Cancer Chemother Pharmacol (2005) 56: 361–369
DOI 10.1007/s00280-005-1026-z

ORIGINAL ARTICLE

Rene Bruno · Carla B. Washington · Jian-Feng Lu
Grazyna Lieberman · Ludger Banken · Pamela Klein

Population pharmacokinetics of trastuzumab in patients With HER2+ metastatic breast cancer

Received: 26 October 2004 / Accepted: 12 January 2005 / Published online: 3 May 2005

Abstract *Purpose*: To characterize the population pharmacokinetics of trastuzumab in patients with metastatic breast cancer. *Methods*: A nonlinear mixed effect model was based on pharmacokinetic data from phase I, II, and III studies of 476 patients. The phase I study enrolled patients with advanced solid tumors. The phase II and III studies enrolled patients with HER2-positive metastatic breast cancer. Patients in the pivotal phase II and III studies were treated with a 4 mg/kg loading dose of trastuzumab followed by 2 mg/kg weekly for up to 840 days. The model adequately predicted observed trastuzumab concentrations. Model stability and performance were verified using bootstrap simulations. Percentiles, mean, and standard deviation of observed levels were compared with their distributions from 100 replicates of datasets simulated under the model. *Results*: A two-compartment linear pharmacokinetic model best described the data and accounted for the long-term accumulation observed following weekly administration of trastuzumab. Population estimates from the base model for clearance (CL) and volume of distribution of the central compartment (V_1) of trastuzumab were 0.225 L/day, and 2.95 L, respectively. Estimated terminal halflife ($t_{1/2}$) based on the population estimate was 28.5 days. Interpatient variabilities in clearance and volume were 43 and 29%, respectively. The number of metastatic sites, plasma level of extracellular domain of the HER2 receptor, and patient weight were significant baseline covariates for clearance, volume, or both ($P < 0.005$). However, these covariate effects on trastuzumab exposure were modest and not clinically important in comparison with the large inter-patient variability of CL. Concomitant chemotherapy (anthracycline plus cyclophosphamide, or paclitaxel) did not appear to influence clearance. *Conclusion*: This population pharmacokinetic model can predict trastuzumab exposure in the long-term treatment of patients with metastatic breast cancer and provide comparison of alternative dosage regimens via simulation.

Keywords Pharmacokinetics · Trastuzumab · Metastatic breast cancer · HER2

R. Bruno · C. B. Washington · J.-F. Lu (✉)
Pharmacokinetic and Pharmacodynamic Sciences,
MS 70 Genentech, Inc, 1 DNA Way, South San Francisco,
CA 94080, USA
E-mail: lu.jianfeng@gene.com
Tel.: +1-650-2255876
Fax: +1-650-2256452

G. Lieberman
Departments of Biostatistics, Genentech Inc,
South San Francisco, CA, USA

P. Klein
Oncology Departments, Genentech Inc,
South San Francisco, CA, USA

L. Banken
Biostatistics Department, Roche, Basel, Switzerland

Introduction

Trastuzumab (Herceptin Genentech, Inc., South San Francisco, CA, USA), a recombinant, humanized anti-HER2 monoclonal antibody, is approved for the treatment of women with HER2-positive metastatic breast cancer (MBC) as a single agent or in combination with chemotherapy.

The pharmacokinetics of trastuzumab were investigated with single and multiple weekly administrations in phase I, II [1–3], and III [4] studies as part of the drug's clinical development program. The single-dose, dose-escalation, phase I study in 16 patients was the only one to give a dense-sampling pharmacokinetic profile of trastuzumab. Mean clearance and half-life estimates were dose dependent. Terminal half-life estimates ranged from 1.1 days (using a one-compartment model at 10 mg) to 23 days (using a two-compartment model at 500 mg; Genentech Inc., data on file).

In multiple-dose studies, the pharmacokinetic samples from the 494 patients were limited to peak and trough samples from multiple weekly administrations.

Pivotal phase II and phase III studies used a loading dose of 4 mg/kg followed by 2 mg/kg/week, and trastuzumab was given either as a single agent [3] or in combination with chemotherapy [4]. Based on a one-compartment model, the half-life estimate averaged 5.8 days (range 1–32 days) [5]. The dosage regimen was designed to achieve and maintain serum trough concentrations greater than 20 μg/mL. With repeated administration, the trough levels tended to increase through weeks 16–32 and then reached a steady state with a mean trough concentration of 79 μg/ml [5].

Nonclinical studies have suggested that the complex formed between trastuzumab and the extracellular domain (ECD) of HER2 has a greater clearance than free trastuzumab (Genentech Inc., data on file). Patients with a high level of ECD tended to have a shorter trastuzumab half-life and lower trastuzumab trough concentrations. Thus the association of ECD with trastuzumab clearance was investigated.

