

Longitudinal Changes in Serum HER-2/*neu* Oncoprotein Levels in Trastuzumab-treated Metastatic Breast Cancer Patients

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Abstract. *Background:* To evaluate longitudinal variations of serum HER-2/*neu* extracellular domain (sHER-2) in metastatic breast cancer patients receiving combined trastuzumab treatment. *Patients and Methods:* Thirty-three patients were monitored by serial sHER-2 ELISA (Oncogene Science). Results were compared to time to progression (TTP) and survival from treatment initiation. Non parametric statistical tests were used. *Results:* Median sHER-2 before first injection was 41.37 ng/ml (range 7.54-1597.00 ng/ml, n=32). Mean sHER-2 levels differed significantly between responders (n=20) and non responders (n=13) (p<0.0001). Median TTP (266 days, range 35-1000 days) was unrelated to clinicobiological variables at diagnosis or number and site of metastases before treatment. Patients with pre-treatment sHER-2 levels ≤30 ng/ml (n=14) had a significantly longer TTP than the group with sHER-2 > 30 ng/ml (n=18) (p=0.0346) and sHER-2 levels were of prognostic value for overall survival from first injection (p=0.0150). *Conclusion:* Our results show that monitoring serum HER-2/*neu* levels during metastatic breast cancer can provide a real time assessment of a woman's HER-2/*neu* status and can provide important information for therapeutic decisions.

Oncogenes and the overexpression of their oncoproteins have been considered to play an important role in the initiation and progression of human cancer (1). Human Epidermal Growth Factor Receptor-2 (HER-2/*neu*) or c-

erbB-2 is an important oncogene involved in cancer development and belongs to a family of tyrosine kinase cell surface receptors (2). This family of kinase receptors also includes c-erbB-1 (also known as HER-1 or EGFR (epidermal growth factor receptor)), erbB-3 (HER-3) and erbB-4 (HER-4). Recent studies indicate that HER-2/*neu* tyrosine phosphorylation and signal transduction is triggered by homo and heterodimerization between the extracellular domains of HER-2/*neu* and the extracellular domains of HER1, HER3 or HER4 (3).

The HER-2/*neu* oncoprotein is a transmembrane growth factor receptor protein that is overexpressed in some breast cancer cells (4) and is a major target for cancer therapies such as trastuzumab (Herceptin[®]) (5). The full length HER-2/*neu* oncoprotein has a molecular weight of 185,000 daltons (p185) and a 97,000-115,000 daltons extracellular domain (ECD) that is shed from the cell surface (6). Many studies have shown that the ECD is shed into the circulation of normal individuals and can be elevated in the serum or plasma of patients with metastatic breast cancer (7-9).

Due to therapeutic interventions with anti-HER-2/*neu* drugs like Herceptin[®], the HER-2/*neu* status of a woman with metastatic breast cancer has become an important factor for managing her treatment (10). However, the typical tissue tests of fluorescent *in situ* hybridization (FISH) and immunohistochemistry (IHC), used to determine HER-2/*neu* status, (11) are not practical once the original tumour is removed and therefore the immunoassay method serves as the only practical way to assess HER-2/*neu* status post-surgery. In this report, we employed a standardised and quantitative manual microtiter plate ELISA (7, 12) to measure HER-2/*neu* ECD. The serum HER-2/*neu* (sHER-2) assay uses monoclonal antibodies directed to epitopes on the HER-2/*neu* ECD and a soluble ECD standard for the accurate

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quantitation of the ECD in serum (13). This immunoassay was used to monitor the longitudinal changes in sHER-2/neu values of women with metastatic breast cancer who were being treated with trastuzumab and chemotherapy. The serum values were then compared to the changes in the clinical course of disease of these metastatic breast cancer patients. Our studies showed that the changes in sHER-2/neu values reflected the clinical course of disease and that increasing values of sHER-2/neu indicated breast cancer progression while decreasing serum values were indicative of response to therapy. This report illustrates that monitoring sHER-2/neu levels during metastatic breast cancer can provide a real time assessment of a woman's HER-2/neu status and can provide important information for therapeutic decisions.

