

Kinetics of HER2/neu ECD in 45 Patients Treated with Trastuzumab (Herceptin®) between January 2001 and June 2005 at the Grenoble University Hospital

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Abstract. The aim of this study was to evaluate the utility of HER2/neu ECD concentration as a marker of the efficacy of clinical response to Herceptin®. Patients and Methods: Iterative measurements of HER2/neu ECD (ELISA c-erbB2/c-neu Rapid Format Elisa kit QIA10™ Calbiochem®) concentrations in 45 patients treated with Herceptin® between January 2001 and June 2005 at the Grenoble University Hospital. Results: Changes in HER2/neu ECD concentrations were observed in 21 patients (47%). The baseline concentration was the concentration of circulating HER2/neu ECD before treatment with Herceptin®. In 15 patients, the mean baseline concentration was 52 ng mL⁻¹ (extreme values 13-170), which normalized no later than at the time of the 3rd administration of Herceptin®. Nine patients (60%) were still alive 5 years later ($p<0.05$). For 6 patients, the mean baseline concentration was 800 ng mL⁻¹ (extreme values 140-2000) which persisted and even increased during Herceptin® therapy; fewer than 25% were alive 30 months later ($p<0.05$). In the case of the 24 patients whose HER2/neu ECD concentration remained <5 ng mL⁻¹, survival time was intermediate. Conclusion: The study confirmed the utility of HER2/neu ECD in predicting therapeutic response. However, as in the case of other circulating tumor markers, it is only useful when there is a variation in concentration. This marker should now be evaluated in multi-center studies covering a large number of homogeneous subjects.

Twenty percent of breast cancers are characterized by overexpression of the human epidermal growth factor receptor 2, HER2/neu, oncogene. This overexpression is predictive of overall survival and disease-free survival, of how the disease progresses in the early or advanced stages and has been found to correlate with high-grade breast carcinomas and negative hormone receptors (1). In addition to its prognostic value concerning disease progression, HER2/neu expression can be a useful indicator of the efficacy of anti-tumor therapy since it has been shown to be predictive of poorer response to chemotherapy and hormonal therapy. Furthermore, HER2/neu overexpression is predictive of the effectiveness of treatment with trastuzumab (Herceptin®), a monoclonal antibody that specifically targets the HER2 receptor (2). But despite this specific targeting, only 30% of patients selected for this treatment have an objective response to first-line single-agent treatment and only 18% respond after second-line chemotherapy, confirming the existence of resistance mechanisms (3). In all cases, response to trastuzumab is limited in time in terms of the efficacy and duration of response (4).

Studies that have used the extracellular domain (ECD) of HER2/neu as a biological marker have shown that 20 to 40% of metastatic breast cancers present high concentrations of HER2/neu ECD (5). Measurements of serum HER2/neu provide the opportunity to monitor clinical response to Herceptin® and can enable early detection of any decreasing response to therapy (6). This is beneficial in terms of quality of patient follow-up, as well as optimization of costs related to the use of an expensive molecule. Measurement of HER2/neu ECD concentration is useful not only for monitoring patients with metastatic breast cancer, but also as a predictive marker for evaluating targeted therapy (7). Biological monitoring can be used to predict response to trastuzumab therapy and improve disease-free survival (8). On a prospective cohort of 45 patients with breast cancer,

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Table I. Anatomopathological characteristics of the study population treated with Herceptin® (n=45).

	Study population, patients treated with Herceptin® n=45	
Histological type		
IDC ⁽¹⁾	38	84.5%
ILC ⁽²⁾	2	4.5%
DCIS ⁽³⁾	1	2%
CI ⁽⁴⁾	3	7%
OTHER	1	2%
Hormone receptor status		
ER ⁽⁵⁾ and/or PR ⁽⁶⁾ >10%	21	47%
ER and PR <10%	24	53%
Histological grade SBR ⁽⁷⁾		
I	3	6.5%
II	13	29%
III	26	58%
ND	3	6.5%
Her2 status in immunohistochemistry (IHC)		
2 +	3	6.7%
3 +	42	93.3%

⁽¹⁾ Infiltrating ductal carcinoma, ⁽²⁾ infiltrating lobular carcinoma, ⁽³⁾ ductal carcinoma *in situ*, ⁽⁴⁾ inflammatory carcinoma, ⁽⁵⁾ estrogen receptors, ⁽⁶⁾ progesterone receptors, ⁽⁷⁾ Scarff, Bloom and Richardson.

treated with Herceptin® between January 2001 and June 2005 at the Grenoble University Hospital, the kinetics of HER2/neu ECD, were evaluated and were correlated with the clinical course of the patients.