The objectives of this population pharmacokinetic (PK) analysis were to: (1) characterize the structural pharmacokinetic model for trastuzumab in a representative population; (2) estimate the magnitude of interpatient variability in pharmacokinetic parameters; and (3) assess the relationship of patho-physiological covariates and concomitant chemotherapy to the pharmacokinetics of trastuzumab.

Methods

Patient population and data collection

Data were prospectively collected in patients with HER2-positive MBC entered in three phase II and III clinical studies. In addition, data from one phase I study in patients with advanced solid tumors were included. A summary is given in Table 1. Expression of HER2 was determined by immunohistochemical analysis of tumor tissue. Local ethics committees approved the studies and all patients gave written, informed consent. The phase I, phase II, [1] and pivotal phase II [3] studies were non-randomized studies of trastuzumab as a single agent, and the pivotal phase III trial H0648 g was a randomized comparison of chemotherapy versus chemotherapy plus trastuzumab [4]. In all studies, trastuzumab was given by intravenous infusion for 30–90 min every week until disease progression. In the randomized phase III trial, the chemotherapy regimen for both treatment groups was either a combination of anthracycline and cyclophosphamide, or paclitaxel, depending on the patient's history of exposure to adjuvant anthracyclines. In accordance with institutional protocol, cyclophosphamide (600 mg/m^2) was given either by intravenous bolus for at least 3 min or by infusion for up to 2 h, doxorubicin (60 mg/m^2) or epirubicin (75 mg/m^2) was given either by slow intravenous bolus for 3–5 min or by infusion for up to 2 h, and patients were adequately hydrated. Paclitaxel (175 mg/m^2) was given over 3 h by

Table 1 Study details

	Study			
	Phase I	Phase II	Pivotal phase II	Pivotal phase III
Regimen	Single dose 10–500 mg	250 mg loading 100 mg weekly	4 mg/kg loading 2 mg/kg weekly	4 mg/kg loading 2 mg/kg weekly plus chemotherapy[a]
Patient population	Advanced cancer (IHC 1+, 2+, 3+)	MBC (IHC 2+, 3+)	MBC 2nd-, 3rd-line therapy (IHC 2+, 3+)	MBC 1st-line therapy (IHC 2+, 3+)
No. of patients treated with trastuzumab	16	46	213	235
No. of patients with evaluable PK data	16	44	205	211
Evaluable PK data points	179	938	1,509	1,223
Sampling design	Full profile over 21 days	Weekly peaks and troughs	Weekly troughs	Weekly troughs
No of samples per patient Median (range)	12 (8 – 13)	21 (1 – 63)	7 (1 – 24)	5 (1 – 17)
Observation duration (days)				
Median	17.4	77.0	64.0	160
90th percentile	27.9	188	330	320

[a]Either a combination of anthracycline and cyclophosphamide, or paclitaxel

intravenous infusion. All patients receiving paclitaxel were premedicated with dexamethasone, diphenhydramine, and cimetidine.

For patients to be evaluable, associated sampling times and dosing times had to be available. For trough levels, a 5-min interval was assumed between sampling and dosing when the time for one of these was not documented. Peak levels (sample to be drawn within 1 h of the end of infusion) were omitted when the sampling times were not documented. Data from 476 patients were evaluable for the pharmacokinetic analysis. A total of 3,249 serum samples were available for the 476 patients but the number and timing of samples depended on the study (Table 1). The dataset included full profiles of data after single doses (from the phase I study), and multiple weekly peak and trough samples from long-term treatment (up to 1 year in approximately 10% of patients). Trastuzumab serum concentrations were determined by a plate-binding enzyme-linked immunosorbent assay (ELISA) with a lower limit of quantitation of 0.156 μ g/ml [6]. All trastuzumab assays were performed by the BioAnalytical Assay Department at Genentech, Inc. Concentrations below the lower limit of quantitation of the assay were omitted from the analysis dataset.

Baseline serum levels of the ECD of HER2 receptors, proteolytically cleaved from the tumor cell surface, were tested using ELISA-based assays with lower limits of quantitation of 5.5 and 3.4 ng/ml for the two assays used [7].

Baseline covariates evaluated in the analysis for their effect on trastuzumab clearance and volume of distribution were age, patient weight, Karnofsky performance status, creatinine clearance, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALK), total bilirubin, total protein, albumin, number of metastatic sites, creatinine clearance (calculated from serum creatinine, age and gender using the Cockcroft and Gault equation [8], HER2 overexpression by immunohistochemistry (IHC), and baseline level of HER2 receptor ECD. No time-varying covariates were used. ECD concentrations below the lower limit of quantitation of the assay were set to half the lower limit of quantitation. The effect of concomitant chemotherapy was also evaluated. Patient characteristics are summarized in Table 2.

