clinical trials.

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