Patients and Methods

Biological monitoring was performed throughout the period of care and treatment of 45 patients with breast cancer who received Herceptin® therapy between January 2001 and June 2005 in the oncology department of the Grenoble University Hospital. By consulting each patient's medical dossier, biological measurements were regularly compared with the clinical evaluation according to RECIST (*Response Evaluation Criteria In Solid Tumor*) criteria. Patients were informed prior to blood sampling and their signed consent was obtained.

Blood samples. Blood samples were taken from each patient before Herceptin® therapy then, whenever possible, further samples were obtained each time a patient was admitted to the day-hospital for administration of Herceptin®. After centrifugation, the serum aliquots were stored at -20°C prior to measurement of HER2/neu ECD concentrations (ELISA c-erbB2/c-neu Rapid Format Elisa kit QIA10™ Calbiochem®).

Statistical analysis. The survival curves were estimated using the Kaplan-Meier method. A log-rank test was used to compare survival between the groups. The level of significance used to test the hypotheses was 5% ($p<0.05$).

Table II. Clinical characteristics of the study population treated with Herceptin®.

	Patients treated with Herceptin® n=45
Age (years)	
Median	53.7
Mean±SD	53.2±13.3
Extremes	25-80
Free interval (months)	
<12	17
12-24	8
>24	11
No metastases	9
Adjuvant chemotherapy	23
Herceptin as neoadjuvant	9
Metastatic chemotherapy	
1 line	8
≥2 lines	14
Herceptin® from outset	11
Radiotherapy	26
Hormonal therapy	13

Results

Forty-five patients were treated with Herceptin® at the Grenoble University Hospital between January 2001 and June 2005. The median age of these patients was 53.7 years (extremes 25-80 years). The anatomopathological aspects obtained after analysis of the excised breast tumor indicated that over 84% of the study population presented an infiltrating ductal carcinoma. The hormone receptors were positive in 47% of cases and 58% of the tumors were grade III according to Scarf and Bloom criteria. HER2 overexpression in immunohistochemistry (IHC) was found to be 3+ in 93% (42/45) of cases and 2+ in 7% of cases (Table I).

The effect of Herceptin® is potentialized when combined with certain chemotherapies. Combined therapy with Navelbine represented the majority of such cases in the study population in terms of the duration of the combination. Twenty-three patients received adjuvant chemotherapy, not including the combination containing Herceptin® (Table II).

Profiles of HER2/neu ECD kinetics (Figure 1). In the case of 21 patients (47%), changes in HER2/neu ECD concentrations were observed during Herceptin® treatment. These patients could be divided into 2 kinetic profiles predictive of response to Herceptin®: The first profile (P1) concerns patients for whom the mean baseline concentration of HER2/neu ECD was 52 ng mL⁻¹ (extremes 13-170). No later than the 3rd administration of the molecule, this concentration dropped below the threshold defined in the measurement technique used, namely 5 ng mL⁻¹ (9). The 15