Table 2 Patient characteristics ($n = 476$)

	Median	Range	n (%)
Age (years)	50	25–81	
Weight (kg)	70	42–119	
Karnofsky score $< 80\%$			55 (12)
Creatinine clearance (mL/min)	101	11–284	
AST (U/L)	29	9–667	
ALT (U/L)	27	4–595	
Alkaline phosphatase (U/L)	91	21–2040	
Bilirubin (mg/dL)	0.40	0.1–8.9	
Total protein (g/dL)	7.1	5.0–9.4	
Albumin (g/dL)	3.9	1.9–4.8	
Number of metastatic sites ≥4			53 (11)
HER2 IHC status			
1+			6 (1)
2+			107 (22)
3+			363 (76)
HER2 ECD (ng/mL)	9.33	1.70–2431	
Concomitant chemotherapy			
None			265 (56)
Anthracycline plus cyclophosphamide			133 (28)
Paclitaxel			78 (16)

Data analysis

The analyses were performed with the nonlinear mixed-effect modeling (NONMEM) program (version V, GloboMax, Hanover, MD, USA) using the first-order method [9]. One-, two-, and three-compartment models with first-order elimination and zero-order input (constant rate infusion) were fit to trastuzumab concentration–time data. Interpatient variability in the pharmacokinetic parameters of clearance (CL) and the volume of the central compartment (V_1) was assumed log-normally distributed (exponential model)

For CL:

$$\mathrm{CL}_j = \hat{CL}_j \exp(\eta_{\mathrm{jCL}})$$

A multiplicative covariate regression model was implemented as follows (eg, for CL):

$$\hat{CL}_j = \theta_1 \left(\frac{WT_j}{\mathrm{med}(WT)}\right)^{\theta_{WT}} (1 + \theta_{\mathrm{MET}} MET_j) \times (1 + (\theta_{\mathrm{CHEM1}}\mathrm{CHEM1_j}) + (\theta_{\mathrm{CHEM2}}\mathrm{CHEM2}_j))$$

where η_{jCL} denotes the proportional difference between the 'true' parameter (CL_j) of individual patient j and the typical value ($\hat{CL}_j$) in the population, adjusted for values of covariates equal to those of the individual patient. The ηs represented random effects with mean zero and variance ω^2. The θs were the regression coefficients to be estimated for continuous (eg, weight [WT]) or dichotomous (eg, number of metastatic sites [MET]) covariates. Continuous covariates were centered on their median (med[WT]) values, allowing θ_1 to represent the clearance estimate for the typical patient with median covariates. Dichotomous covariates were coded 0 or 1 (eg, MET = 1 if number of metastatic sites = 4 or greater; otherwise MET = 0). θ_m took a value between –1 and +1 and represented the fractional change $\hat{CL}$ when MET = 1. Chemotherapy was coded CHEM1 = 1 when patients received the anthracycline plus cyclophosphamide combination (0 otherwise) and CHEM2 = 1 when patient received paclitaxel (0 otherwise).

Residual error was modeled as proportional, i.e., assuming a constant coefficient of variation for error over the range of measured concentrations.

Individual estimates of random effects (η) and pharmacokinetic parameter values were obtained using post-hoc empirical Bayesian estimation based on the population parameters and the patients' observed concentrations [9]. Goodness-of-fit plots and graphical

representations were produced from NONMEM-generated tables using S-PLUS (version 2000; MathSoft Inc., Cambridge, MA, USA). Plots were made of individual random effects versus covariates (base and final models) to judge the appropriateness of the covariate model.

Comparisons of alternative structural models and construction of the regression model for trastuzumab were based on the objective function values (OFV) and likelihood ratio test [9]. Differences (δ) in OFV of greater than 7.9 for 1 degree of freedom and 10.6 for 2 degrees of freedom (corresponding to a significance level of $P < 0.005$), were used to discriminate between hierarchical models. A nominal P value of 0.005 was chosen, as the *P* values from the first-order method were known to be anticonservative [10, 11]. The structural model and baseline covariates were first assessed using single-agent data ($n = 265$ patients). Covariates that were significant individually were included in the full model. Backward elimination was applied to obtain the final model. Data from the combination trial were then added and the effect of concomitant chemotherapy was assessed using the previous model (i.e., after adjustment for baseline covariate effects).

A bootstrap resampling technique was used to evaluate the stability of the final model [12] and estimate the confidence intervals (CI) of parameters. The model evaluation consisted of repeatedly fitting the model to 500 bootstrap replicates of the dataset. The datasets were created by randomly sampling the patient data (including concentration–time data, dosing history, and covariates) with replacement up to the total number of patients in the original dataset, using the Wings for NONMEM bootstrapping program [13]. The median of the 500 parameter estimates was compared with the point estimates from the original database. The 95% CI of parameter estimates was defined as the 2.5%, 97.5% percentiles of bootstrapped parameter estimates.

