Serum HER-2/*neu* and Relative Resistance to Trastuzumab-based Therapy in Patients With Metastatic Breast Cancer

Suhail M. Ali, MD[1,2]
Walter P. Carney, PhD[3]
Francisco J. Esteva, MD, PhD[4]
Monica Fornier, MD[5]
Lyndsay Harris, MD[6]
Wolfgang. J. Köstler, MD[7]
Jean-Pierre Lotz, MD[8]
Diana Luftner, MD[9]
Marie-France Pichon, PharmD, DSc[10]
Allan Lipton, MD[1]
the Serum HER-2/*neu* Study Group

[1] Department of Hematology-Oncology, Penn State Hershey Cancer Center, Penn State University/Hershey Medical Center, Hershey, Pennsylvania.

[2] Department of Medicine, Lebanon Veterans Administration Medical Center, Lebanon, Pennsylvania.

[3] Oncogene Science/Siemens HealthCare Diagnostics, Cambridge, Massachusetts.

[4] Departments of Breast Medical Oncology and Molecular and Cellular Oncology, Breast Cancer Translational Research Laboratory, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

[5] Breast Cancer Medicine Service, Memorial Sloan-Kettering Cancer Center, New York, New York.

[6] Breast Disease Unit, Yale University, New Haven, Connecticut.

[7] Department of Medicine I, Division of Oncology, Medical University of Vienna, Vienna, Austria.

[8] Service d'Oncologie Médicale, Tenon Hospital, Paris, France.

[9] Medizinische Klinik und Poliklinik II, Schwerpunkt Onkologie and Hëmatologie, Humboldt University, Berlin, Germany.

[10] Laboratoire d'Oncobiologie, René Huguenin Cancer Center, Saint-Cloud, France.

Additional authors in the International Serum HER-2/*neu* Study Group: Didier Brault (Tenon Hospital, Paris, France), Harold Burstein (Dana-Farber Cancer Institute, Boston, Mass), Joseph Gligorov (Tenon Hospital, Paris, France), Kim Leitzel (Penn State University/Hershey Medical Center, Hershey, Penn), Rainer Neumann (Siemens HealthCare Diagnostics GmbH, Fernwald, Germany), Christopher P. Price (University of Oxford, Oxford, United Kingdom), Robert P. Thiel (Thiel Statistical Consultants, Oxford, Conn), Chantal Tse (Tenon Hospital, Paris, France), and Jennifer Wheler (Memorial Sloan Kettering Cancer Center, New York, NY). Of these, Robert Thiel is a paid consultant to Oncogene Diagnostics, Rainer Neuman is an employee of Siemens, and Kim Leitzel receives research funds from Oncogene Science, Siemens, Monogram Sciences, Novartis, and Pfizer; and receives honoraria from Oncogene Science, Siemens, and Monogram.

Allan Lipton is on the Speakers Bureau of Amgen and Novartis, is a consultant to Amgen, Novartis, Incyte, Monogram Biosciences, and Acceleron; he has provided expert testimony for Novartis; in addditon, Dr. Lipton receives research support from Oncogene Sciences/Siemens, Monogram Biosciences, and Pfizer.

Walter P. Carney is an employee of Siemens.

Address for reprints: Kim Leitzel, MSc, Section of Hematology/Oncology, Penn State University, Hershey Medical Center, Room C6820, 500 University Avenue, Hershey, PA 17033; Fax: (717) 531-5076; E-mail: kleitzel@psu.edu

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BACKGROUND. Previous reports based on small patient numbers suggested that changes in serum HER-2/*neu* levels may predict response or lack of response to trastuzumab-based therapies in metastatic breast cancer (MBC). The objectives of this study were to pool data from 307 patients with MBC from 7 medical institutions to validate that the serum HER-2/*neu* profile predicts patient resistance to trastuzumab and to establish a clinically relevant cutoff.