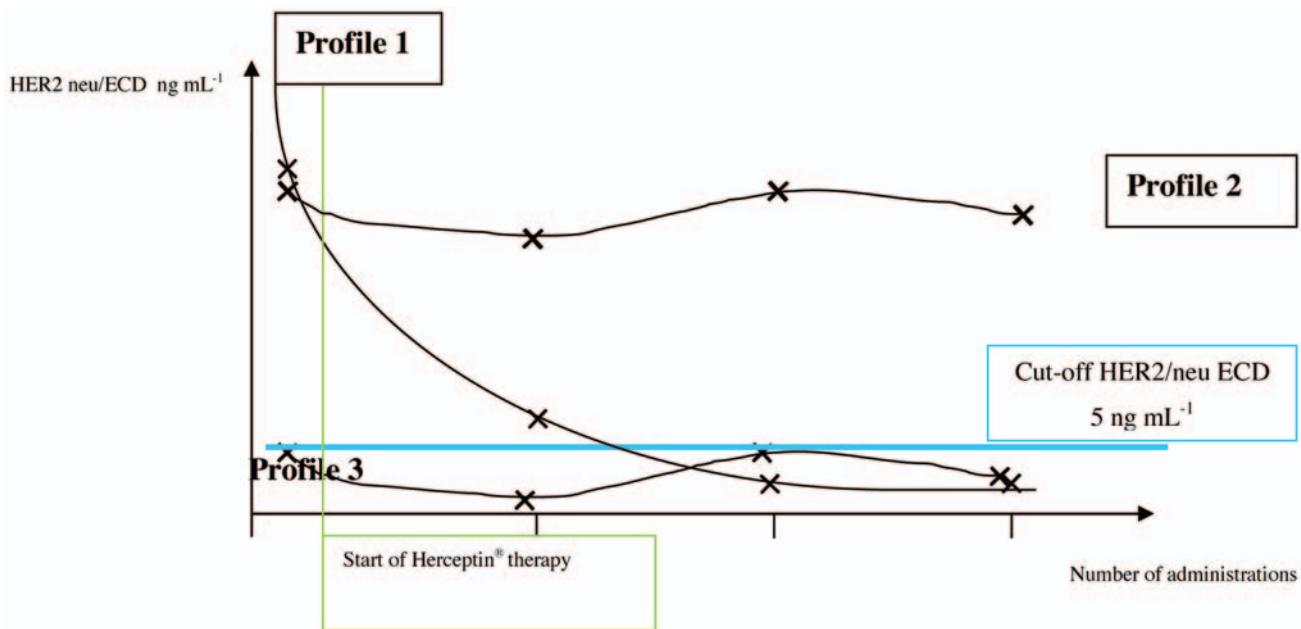


Figure 1. Dynamics of HER2/neu ECD serum concentrations in patients treated with Herceptin®.