Predictive performance of the model was evaluated by simulation [14]. Test statistics of trough concentrations from the phase III studies (one value per patient, either the last or a random one) were chosen and the 25th, 50th (median), 75th percentiles, mean, and standard deviation calculated. Trough concentrations were simulated from the model under the same design as the observed data.

Parameters were drawn from their estimated distribution in the population (maximum likelihood parameter estimates) [15]. Observed statistics were compared with the distribution from 100 replicates of simulated datasets.

Finally, steady-state exposure profiles following 2 mg/kg weekly (the current recommended regimen) and 6 mg/kg every-3-week dosing (an investigational regimen) [16] were simulated for a patient population representative of the patient population from the two pivotal studies, using the final population pharmacokinetic model. Covariates were obtained by randomly sampling patients' data from the dataset of the two phase III studies, with replacement of up to 2,000 patients.

Results

Determination of the structural pharmacokinetic model

The two-compartment model described the single-agent data satisfactorily. Residual variability was 24% and goodness-of-fit plots gave no evidence for model misspecification (data not shown). Figure 1 shows the fit of the model to the data of typical individuals, indicating that the model accounted for the trough concentrations after long-term trastuzumab treatment. The fit of the one-compartment model was unconvincing ($\delta = +1025$) and the three-compartment model significantly im-

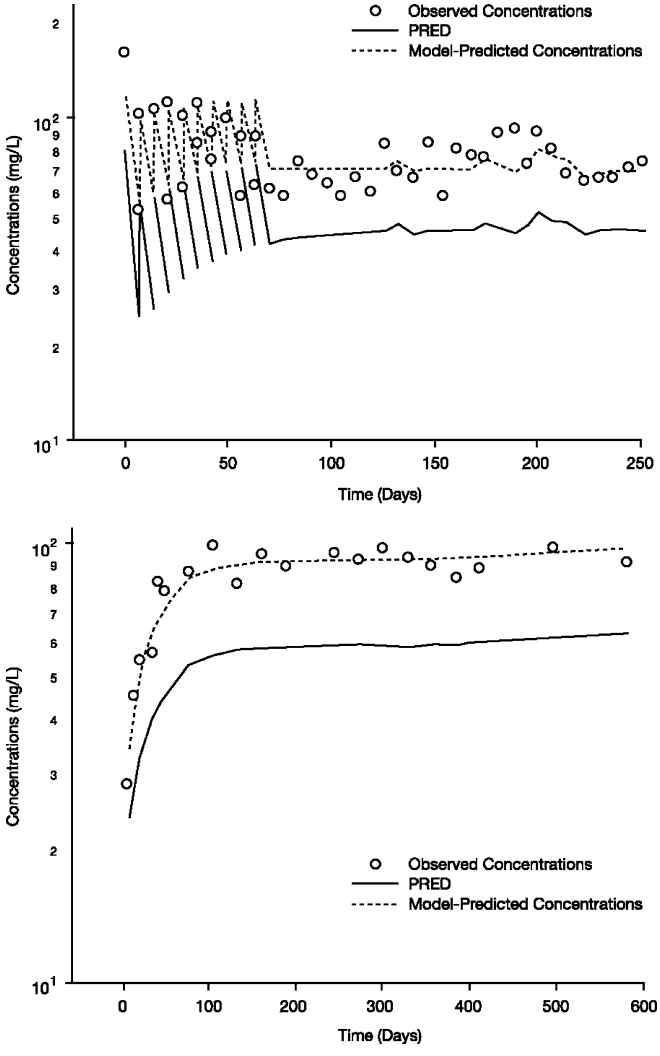


Fig. 1 Typical concentration–time profiles of patients treated in the phase II study (*panel A*) and in the pivotal phase II study (*panel B*). *Solid lines* represent population prediction (PRED); *dashed lines* represent individual prediction

proved the fit ($\delta = -79$) but this model exhibited convergence difficulties. Model parameter estimates yielded large standard errors, and strong correlations among the parameters. The two-compartment model was therefore retained as the structural model. All model parameters were well estimated (%CV12 and K_{21}).

Covariate effects on pharmacokinetic parameters

ECD effect was evaluated during the analysis since trastuzumab binds to ECD and forms a trastuzumab-ECD complex that was found to have faster clearance than free trastuzumab. Exploratory analyses showed that trastuzumab clearance increased with ECD up to approximately 200 ng/ml. However, the relatively few ECD levels above 200 mg/ml were not associated with further increases in clearance (Fig. 2, panel A). Similarly, there was a modest increase in clearance in patients with four or more metastatic sites (Fig. 2, panel B). In univariate analyses, several covariates significantly influenced clearance and V_1. The most influential was the number of metastatic sites, MET ($\delta = 92$), followed by ALT, ECD, and total protein ($\delta > 15$). The most influential covariate for V_1 was ECD ($\delta = 32$), followed by weight, ALT, HER2 overexpression ($\delta > 15$), and ALK and MET ($\delta > 8.0$). In multivariate analysis, all covariates (except ALT, which was strongly correlated with ECD) were found to be significant. In the final model, the only significant covariates were MET and ECD on clearance, and weight and ECD on volume The final model for baseline covariates based on single-agent data was:

$$\hat{CL} = \theta_{\mathrm{CL}}[\min(\mathrm{ECD}, 200)/8.23]\theta_{\mathrm{ECD}}, \quad \mathrm{CL} \times [1 + (\theta_{\mathrm{MET}}\mathrm{MET})]$$

$$\hat{V}_1 = \theta_{\mathrm{V}}(\mathrm{WT}/65)\theta_{\mathrm{WT}} \times [\min(\mathrm{ECD}, 200)/8.23]\theta_{\mathrm{ECD,V}}$$

When data from the combination phase III trial were included in the analysis database, concomitant chemotherapy did not influence clearance or V_1 ($\delta = 7.8$ for clearance, 2 degrees of freedom). The estimated effects were small (less than 10% change in parameters with co-administered chemotherapy); confidence intervals included zero. The previous model was therefore kept as the final model. All parameters were well estimated and stable after bootstrapping. The median parameters from the 500 bootstrapped datasets were within 5% of the corresponding point estimates from the original database (data not shown). Table 3 shows the final parameter estimates.

The typical clearance (i.e., the clearance of a patient with median WT, median baseline ECD and < 4 METs) for the final model was 0.225 L/day, and the typical volume of distribution was 2.95 L. The terminal half-life ($t_{1/2\ \lambda\ z}$) was 28.5 days. The interpatient variabilities for clearance and volume of distribution were 43% and 29%, respectively. The residual variability was 23% (CV). With this model, after the 2 mg/kg weekly maintenance dose, the steadystate exposure for a typical patient would be 578 mg day/l, with steadystate predicted peak and trough concentrations of 110 and 66 mg/l, respectively.

As already mentioned, the number of metastatic sites was the most influential covariate for clearance, which was 22% higher in patients with four or more sites. This difference in clearance would result in a 18% lower steady-state exposure. Shed HER2 ECD was also included in the final model for clearance. Its effect was small; clearance was 14% higher in patients with baseline shed antigen levels of 200 ng/ml or greater, compared with a patient with a baseline level of 8.23 ng/ml (median). Shed ECD had more effect on V_1. Patients with baseline ECD levels of 200 ng/ml or greater had a 40% greater volume of distribution. The combined effect of shed antigen on clearance and on V_1 would result in a 12% lower steady-state exposure in patients with high baseline ECD. Finally, V_1 would range from 2.5 L to 3.7 L in patients with extreme body weight (i.e., from 49 kg [5th percentile] to 96 kg [95 th percentile]). The preceding covariate effects would give modest changes in the terminal half-life, ranging from 24.3 days in patients with four and more metastatic sites to 33.9 days in patients with high ECD levels (200 ng/ml or greater).

Assessment of model predictive performance and model simulation

Figure 3 shows that the model adequately predicted the mean and also the variability (25th and 75th percentiles) of the last-trough level for each patient treated in the phase II and III studies ($n = 416$). The mean predicted last-trough level (60.2 mg/l) was similar to the mean observed value (59.4 mg/l). However, there was a trend for the model to under-predict high levels (75th percentile predicted 76.1 mg/l while the observed value was 81.4 mg/l). The model also predicted the standard deviation of last-trough level (not shown). The model adequately predicted trough levels over treatment durations ranging from 6 days to 840 days. The model performed the same in predicting a trough level randomly selected from all observed samples for a given patient.

The model was used to simulate steady-state trough levels following dosing with the recommended regimen: 4 mg/kg followed by 2 mg/kg/week, and an every-3-week regimen: 8 mg/kg followed by 6 mg/kg every 3 weeks (Fig. 4). These simulations show that the minimum target level of 20 mg/l, would be achieved in 91.9 and 80.2% of the patients in the weekly and the every-3-weeks regimen, respectively. Figure 4 shows both the expected (median) profile and the predictability/variability of this population PK analysis based on the known and unknown sources of variability.