METHODS. This was an international, multicenter, retrospective analysis of individual pooled data from 307 patients with MBC who were treated with first-line trastuzumab-based therapy. Serum was collected at baseline and 30 to 120 days after the initiation of trastuzumab therapy. A serum HER-2/*neu* decrease $\geq$20% (receiver operating curve analysis) was defined as a significant HER-2/*neu* change.

RESULTS. Of the 307 patients with MBC, 191 patients (62%) had a significant decline (>20%) in serum HER-2/*neu* and 116 patients (38%) did not. The objective response rate was 57% for patients who achieved this decline in serum HER-2/*neu* (>20%) compared with 28% for patients who did not. Patients who achieved this decline in serum HER-2/*neu* also had a significantly longer time to disease progression (320 days vs 180 days; $P < .0001$), longer duration of response (369 days vs 230 days; $P = .008$), and longer overall survival (898 days vs 593 days; $P < .018$).

CONCLUSIONS. In this pooled analysis of 307 patients with MBC, individuals who did not achieve a significant decline ($\geq$20%) in serum HER-2/*neu* levels had decreased benefit from trastuzumab-based therapy, and these patients should be considered for clinical trials evaluating additional HER-2/*neu*-targeted interventions. ***Cancer*** **2008;113:1294–301.** © *2008 American Cancer Society.*

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The human epidermal growth factor receptor 2 proto-oncogene (HER-2, *neu*, ErbB-2) is a transmembrane receptor that has intracellular tyrosine kinase activity.[1,2] It is overexpressed by immunohistochemistry (IHC) or amplified by fluorescence in situ hybridization (FISH) analysis in approximately 20% to 25% of invasive primary breast cancers[1,3] and is associated with a poor prognosis and more aggressive disease.[4] The HER-2/*neu* extracellular domain (ECD) is cleaved by the ADAM metalloproteinases, and the remaining membrane-bound internal domain is activated constitutively.[5] The ECD (p97-115 kD) of the HER-2/*neu* protein is released into the circulation, and serum HER-2/*neu* levels are elevated in 30% to 70% of patients with metastatic breast cancer (MBC).[6] Rising serum HER-2/*neu* concentrations have been associated with progressive metastatic disease and a poor response to chemotherapy.[7] Furthermore, studies have demonstrated that an elevated pretreatment HER-2/*neu* level is associated with decreased response to both first- and second-line endocrine therapy.[8,9] Numerous studies also have demonstrated that changes in serum HER-2/*neu* levels parallel the clinical course of disease; and, in several publications, rising levels were observed before actual clinical diagnosis.

Trastuzumab (Herceptin; Genentech, South San Francisco, Calif) was the first HER-2/*neu*-targeted therapy approved by the U.S. Food and Drug Administration (FDA) for the treatment of patients with MBC. Trastuzumab is a humanized monoclonal antibody directed against the HER-2/*neu* ECD. Single-agent response rates range from 12% to 30%, depending on the HER-2/*neu* status of the tumor and the patient's prior treatment.[10,11] Trastuzumab improves response rates, time to disease progression, and survival in patients with MBC when it is added to chemotherapy.[12,13] It has been demonstrated that trastuzumab is synergistic with a variety of commonly used chemotherapy agents, such as paclitaxel, docetaxel, platinum salts, and vinorelbine.[12-15]

The most commonly used methods for selecting patients for trastuzumab monoclonal antibody therapy are IHC and FISH.[16] However, only 30% to 61% of these patients will respond to trastuzumab-based therapy. Studies also have indicated that the rise and fall of serum HER-2/*neu* are correlated with the clinical course of disease in patients with MBC who have received trastuzumab and chemotherapy.[17-18] In a 2004 pilot study, Kostler et al demonstrated that a significant decrease in serum HER-2/*neu* from the pretreatment baseline level within 30 days after starting treatment was an early predictor of outcome after trastuzumab therapy.[19] Subsequent reports from Esteva et al,[20] Fornier et al,[21] Bethune-Volters et al,[22] and Tse et al,[23] using small numbers of patients, supported the observation that a significant decrease in serum HER-2/*neu* from pretreatment level was a predictor of outcome after trastuzumab-based therapies. Because all of those earlier reports used small patient cohorts and reported different serum HER-2/*neu* cutoff levels, we coordinated a multicenter/multinational study of 307 patients with MBC that analyzed serum HER-2/*neu* levels at baseline and compared those levels with serum HER-2/*neu* levels from blood drawn a median of 30 days after the initiation of trastuzumab-based therapies.