Patients and Methods

Patients. Thirty-three breast cancer patients, treated with trastuzumab and various chemotherapies, were studied from January 1997 to August 2002. At the end of the study (January 2003), 17 (51.5%) patients were still under trastuzumab treatment. From November 2000 to August 2002, all patients received weekly injections of 2 mg/kg, after a first loading dose of 4 mg/kg. After August 2002, 16/17 patients continued to be treated by 3 weekly injections of 6 mg/kg after a loading dose of 8 mg/kg.

Serial serum samples were drawn before and during trastuzumab treatment and assayed for tumour markers CA 15.3 and CEA and the oncoprotein HER-2. All patient samples were analysed without knowledge of the clinical status of individual patients. For 22 patients, sera taken at diagnosis were retrieved from our blood bank. The cumulated trastuzumab dose (mg) at each date of blood sampling was calculated.

Evaluation of response to trastuzumab was based on serial measurements by medical imaging techniques (CT scan or echography) and on regular clinical evaluations during treatment. Responses were classified according to the WHO recommendations as progressive disease (PD), no change (NC), partial or dissociated response (PR) and very important or complete response (CR). For the statistical comparisons, treatment responses were scored at each date of blood sampling by a clinician unaware of the results of biological markers. A binary classification (responders: NC + PR + CR), versus non-responders (PD) was also used for comparisons and at the endpoint of the study for overall response.

CA 15.3 assays. Serum CA 15.3 was measured in duplicate by an immunoradiometric technique (ELSA, Cis Bio International, Gif sur Yvette, France). Quality control was ensured by assaying two levels of control sera in each series. Normal and elevated CA 15.3 concentrations were defined with reference to the 30 U/ml cut-off recommended by the manufacturer.

CEA assays. Serum CEA was measured in duplicate by an immunoradiometric technique (ELSA, Cis Bio International). Quality control was ensured by assaying two levels of control sera in each series. Normal and elevated CEA concentrations were defined with reference to the 5 ng/ml cut-off recommended by the manufacturer.

Serum HER-2 assays. sHER-2 concentrations were measured by ELISA (HER-2/neu Manual ELISA, Oncogene Science, Cambridge, MA, USA). All samples were analysed in duplicate. This sandwich enzyme immunoassay utilises two mouse monoclonal antibodies binding to independent sites on the HER-2 extracellular domain (13). Recombinant human HER-2/neu ECD secreted by NIH 3T3 cells was used to prepare calibrators and controls to track day-to-day assay performance. Quality control was ensured by assaying three levels of control sera in each series, which included high (15-22 ng/ml), medium (7.37-11.1 ng/ml) and low (3.09-4.63 ng/ml) HER-2/neu ECD.

Immunohistochemistry. Oestrogen (ER) and progesterone receptors (PgR) status were determined by using antibodies M 7047 for ER and M 3569 for PgR (Dako, Glostrup, Denmark). ErbB-2 overexpression was assessed with HercepTest (Dako) and CB 11 antibody (Novocastra, Newcastle, UK). When tumours were found to be 2+, the result was verified by FISH technique.

Statistical methods. All statistical analyses were performed using SAS statistical packages (Version 8.2, SAS Institute, Cary, NC, USA).

Differences in the distribution of the characteristics between patients' subgroups were analysed by using the Chi-square test. Differences between two independent groups were determined by Mann-Whitney *U*-test. Kruskal-Wallis one-way analysis of variance was used to test for overall homogeneity and, when the test was significant ($p < 0.05$), pairwise comparisons were performed by using the Mann-Whitney *U*-test. For the continuous variable of cumulated trastuzumab dose, an optimal cut-off point was determined by the method of Miller and Siegmund (14). To evaluate time to progression, the Kaplan-Meier (15) estimation was used and comparisons were made with the log-rank test (16).

The probability of disease progression was analysed longitudinally with the Generalised Estimating Equations (GEE) model. Let y_{ij} be the binary clinical response determined at the j th trastuzumab dosage for the patient i , $i = 1, \dots, n$, $j = 1, \dots, n_j$, and coded as 1 = disease progression, 0 = stable, partial or complete regression. We assume that observations on different patients are independent, though we allow for correlation between outcomes observed on the same patient. Each patient has covariates x_{ij} , which may be both time-stationary and time-varying covariates, including interaction terms. The marginal distribution of y_{ij} is Bernoulli with:

$$\pi_{ij} = \pi_{ij}(\beta) = P(y_{ij} = \text{disease progression} \mid x_{ij}, \beta) = \exp(\theta_{ij}) / (1 + \exp(\theta_{ij}))$$

where $\theta_{ij} = \ln(\pi_{ij} / (1 - \pi_{ij}))$ and β a vector of parameters to be estimated.