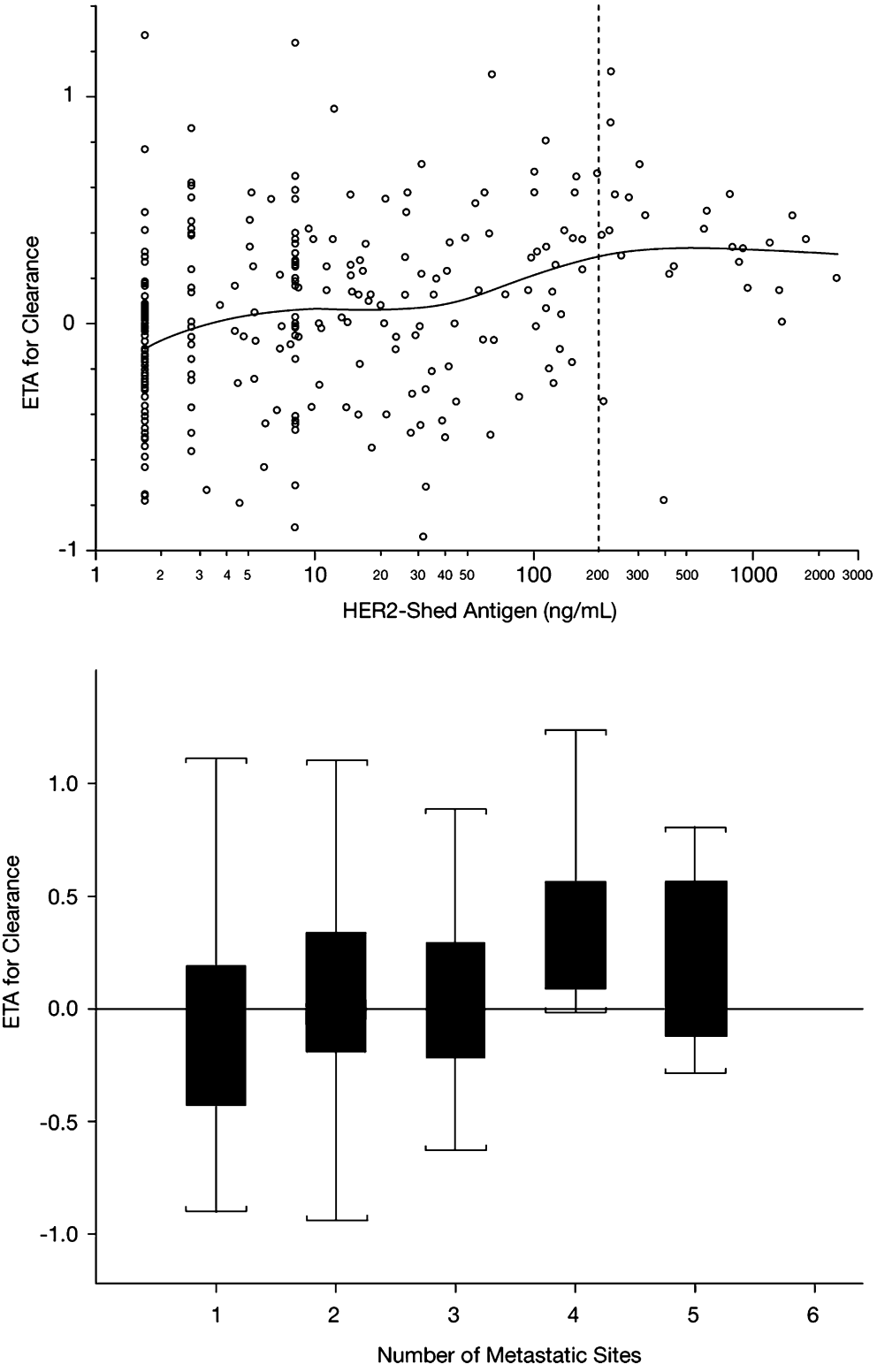


Fig. 2 Random effect (ETA) for clearance obtained from base model versus baseline ECD level. The solid line shows spline fit and the dotted line shows 200 ng/ml (*panel A*). Box plot of ETA of clearance versus number of metastatic sites (*panel B*) Boxes, interquartile ranges (third quantile to first quartile); whiskers, 1.5 times the interquartile ranges; *white horizontal lines*, medians (50th percentile)

Discussion

This is the first population pharmacokinetic model for trastuzumab. The population approach combined the pharmacokinetic data from the phase I single-dose study (intensive sampling) with the peak and trough plasma levels from the later clinical studies. The database of 476 patients included 416 from the phase II and III studies [3, 4] that supported the Food and Drug Administration approval for trastuzumab. Based on single-agent data ($n = 265$ patients), a two-compartment linear model fit the data best and adequately predicted trastuzumab

Table 3 Parameter estimates of the final population pharmacokinetic model

	Point estimate[a]	95% CI[b]
Parameters and covariate effects		
CL (L/day)	0.225	0.213, 0.238
ECD on CL	0.041	0.013, 0.071
MET on CL	0.221	0.0611, 0.429
V (L)	2.95	2.67, 3.27
WT on V	0.556	0.211, 0.824
ECD on V	0.105	0.0726, 0.146
K_{12} (day^{1})	0.164	0.135, 0.191
K_{21} (day^{1})	0.101	0.0826, 0.122
$t_{1/2\lambda z}$ (day)	28.5	25.5, 32.8
Random effects		
ω_{CL}(%)	43	39, 47
ω_{V1}(%)	29	21, 38
ω_{K12}(%)	54	43, 62
ω_{K21}(%)	67	57, 78
σ (%)	23	21, 24