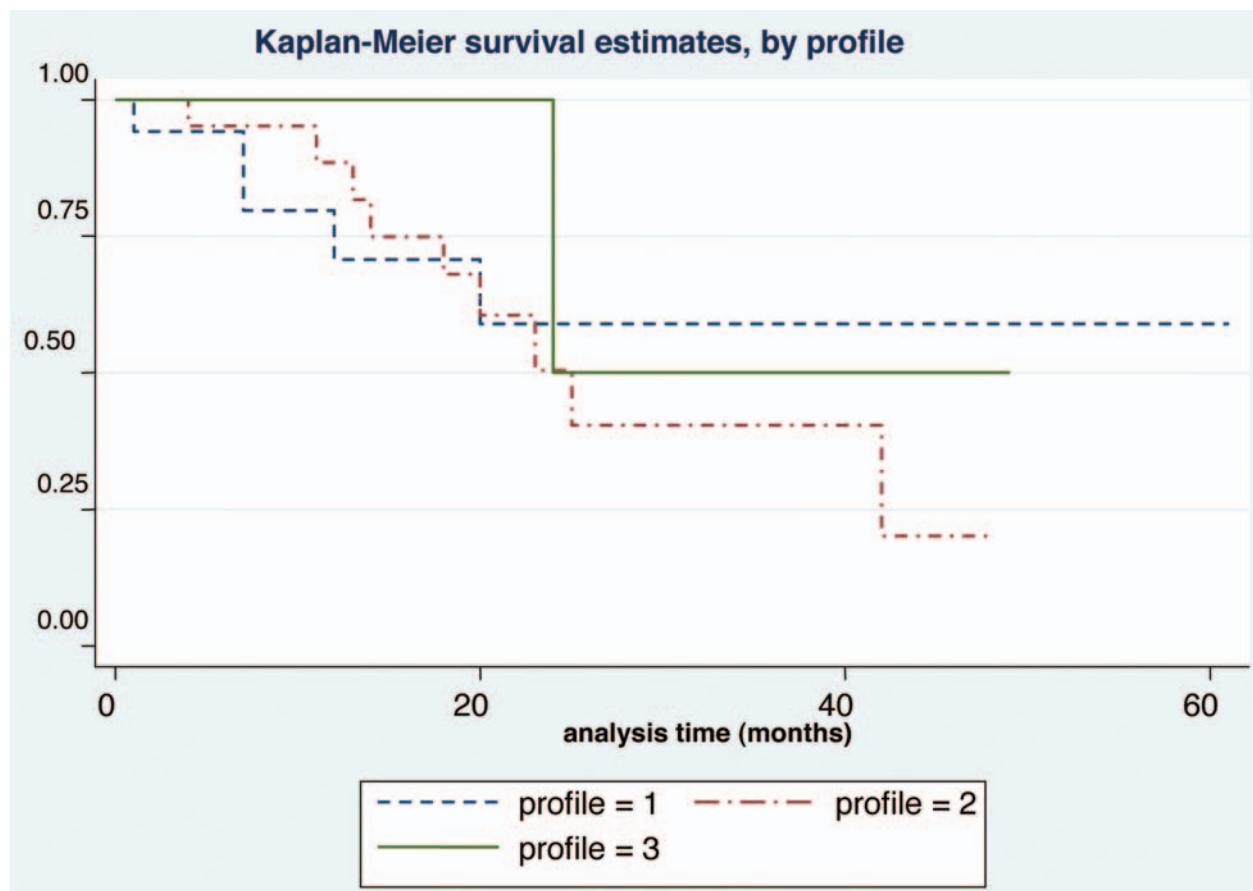


Figure 2. Survival curves according to HER2/neu ECD serum kinetic profiles.

patients concerned had an objective response to Herceptin®, with partial or complete remission. In this case, a correlation was observed between normalization of the HER2/neu ECD concentration and the efficacy of Herceptin®. Concentrations returned to normal even before a clinical response was obtained, strongly suggesting the role of an early marker for evaluating response to treatment. After between 6 months and a year, four of the patients presented an increase in HER2/neu ECD concentration, the average concentration being 300 ng mL⁻¹ (extremes 47-900), with accompanying clinical relapse.

The second profile (P2) concerns patients with a very high mean baseline concentration of 800 ng mL⁻¹ (extremes 140-2000) which persisted and even increased with Herceptin® therapy. This profile concerned six patients who were resistant from the outset to Herceptin®. The persistently high concentration of circulating HER2/neu ECD and the lack of response to treatment were thus correlated in this case too. The absence of a reduction in concentration of the circulating marker at the start of Herceptin® therapy was objectivitated by the absence of clinical response observed. These two profiles provided information concerning patients' response to Herceptin®.

However, in 24 patients, *i.e.* 53% of the study population, the concentration of circulating HER2/neu ECD remained <5 ng mL⁻¹ and thus did not provide an indication of response to Herceptin®. The patients in this third profile (P3) responded in a variable way to Herceptin®.

Analysis of the survival curves of the study population according to HER2/neu ECD serum profile showed that over 60% of patients presenting P1 were alive more than 60 months later. On the other hand, less than 25% of the patients presenting P2 were alive after 30 months. Survival of P3 patients was intermediate (Figure 2).

Discussion

Herceptin® received approval in 2000 for treatment of patients with metastatic breast cancer, but at the time only women who had failed to respond to approved protocols were eligible for treatment, either as monotherapy in patients already pretreated with at least 2 chemotherapy protocols or in combination with paclitaxel in patients not pretreated by chemotherapy and in whom anthracycline-based treatment was unsuitable. Almost half of the study population (22/45 *i.e.* 49%) corresponded to these criteria. The HER2/neu ECD concentration was of value in predicting therapeutic response in almost half of the study group (21/45). Like other circulating tumor markers, HER2/neu ECD is useful only when there is a variation in concentration. The present study concerns only a small population who were not a homogeneous group on recruitment. Nevertheless, statistically significant survival ($p<0.05$) between profiles 1 and 2 confirmed the results in the literature. Patients who presented normalized concentrations

of HER2/neu ECD 12 weeks after the start of trastuzumab therapy had a better response than those in whom the concentration remained high ($p=0.005$) (10). At the 2005 ASCO meeting, the value of predicting therapeutic response at the individual level was emphasized, as well as the general interest of optimizing treatment costs (11).

The dynamic approach to measuring HER2/neu ECD by analyzing changes in concentrations of the marker is very attractive since it provides the possibility of defining different indicators of therapeutic efficacy and of relapse. A prospective multi-center study on larger populations must now be conducted and should include other lines of investigation into response to Herceptin® and the mechanisms involved in resistance to Herceptin® therapy (12-14). When choosing targeted therapy, criteria must include biological exploration of the target or signaling pathways involved. Biological parameters must be validated in the context of research programs led by the clinicians concerned, who will be able to evaluate the role and contribution of each examination in disease management strategies and thus optimize the cost/efficiency ratio.

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