Our interest lies in making inferences about the parameters β associated with the marginal probabilities $\pi_{ij}(\beta)$. Since y_{ij} is binary, a natural choice of link function relating $\pi_{ij}(\beta)$ to the covariates is the logit link function:

$$\log(\pi_{ij} / (1 - \pi_{ij})) = x_{ij}' \cdot \beta$$

In addition to this marginal mean model, we need to model the covariance structure of the correlated observations on a given subject. The $n_i \times n_i$ covariance matrix of Y_i is modelled as:

$$V_i = \varphi \cdot A_i^{1/2} \cdot R(\alpha) \cdot A_i^{1/2}$$

where φ is the dispersion parameter, A_i is a diagonal matrix of variance functions, and $R(\alpha)$ is the working correlation matrix of Y_i indexed by a vector of parameters α , which are unknown and must be estimated.

Table I. Clinico-biological characteristics of the patients at first diagnosis.

Variable	Categories	n (%)
Age (years)	≤35	5 (15.1)
	36-45	11 (33.3)
	46-55	10 (30.3)
	> 55	7 (21.2)
Tumour size	T0	1 (4.2)
	T1	3 (12.5)
	T2	12 (50.0)
	T3	7 (29.2)
	T4	1 (4.2)
	unknown	9
Number of invaded lymph nodes (pN)	0	5 (18.5)
	1-3	7 (25.9)
	>3	15 (55.6)
	unknown	6
Metastases (M)	absence	24 (72.7)
	presence	9 (27.3)
Histology	ductal	28 (84.8)
	lobular	1 (3.0)
	undifferentiated	4 (12.1)
Histological grading*	II	16 (51.6)
	III	15 (48.4)
	unknown	2
Carcinoma <i>in situ</i>	absence	12 (38.7)
	presence	19 (61.3)
	unknown	2
Immunohistochemistry	2+	3 (9.1)
	3+	30 (90.9)
ER	negative	19 (57.6)
	positive	14 (42.4)
PgR	negative	21 (63.6)
	positive	12 (36.4)
Baseline CA 15.3 (U/ml)	median 18.5	range 11-4720
Baseline CEA (ng/ml)	median 2.0	range 1-1152
Baseline sHER-2 (ng/ml)	median 15.62	range 7.69-345.60

*Scarff Bloom Richardson up to January 2001 (n=25) Elston and Ellis after (n=6)

There are several specific choices of the form of working correlation matrix. The assessment of the best model and of the appropriate covariance structure was made according to two criteria: the *p*-value associated with the estimated regression coefficients β and the goodness of fit, determined by the measures of scaled deviance and scaled Pearson provided by the GENMOD SAS procedure including the GEE support.

Responses to trastuzumab treatment and survival. Time to disease progression (days) was defined as the time from first trastuzumab

injection to disease progression or death (whichever occurred first) and was censored at the endpoint date for patients who were still responding to treatment. Survival was defined as the time from first trastuzumab injection to death and was censored at the last visit for patients who were alive.