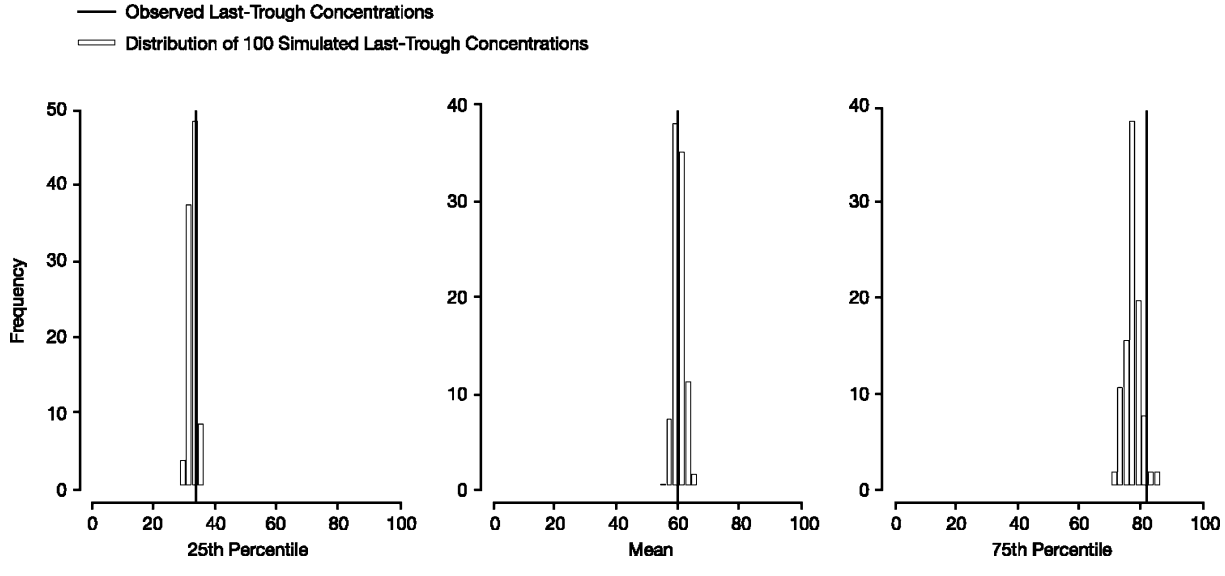
[a]By fitting the original database
[b]2.5th to 97.5th percentiles of 500 bootstrap estimates

accumulation with long-term weekly dosing. The baseline covariates (number of metastatic sites, level of shed ECD of the HER2 receptor, and patient weight) were significant covariates for either clearance or V_1, or both ($P < 0.005$). Combination-trial data ($n = 211$ patients) were included with the single-agent database to assess drug–drug interactions. Giving anthracycline plus cyclophosphamide or paclitaxel chemotherapy concomitantly did not appear to influence clearance and V_1.

Covariate effects modestly influenced pharmacokinetic parameters and there were modest changes in exposure to trastuzumab compared with the typical patient. In this dataset, patient clearance and V_1 increased with the level of baseline ECD up to 200 ng/ml and then reached a plateau at an ECD level of 200 ng/ml with maximum increases of 14% and 40%, respectively, compared with a typical patient with a median ECD of 8.23 ng/ml. The positive association of clearance of trastuzumab with ECD is consistent with the faster clearance of trastuzumab-ECD complex compared to free trastuzumab seen in nonclinical studies (Genentech Inc., data on file). Circulating ECD is only a small fraction of the total load of HER2 receptor antigen. These trials did not give a quantitative measure of tumor burden and the relationship between circulating ECD and tumor burden is unknown (although we might expect higher ECD levels to be associated with higher tumor burdens). The limited influence of these effects on trastuzumab exposure is consistent with the low level of circulating ECD relative to trastuzumab level. Patients with four or more metastatic sites (a covariate related to tumor burden) had a statistically significantly faster clearance independent of the HER2–ECD level, but this covariate had little influence on trastuzumab exposure.