MATERIALS AND METHODS

Patient Population

Patients who were eligible for participation in the current study were women with MBC who received trastuzumab therapy with or without chemotherapy according to the established practice of the treating physician. Patients were trastuzumab-naive at the time of entry into this study. The first (baseline) serum sample for each patient was taken before trastuzumab therapy was started. The second serum sample was obtained from patients 16 to 120 days after trastuzumab therapy was started. Data from all solicited investigators were included to avoid bias. One center collected the second serum sample 120 days after treatment was started; however, only 67 patients (22%) had the second serum sample drawn 60 days after treatment was started. Individual patient data were obtained from participating institutions, and an analysis of the pooled individual data was performed. Each participating institution received approval from their internal Institutional Review Board to contribute patients to this study.

Evaluation of Tumor Response and Clinical Endpoints

Response to treatment was assessed according to the criteria of the World Health Organization[24] or Response Evaluation Criteria in Solid Tumors.[25] The objective response rate (ORR) was defined by including patients who achieved a complete response and patients who achieved a partial response. The time to progression (TTP) was defined as the time from the start of trastuzumab therapy to the time of tumor

TABLE 1
Patient Demographics

Variable	No. of Patients (%)		
	≤20% Serum HER-2 Decline	>20% Serum HER-2 Decline	Total
Hormone receptor status			
Positive	61 (52.59)	95 (49.74)	156 (50.81)
Negative	45 (38.79)	88 (46.07)	133 (43.3)
Unknown	10 (8.62)	8 (4.19)	18 (5.86)
Line of chemotherapy			
First line	56 (48.28)	112 (58.64)	168 (54.7)
Second line	39 (33.62)	54 (28.27)	93 (30.29)
Unknown	21 (18.1)	25 (13.09)	46 (14.98)
Concurrent chemotherapy*			
No	32 (27.59)	22 (11.52)	54 (17.59)
Yes	84 (72.41)	169 (88.48)	253 (82.4)
Baseline HER-2/*neu* >15 ng/mL*	63 (54.3)	132 (84.8)	195 (73.3)

HER-2/*neu* indicates human epidermal growth factor receptor 2 proto-oncogene.
* $P < .05$.

progression. The duration of response (DRP) was also defined as the time from the start of trastuzumab therapy to tumor progression in the subgroup of patients who had a complete or partial response to therapy. Overall survival (OS) was calculated from the start of trastuzumab therapy until death.

Serum HER-2/*neu* Assay

Serum HER-2/*neu* testing was performed by using either the Siemens Immuno-1 automated assay or the manual microtiter enzyme-linked immunosorbent assay. The FDA has cleared both methods with an indication for follow-up and monitoring of patients with MBC. Previous studies have demonstrated similar diagnostic performance of the automated and manual methods, with very high correlation between the methods (r2 = 0.99), because the antibodies used for capture and detection of the circulating HER-2/*neu* antigen are identical for both assays. The study published by Payne et al demonstrated that trastuzumab does not interfere with the serum HER-2/*neu* assay.[26]

Statistical Analysis

Receiver operating curve (ROC) analysis was performed to determine the optimal serum HER-2/*neu* cutoff level. From that ROC analysis, a significant serum HER-2/*neu* decline was defined as a decrease >20% at the follow-up visit. This definition of a significant change was similar to that derived from the FDA-cleared cutoff value for nontrastuzumab therapies.