Results

Clinico-biological characteristics of the patients and treatment modalities. All patients were selected for trastuzumab treatment on the basis of tumour erbB-2 overexpression and consequently differed from the general population of breast cancers by several characteristics. The main characteristics of the patients are summarised in Table I. The mean age of the patients was 45.3 ± 10.5 years with a high proportion of women under 45 years. Fifty-five percent of patients had more than 3 invaded lymph nodes and 27% metastases at presentation. Among metastatic sites, there was a high proportion of visceral metastases (liver 33%, lung 12%). There was also a high proportion of tumours with *in situ* carcinoma, including 6 comedocarcinomas and 8 high-grade *in situ* carcinomas, and of grade III histological grading. The proportion of ER- or PgR-positive tumours was significantly less than in the general population of breast cancers. Twelve patients received neoadjuvant treatment, 26 underwent surgery, 3 received adjuvant tamoxifen therapy and 15 adjuvant chemotherapy. Before trastuzumab treatment, 9 patients presented a local recurrence and all developed metastases, as single metastasis in 8 cases. The first metastatic site was in lymph nodes for 12 patients, liver for 11 patients, bone for 5 patients, lung for 4 patients and skin metastases for one patient. Trastuzumab treatment was the first-line therapy for 10 (30%) patients, one patient received a previous line of therapy and 14 (42%) from 2 to 8 lines of treatment. All but 3 patients were treated by trastuzumab combined to chemotherapy or hormone therapy. Trastuzumab therapy was used in conjunction with docetaxel for 11 patients, paclitaxel for 15 patients, 5-fluorouracil and docetaxel for 2 patients, cis-platinum or anti-aromatase for one patient. The median follow-up time from trastuzumab treatment was 312 days (interquartile 168 days). The median number of trastuzumab injections was 24 (range 2-88), with a median cumulated dose of 3150 mg (range 780-9900). At the endpoint of the study, treatment with trastuzumab was discontinued for 17 (51%) patients, 13 patients because of disease progression and 4 because of toxicity, while 9 (27%) patients died.

Baseline serum sHER-2, CA 15.3 and CEA. The mean and standard deviation (SD) of baseline sHER-2 was 38.25 ± 72.24 ng/ml (range 7.69-345.60 ng/ml). The sHER-2 concentration was ≤ 15 ng/ml in 10 cases (45.5%) and > 15 ng/ml in 12 (54.5%) cases. Baseline sHER-2 was positively correlated with tumour size in 3 groups (T0+T1, T2, T3+T4) ($p=0.025$), but was unrelated to the number of metastatic

Table II. Biological markers according to treatment responses at each blood sampling.

	Progressive disease n=29	No change n=16	Partial response n=47	Complete response n=72
sHER-2 (ng/ml)				
Mean ± SD	292.35 ± 585.66	83.92 ± 106.94	40.15 ± 48.43	11.53 ± 4.57*
Median	99.6	36.22	20.64	10.20
Range	9.88-2565	9.48-417.40	5.80-207.82	6.14-31.75
CA 15.3 (U/ml)				
Mean	646 ± 1226	544 ± 1086	53 ± 60	21 ± 11
Median	76	101.5	31	20.5
Range	10-4440	20-3540	6-322	5-68
CEA (ng/ml)				
Mean	415 ± 1226	867 ± 2270	40 ± 126	5 ± 13
Median	10	9	3	1
Range	1-6400	1-6950	1-800	1-82

* n=71 for sHER-2 in this group.

Table III. Pattern of sHER-2 variations according to endpoint clinical responses.

	Non-responding patients n=13	Responding patients n=20
Continuous increase of sHER-2	2	0
Fluctuations of sHER-2	2	0
Continuous decrease of sHER-2	3	15
Transient sHER-2 decrease :	6	3
With at least one sHER-2 level ≤15 ng/ml	1	1
sHER-2 decrease without reaching 15 ng/ml	5	2

sites before treatment. CA 15.3 was found to be positively correlated only with the presence of metastases at diagnosis ($p=0.010$). Serum CEA showed no significant relationships with clinical or histological variables of the initial evaluation or from follow-up (Table I). Spearman rank correlation showed that pre-treatment sHER-2 was positively correlated with CEA ($p=0.002$), but not with CA 15.3.

Tumour biological markers before first injection and during trastuzumab treatment. The mean number of serum samples per patient was 6.6 ± 3.5 . The mean interval between consecutive blood sampling was 46.7 ± 37.6 days. For one patient a blood sample taken less than 6 weeks before first trastuzumab injection was not available.

Mean±SD sHER-2 before first trastuzumab injection was 165.94 ± 342.41 ng/ml (median 41.37, range 7.54-1597.00 ng/ml, n=32). Mean±SD CA 15.3 was 539 ± 1502 U/ml (median 36, range 11-6540 U/ml). Thirteen out of 32

(40.6%) pre-treatment blood samples were ≤ 30 U/ml for CA 15.3. Mean±SD CEA was 238 ± 939 ng/ml (median 7, range 1- 5250 ng/ml). When grouped according to IHC scores, mean±SD sHER-2 before trastuzumab was 23.61 ± 13.71 ng/ml for 2+ tumours (n=3) and 175.80 ± 352.29 ng/ml for 3+ tumours (n=30). Due to the small number of cases this difference is not statistically significant.