Further data analysis was performed by comparing the group of patients who achieved this significant decline in serum HER-2/*neu* level (>20%) with patients who did not achieve this decline in serum HER-2/*neu*. The chi-square and logistic regression statistical tests were used to analyze categorical data. The log-rank test and a Cox proportional-hazards multivariate model were used to analyze the time to event variables. Kaplan-Meier analysis and graphs were generated using SPSS (Statistical Package for the Social Sciences) for Windows (version 11.0).

TABLE 2
HER-2/*neu* Status of the Primary Breast Cancer

HER-2/*neu* Tissue Status	No. of Patients (%)		
	≤20% Serum HER-2/*neu* Decline	>20% Serum HER-2/*neu* Decline	Total
FISH positive or IHC 3+	84 (72.41)	151 (79.06)	235 (76.55)
IHC 2+	14 (12.07)	18 (9.42)	32 (10.42)
IHC 1+	3 (2.59)	4 (2.09)	7 (2.28)
IHC 0	7 (6.03)	7 (3.66)	14 (4.56)
Unknown	8 (6.9)	11 (5.76)	19 (6.19)

HER-2/*neu* indicates human epidermal growth factor receptor 2 proto-oncogene; FISH, fluorescence in situ hybridization; IHC, immunohistochemistry.

RESULTS

Patient Characteristics

This was a retrospective analysis of pooled data from 7 institutions comprising 307 patients with MBC who received trastuzumab-based therapy. Table 1 provides the demographics of the patient population. All demographic variables were balanced, except that fewer patients who achieved a significant decline in serum HER-2/*neu* levels (>20%) had received trastuzumab monotherapy; those patients also had higher baseline serum HER-2 levels (median, 52.9 ng/mL; range, 7.4-6076 ng/mL) compared with patients who did not have a decline in serum HER-2/*neu* levels (median, 15.9 ng/mL; range, 5.2-4180 ng/mL). Table 2 shows the HER-2/*neu* status of the primary breast tumor and indicates that 76.5% of patients had 3+ IHC HER-2/*neu* overexpression or had HER-2/*neu* gene amplification documented by FISH analysis. Table 2 also shows that 10% of patients had 2+ IHC overexpression, 2% had 1+ IHC overexpression, 4.5% had negative IHC results for antibody staining, and 6% had unknown HER-2/*neu* status. Statistical analyses were conducted on the entire patient population, and on the patient subset with 3+ IHC overexpression and/or FISH-amplified HER-2/*neu*, and on the patient subset baseline serum HER-2/*neu* levels >15 ng/mL or <15 ng/mL (15 ng/mL is the upper limit of normal for serum HER-2).[9]

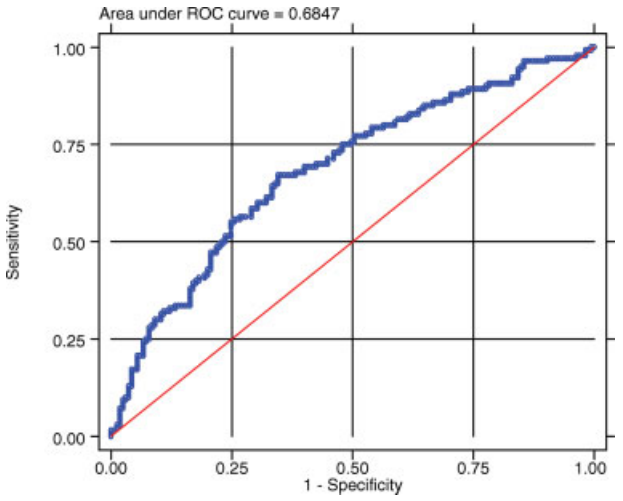

FIGURE 1. This chart illustrates the area under the receiver operating characteristic (ROC) curve for changes in serum levels of HER-2/*neu* and patient response to trastuzumab (n = 307 patients).

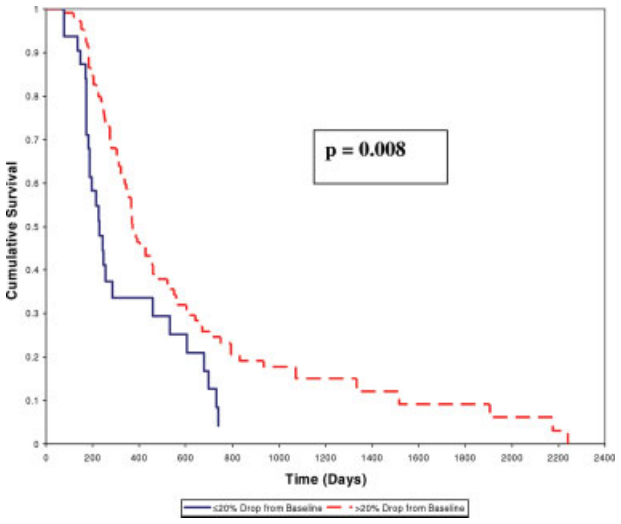

FIGURE 2. This chart illustrates the duration of response (n = 142) in patients who achieved a complete or partial response grouped by patients who achieved a significant decline (>20% decrease) in serum levels of HER-2/*neu* from baseline and those who did not (<20% decrease).

A follow-up serum sample was collected at a median of 30 days after the start of trastuzumab therapy (range, 16-120 days). One center collected the follow-up sample at 120 days after the start of trastuzumab therapy. Fifty-eight percent of the patients who achieved a significant decline in serum HER-2/*neu* levels (<20%) had follow-up samples collected by Day 30 compared with 53% of patients who did not have a significant decline in serum HER-2/*neu* levels. There was no significant difference in the time from pretreatment to posttreatment blood draw between these 2 groups.

Change in Serum HER-2/*neu* Levels

ROC analysis was performed to determine the cutoff that yielded the optimal patient response to trastuzumab treatment (Fig. 1). From that ROC analysis, the optimal serum HER-2/*neu* decline was defined as a decrease >20% at the follow-up blood draw (ROC sensitivity, 76%; specificity, 50%). This definition of a significant change was similar to that derived from the FDA-cleared cutoff for nontrastuzumab therapies.

According to this ROC-selected, optimized serum HER-2/*neu* change cutoff, 191 of 307 patients (62%) achieved a significant decline in serum HER-2/*neu* (levels decreased >20%), whereas 116 of 307 patients (37.8%) did not achieve a significant decline in serum HER-2/*neu* (levels decreased <20%).

Overall Response Rate and Duration of Response

The ORR (complete plus partial responses) to trastuzumab-based therapy in patients who achieved a significant decline in serum HER-2/*neu* levels (>20%) was more than double (57%) compared with the ORR for patients who did not achieve this decline in serum HER-2/*neu* (28%; $P < .001$). In the subgroup of 142 patients who had an objective response, the median DRP was significantly longer for patients who achieved a significant decline (>20%) in serum HER-2/*neu* compared with patients who did not (369 days vs 230 days; $P = .008$) (Fig. 2).

Time to Progression

Figure 3 compares the TTP in patients who had declines <20% or >20% in serum HER-2/*neu* levels from baseline and indicates that patients who achieved a significant decline (>20%) had a longer TTP (320 days) compared with patients who did not achieve a significant decline (180 days; $P < .0001$). A multivariate model was performed adjusting for baseline variables, and a significant decline (>20%) in serum HER-2/*neu* was an independent predictive factor for longer TTP (Table 3).

Overall Survival

Figure 4 compares the survival of patients who had declines <20% and >20% in serum HER-2/*neu* levels from baseline. Survival data were available for 241 of 307 patients: One hundred forty-three of 241 patients (59%) died, and the median follow-up for survivors was 860 days. OS was significantly longer for patients who achieved a significant decline (>20%) in serum HER-2/*neu* (898 days vs 593 days; $P = .018$) (Fig. 4).