During trastuzumab treatment, the distributions of sHER-2, CA 15.3 and CEA were divided into the following quartiles: – represents the $\leq 50\%$ quartile (median), + represents the 50% quartile to the $\leq 75\%$ quartile, and ++ represents the $> 75\%$ quartile. The pairwise comparisons revealed significant statistical relationships (Chi-square tests, $p < 0.0001$ in all instances). However, their trough and maximum concentrations were found to be generally not synchronous. Six out of 33 cases never show elevated CA 15.3 during monitoring although sHER-2 and CEA were elevated.

sHER-2 before and during trastuzumab treatment and clinical responses. The mean and median concentrations of sHER-2, CEA and CA 15.3 according to the four different groups of responses at each sampling date during treatment are presented in Table II. The general trend is a decrease of the concentration of all biological markers as the quality of treatment response increases. When dichotomised according to the endpoint overall response, 15 out of 20 responding patients showed a continuous decrease of sHER-2. Non-responding patients displayed several patterns, but only one out of the 13 patients had sHER-2 levels below 15 ng/ml (Table III). The difference in mean sHER-2 levels of responders versus non-responders is statistically different ($p < 0.0001$).

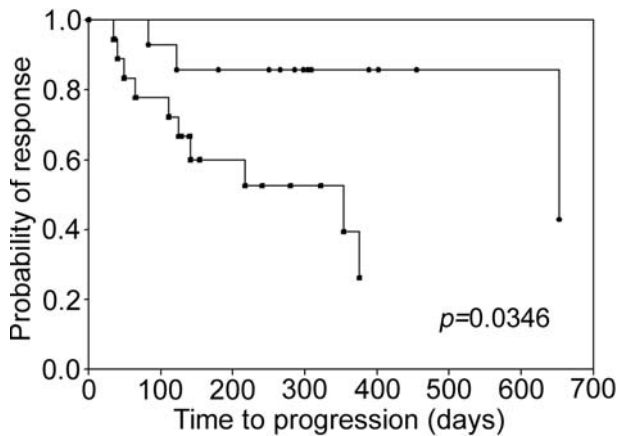


Figure 1. Time to progression of trastuzumab-treated breast cancer patients according to pre-treatment sHER-2 concentration (cut-off 30 ng/ml). ● = sHER-2 ≤ 30 ng/ml, ■ = sHER-2 > 30 ng/ml.

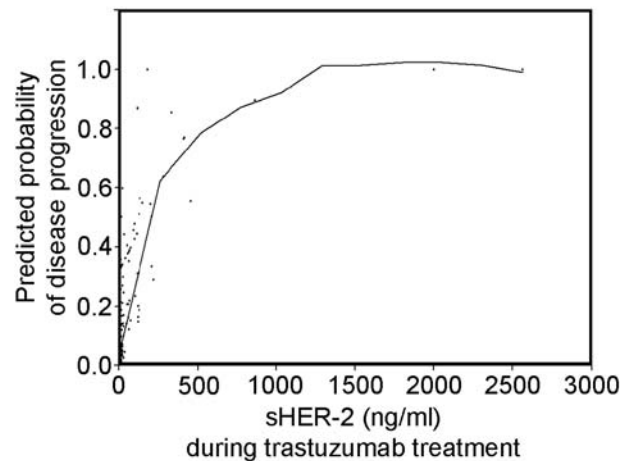


Figure 2. Predicted probability of disease progression as a function of sHER-2 concentration during trastuzumab-based treatment.

Mean CA 15.3 was 645 ± 1226 U/ml for patients with PD and 94 ± 401 U/ml for responsive (NC+PR+CR) patients. The differences are also significant ($p < 0.0001$). On the contrary, non-significant differences were found for CEA (415 ± 1225 ng/ml during PD and 120 ± 814 for responsive patients).

As expected, the cumulated doses were also significantly related to patient response, the highest doses being found in the group of responding patients (3074 ± 2020 mg of trastuzumab versus 1742 ± 1941 mg in the PD group). Using the method of Miller and Siegmund, we found 480 mg of trastuzumab cumulated dose to best discriminate between responding and non-responding patients. This cumulated dose corresponds to the third or fourth week of treatment. For the test based on the usual Chi-square distribution, the required p -value was very small. Because of the well-

known problem of multiple testing, the p -value was corrected and reached a value of less than 0.00000052 when restricting the range of possible cut-off points to the interval between the 1% and 99% quantiles of the cumulated dose.

sHER-2 and time to progression. Patients had a mean time to progression (TTP) of 289 ± 221 days (median 266 days, range 35-1000 days). At the endpoint of the study, 13 patients were classified as non-responders and 20 patients were still responsive to treatment, including 4 censored patients. The time to progression was found to be unrelated to clinical, histological and biological variables recorded at diagnosis (Tables I and II). TTP was also unrelated to the site of the first metastasis or to the number of metastases before trastuzumab treatment.

Table IV. Analysis of GEE parameter estimates.

Variables	Coefficient	Standard Error	Z value	P-value
Intercept	7.1402	1.3290	5.37	< 0.0001
sHER-2 before trastuzumab ^a	1.8917	0.6470	2.92	0.0035
sHER-2 (ng/ml) ^b	0.0047	0.0022	2.10	0.0360
CEA (ng/ml) x ER status ^{b,c}	0.0324	0.0057	5.74	< 0.0001
CA 15-3 (U/ml) x PgR status ^{b,c}	0.0006	0.0002	2.44	0.0147
Cumulated dose (mg) x time to progression ^d	-0.0004	0.0001	-4.86	< 0.0001
Lung or liver first metastasis ^e	-1.1641	0.3727	-3.12	0.0018
IHC ^f	-3.2671	0.6080	-5.37	< 0.0001

^a sHER-2 before trastuzumab is coded as 1 if greater than 30 ng/ml, 0 otherwise.

^b sHER-2, CEA, and CA 15-3 were evaluated during trastuzumab therapy.

^c ER and PgR status are coded as 0 if negative, 1 otherwise.

^d Time to progression expressed in two classes according to the median cut-off (266 days).

^e Existence of a liver or lung first metastasis is indicated by 1, 0 otherwise.

^f IHC score is coded as ++ = 0, +++ = 1.

We tested different cut-off levels of sHER-2 to study the relationship between sHER-2 concentration before first trastuzumab injection and TTP. The best cut-off found was 30 ng/ml ($p=0.0367$), while the median cut-off (41.37 ng/ml) was less significant ($p=0.0458$). Patients with a pre-treatment sHER-2 ≤ 30 ng/ml ($n=14$) had a longer TTP (median 301 days, range 83-1000 days) than the group with sHER-2 > 30 ng/ml ($n=18$, median 147 days, range 35-848), the difference between responders and non-responders being significant by the log rank test ($p=0.0346$, Figure 1).

Survival from initiation of trastuzumab treatment. Median survival time was 312 days (range 50-1027 days). None of the classical variables of the initial evaluation of the cancer were found to have a prognostic value for survival by Kaplan Meier analysis. The response to treatment in two groups (PD versus NC + PR + CR) was highly significant (8 deaths in the PD group versus 1 in the responders, $p<0.0001$). sHER-2 before first trastuzumab injection was also of prognostic value, with only one out of 9 patients having ≤ 30 ng/ml sHER-2 dying, whereas the ratio was 8/9 when sHER-2 was over 30 ng/ml ($p=0.0150$).

Statistical models predicting responses to trastuzumab. All clinical, histological and biological variables and the data concerning the trastuzumab treatment and patients' responses were analysed in the generalised estimating equations (GEE) model. We applied both forward and backward model selection procedures for the mean structures using different working correlation matrices. As expected, since our data are unbalanced, the final model has been constructed by using the exchangeable working correlation matrix, calculated in all cases; exchangeable refers to having a value of 1 on the diagonal and identical off-diagonal elements corresponding to a number estimated by default in the SAS GENMOD procedure. The probability of having disease progression showed a significant association with: high sHER2 levels evaluated before trastuzumab therapy, high sHER2 levels during trastuzumab treatment, the interactions between CEA levels and ER, between CA 15.3 levels and PgR, between the cumulated trastuzumab dose and the time to progression expressed in two modalities according the median cut-off, the existence of a first lung or liver metastasis and finally with the IHC score.