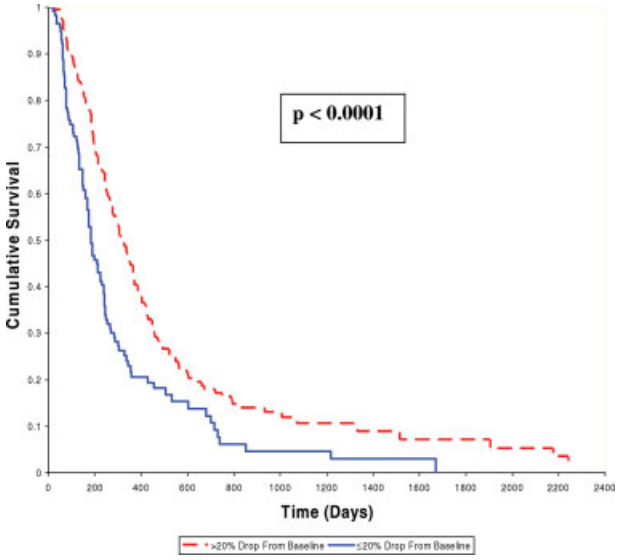

FIGURE 3. This chart illustrates the time to progression grouped by patients who achieved a significant decline (>20% decrease) in serum levels of HER-2/*neu* from baseline, and those who did not (<20% decrease).

TABLE 3
Multivariate Analysis of Time to Progression

Variable	HR	*P*
Serum HER-2/*neu* change (≤20% decrease vs >20% decrease)	1.89	<.0001
Line of metastatic chemotherapy/hormone therapy (first vs other lines vs unknown)	1.02	.84
Concurrent chemotherapy vs trastuzumab monotherapy	0.61	.002
Baseline serum HER-2/*neu* (>15 ng/mL vs <15 ng/mL)	1.53	.007
Follow-up serum sample (<30 d vs >30 d)	0.87	.31

HER-2/*neu* indicates human epidermal growth factor receptor 2 proto-oncogene; HR, hazard ratio.

In a multivariate model that was adjusted for baseline variables, change in serum HER-2/*neu* was an independent prognostic factor for OS ($P = .003$) (Table 4).

Outcome in Patients With 3+ Immunohistochemical Overexpression/Positive Fluorescence in Situ Hybridization Results

Primary breast cancer tissue HER-2/*neu* assay results were available in 94% of patients and indicated that 76.6% of patients had HER-2/*neu* 3+ IHC overexpression or had HER-2/*neu* gene amplification documented by FISH analysis. One center treated some of their patients with trastuzumab although they had only 0 or 1+ IHC HER-2/*neu* overexpression; these patients constituted 7% of the study population. In the analyses described above, all patients who had serial serum HER-2/*neu* values, treatment with trastuzumab, and clinical follow-up were included. A subset analysis also was performed for patients who had HER-2/*neu* 3+ IHC overexpression or had gene amplification documented by FISH analysis. In that subset analysis, the ORR ($P < .001$), TTP ($P < .001$), and OS ($P = .004$) were significantly better in the group of patients who achieved a significant decline (>20%) in serum HER-2/*neu* levels (Table 5). In total, there were 109 responders in the group. The DRP also trended longer in the group of patients who achieved a significant decline in serum HER-2/*neu* levels ($P = .075$).