The final model is displayed in Table IV and Figure 2. It indicates the covariates of the mean structure with a nominal p -value < 0.05 obtained after model selection. The ratios of the estimated coefficients to their standard errors are all quite large, indicating a significant population model effect. Goodness of fit measures of scaled deviance and scaled Pearson were found to be respectively 0.63 and 0.73, both less than 1, suggesting a good fit of the model.

Discussion

The study population was heterogeneous regarding treatment protocols. Trastuzumab-containing therapies were used after classical treatments had failed for 2/3 of the patients. Consequently, the results presented here are preliminary and have to be confirmed by prospective protocols.

At the technical level, it has been shown in a previous study by Payne *et al.* (17) that high trastuzumab concentrations spiked into sHER-2/neu assays did not interfere with quantitation of the HER-2/neu ECD, therefore the data presented here reflect the real sHER-2/neu status. Before trastuzumab treatment, we found median sHER-2 more elevated in IHC 3+ than IHC 2+ tumours. However, the difference was not significant due to the small size of the series. For the same reason, no statistically significant relationship was evidenced between sHER-2 or CA 15.3 concentrations before trastuzumab and tumour burden estimated by the number of metastatic sites. Such relationships were suggested but not firmly demonstrated in a previous work by Ali *et al.* (18). Schoendorf *et al.* found a high proportion of elevated sHER-2 in patients with visceral metastases (19).

Our results show that the variations of sHER-2 during trastuzumab treatment correlate very well with the clinical course of the patients. Similar conclusions were drawn by two previous studies by Esteva *et al.* (20) and Schoendorf *et al.* (19). The kinetics of sHER-2 variations were neither superimposable on those of CA 15.3, since we observed 18% of CA 15.3 under cut-off during patient's monitoring, nor to CEA, especially in the absence of liver metastases. It must be emphasised that sHER2 variations during combined trastuzumab treatment are also partly due, as for tumour markers, to the effect of associated therapy whose contribution cannot be dissociated from sHER-2/trastuzumab interaction.

Apart from biological monitoring during trastuzumab-based treatment, our results show that the pre-treatment sHER-2 level has a predictive value for response to treatment. We constructed a model that can be used to emulate patient response. The significant variables in this model included pre-treatment sHER-2 concentration, sHER-2, CEA and CA 15.3 concentrations at any time during treatment, ER or PgR status, cumulated trastuzumab dose x time to progression, existence of first lung or liver metastasis and initial IHC score. More importantly, we have shown pre-treatment sHER-2 above 30ng/ml to be predictive for treatment failure. Several explanations can be put forward. In a pharmacokinetic study, Baselga *et al.* observed 5 out of 45 patients presenting with a very elevated baseline sHER-2 concentration and a shortened trastuzumab half-life (21). However, the formation of circulating immune complexes, resulting in a reduced trastuzumab action, has not yet been evidenced in large series. It cannot be excluded that elevated levels of shed ECD result in fewer sites for trastuzumab binding to the cell. Trastuzumab

needs to bind the ECD to cause intracellular damage and down-regulation of signal transduction. Therefore, the growth-promoting effect of constitutively active transmembrane receptor tyrosine kinase domain induced by a high rate of erbB-2 cleavage could be incompletely inhibited by trastuzumab.

In accordance with preliminary studies (23, 24), our results show that trastuzumab response can be evaluated early, at nearly 500 mg of trastuzumab cumulated dose, or after the first three weeks of treatment. sHER-2 monitoring of trastuzumab-treated patients could facilitate clinical decisions regarding the need to prolong treatment. Moreover, our work demonstrates sHER-2 to be a powerful predictor of survival even in heavily metastatic and treated patients.

In conclusion, in trastuzumab-treated patients, sHER-2 monitoring is a predictor of therapeutic response and a prognostic factor. If our preliminary observations are confirmed by larger series, this oncoprotein will become an important biological marker in breast cancer.

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