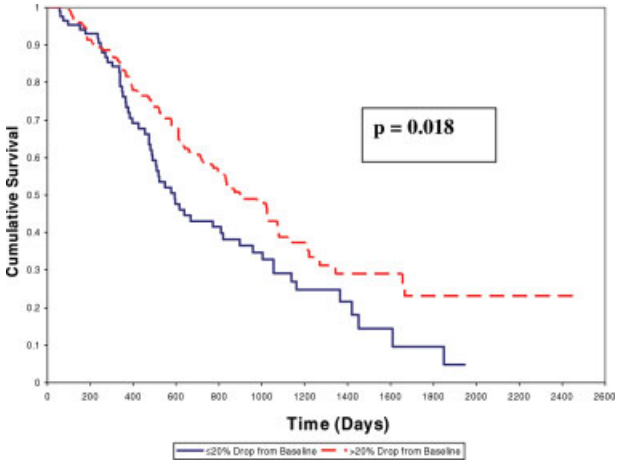

FIGURE 4. This chart illustrates overall survival grouped by patients who achieved a significant decline (>20% decrease) in serum levels of HER-2/*neu* from baseline and those who did not (<20% decrease).

TABLE 4
Multivariate Analysis of Overall Survival

Variable	HR	*P*
Serum HER-2/*neu* change (≤20% decrease vs >20% decrease)	1.72	.003
Line of metastatic chemotherapy/hormone therapy (first vs other lines vs unknown)	1.34	.06
Concurrent chemotherapy vs trastuzumab monotherapy	1.02	.92
Baseline serum HER-2/*neu* (>15 ng/mL vs <15 ng/mL)	1.71	.017
Follow-up serum sample (<30 d vs >30 d)	0.95	.8

HER-2/*neu* indicates human epidermal growth factor receptor 2 proto-oncogene; HR, hazard ratio.

DISCUSSION

The results from this 7-site, pooled analysis establish the optimal serum HER-2/*neu* change cutoff point for patient response to trastuzumab therapy, and

TABLE 5
Decreases in Serum Levels of HER-2/*neu* and Clinical Outcomes After Trastuzumab-based Therapy in Patients With 3+ Immunohistochemistry and Fluorescence In Situ Hybridization-amplified HER-2/*neu*

HER-2/*neu* Levels From Baseline to Follow-up	ORR %	Median DRP, d	Median TTP, d	Median OS, d
Decrease >20%	58.3	403	334	1023
Decrease ≤20%	25	245	173	519
P	<.001	.075	<.001	.004

ORR indicates overall response rate; DRP, duration of response; TTP, time to progression; OS, overall survival.

identify a patient group more likely to be resistant to trastuzumab. Our results confirm several smaller publications in patients with MBC who received trastuzumab-based therapy.[19-23] Kostler et al[19] used multiple logistic regression analyses and identified the kinetics of serum HER-2/*neu* levels as the only factor that allowed for the accurate prediction of response to trastuzumab-based therapy. They reported that serial changes in serum HER-2/*neu* levels not only paralleled the clinical course of disease but also preceded clinical changes, and this allowed for a significant prediction of response, clinical benefit, and progression-free survival in the early weeks of trastuzumab-based treatment.[19] In a report by Esteva et al, patients with MBC were monitored for serum HER-2/*neu* levels over 12 to 20 months, and the results indicated that progression-free survival differed significantly according to the serum HER-2/*neu* decline within the first 2 to 4 weeks of initiating therapy.[20] Fornier et al evaluated HER-2/*neu* levels from patients with MBC at baseline and after 12 weeks of therapy with paclitaxel and trastuzumab, and they reported that patients with elevated serum HER-2/*neu* levels that normalized after 12 weeks of therapy had a higher response rate compared with patients who had persistently high serum levels of HER-2/*neu*.[21] Another report by Bethune-Volters et al concluded that serum HER-2/*neu* monitoring during trastuzumab therapy was an early indicator of patient outcome and was a powerful predictor of survival.[22] In a report by Tse et al, serum HER-2/*neu* levels before and after therapy were predictors of clinical outcome; patients who had the least decline in serum HER-2/*neu* levels had a shorter TTP.[23]

In this multicenter, retrospective analysis of pooled data from 307 patients with MBC who received trastuzumab-based therapies, we performed ROC analysis and identified the optimal cutoff point for serum change in HER-2/*neu* that yielded the maximal specificity and sensitivity for patient response to trastuzumab therapy. This analysis defined a significant decline >20% in serum HER-2/*neu* as the optimal cutoff point for patient response to trastuzumab therapy. Overall, 191 of 307 patients (62%) achieved this significant decline in HER-2/*neu* levels (≥20%) at first follow-up, and 116 of 307 patients (38%) did not achieve this decline (<20% decrease). The ORR (complete responses + partial responses) was doubled (57%) for patients who achieved a significant decline (>20%) in serum HER-2/*neu* levels compared with a 28% response rate for patients who did not. Patients who achieved this significant decline in serum HER-2/*neu* levels also had a significantly longer time to disease progression (320 days vs 182 days; $P < .001$), longer DRP (369 days vs 230 days; $P = .003$), and longer OS (898 days vs 593 days; $P < .012$). The results were similar for the subgroup of patients who had baseline levels above or below the 15 mg/mL cutoff, and for the subgroup of patients who had either 3+ IHC overexpression or FISH amplification.

The current results in the largest patient group studied to date establish a serum HER-2/*neu* decline ≥20% as the optimal cutpoint for predicting the greatest response to trastuzumab and, conversely, indicate that the remaining patient cohort (40%) has relative resistance to trastuzumab therapy. According to the current 2007 American Society of Clinical Oncology/College of American Pathologists guidelines for HER-2 IHC/FISH testing,[27] 50% of HER-2/*neu*-positive patients selected will still be resistant to trastuzumab-based therapy. Proposed mechanisms of trastuzumab resistance, including loss of phosphatase and tensin homolog (PTEN), mutation of phosphoinositide-3 kinase (P13K), activation of insulin-like growth factor 1 receptor signaling, activation of epidermal growth factor receptor (EGFR), and mucin 4 masking of HER-2, are currently being studied.[28,29] Another mechanism of trastuzumab resistance may be full-length p185 HER-2/*neu* cleavage by the ADAM proteases into a cell membrane-bound portion, which contains a constitutively activated tyrosine kinase domain (p95), and the corresponding ECD measured in serum.[30] HER-2 p95-expressing tumors occurred with increased frequency in lymph node-positive breast cancer (23 of 78 tumors) compared with lymph node-negative breast cancers (9 of 63 tumors; $P = .032$).[30] Thus, HER-2 p95 generation may endow the tumor cell with increased metastatic potential, and serum HER-2/*neu* may function as a

surrogate biomarker of HER-2 p95 generation. The HER-2/EGFR tyrosine kinase inhibitor lapatinib, which recently was approved for use in patients with trastuzumab-resistant MBC,[31] has been proposed as an alternative treatment for patients who have tumors that express HER-2 p95, because only 1 of 11 HER-2 p95-positive patients responded to trastuzumab.[32] Recently, it was demonstrated that novel agents, such as an ADAM sheddase inhibitor, enhanced the antitumor effect of trastuzumab in HER-2-overexpressing BT-474 breast cancer cells.[33] Also, a novel heat-shock protein 90 (Hsp-90) inhibitor, tanespimycin (17-AAG; KOS-953), in combination with trastuzumab, was tolerated well and had antitumor activity in patients who had HER-2 + breast cancer with tumors that progressed during treatment with trastuzumab.[34]

In the current report, patients who did not have a significant decline (≥20%) in serum HER-2/*neu* levels had decreased benefit from trastuzumab-based therapy. Monitoring changes in serum HER-2 levels at a median of 1 month after trastuzumab treatment to predict clinical response may be valuable for identifying a patient population that might benefit from additional treatment regimens with other HER-2/*neu*-targeted therapies. Currently, although trastuzumab should not be stopped based on the absence of a decline in serum HER-2 levels, prospective clinical trials evaluating the use of other HER-2-directed therapies (ie, lapatinib, Hsp-90 inhibitor, sheddase inhibitor) with and without continued trastuzumab therapy are warranted for patients who do not achieve a 20% decline in serum HER-2.

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