The terminal half-life was estimated at 28.5 days (95% CI 25.5, 32.8 days) from the final model. This is consistent with the model-independent estimate of 18–27 days in a phase II study with trastuzumab every 3 weeks [16]. This long half-life is similar to that of endogenous IgG1 immunoglobulins (23 days) [17], which constitutes the backbone of trastuzumab. The estimate of clearance is 0.225 l/day in this analysis and is consistent with that estimated following the every-3-weeks regimen of 8.2–9.8 ml/h, i.e., 0.196– 0.235 l/day (cycle 12, week 36) [16] after the use of a maintenance dose of 6 mg/kg compared with 2 mg/kg. The schedule independence of trastuzumab pharmacokinetics is consistent with linear pharmacokinetics at clinical doses. However, there is evidence that trastuzumab may clear faster at low doses. This is consistent with the observation of faster clearance in patients with elevated ECD, but it does not confirm the observation of a shorter

Fig. 3 The model adequately predicted the median but also the variability (25th and 75th percentiles) of the last trough level observed for each patient treated in the phase II and III studies ($n = 416$)



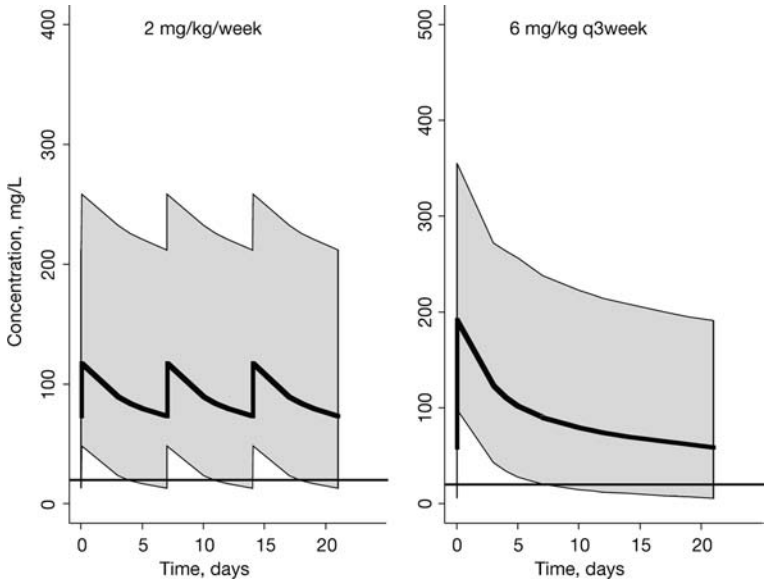


Fig. 4 Simulated (median, 5th, and 95th percentiles, $n = 2{,}000$ patients) steady-state trastuzumab concentrations following weekly and every-3-weeks dosing

half-life in these patients [3, 4]. This analysis is limited to baseline ECD, as the post-dosing measurements of ECD were confounded by excessive trastuzumab in the assays. Trastuzumab is reported to inhibit ECD shedding by breast cancer cell lines overexpressing HER2 [18]. The relationship between baseline ECD and response and the effect of trastuzumab and chemotherapy on ECD levels was investigated in 266 patients in four phase II/III clinical trials. No clear relationship was found between baseline ECD levels and best clinical response [19].

The model adequately predicted 25th, 50th (median), 75th percentiles, mean, and standard deviation of observed trough levels, showing the usefulness of the model in predicting trastuzumab exposure and its variability in long-term therapy. Simulations showed that more than 90% of the patients would achieve or exceed the 20 mg/l trough target plasma level at steady-state with the approved regimen of a 4 mg/kg loading dose followed by 2 mg/kg weekly. The simulation of trastuzumab every 3 weeks at a maintenance dose of 6 mg/mL shows that slightly lower trough and higher peak levels would be expected, as observed by Leyland-Jones et al. [16]. Trough levels would still be greater than 20 mg/l in more than 80% of patients. A longer mean half-life of 18 days (cycle 12, week 36) using noncompartmental pharmacokinetic analysis was reported. This regimen proved safe and appeared to have similar efficacy to the weekly schedule [16]. The peak levels with this regimen were higher than those with the current dosing regimen of 2 mg/kg maintenance. However, they are much lower than the predicted levels with maintenance dosing of 4 mg/kg/week, which proved safe in a previous study of single-agent trastuzumab in patients with HER2-positive MBC [20].

In conclusion, the long-term accumulation of trastuzumab with weekly dosing can be predicted by a linear two-compartment pharmacokinetic model with a long half-life. Both the pharmacokinetics and the observed variability are typical of the IgG1 immunoglobulins. Trastuzumab pharmacokinetics appear to be schedule independent. The proposed population pharmacokinetic model can predict trastuzumab exposure and variability in a large and representative patient population. This model should therefore support pharmacokinetic and exposure comparisons of dosage regimens as well as pharmacokinetic and pharmacodynamic studies of trastuzumab.

Acknowledgements The authors thank Dr Judy A. Fox and Dan Combs (Genentech, Inc.) for sharing their expertise in antibody biology and pharmacokinetics and Drs Virginia Paton, David Allison, Leonard Reyno and Daniel Spyker (Genentech, Inc.) and Dr Jean-Louis Steimer (Roche) for their review of the analysis and insightful comments.

References

1. Baselga J, Tripathy D, Mendelsohn J, Baughman S, Benz CC, Dantis L et al (1996) Phase II study of weekly intravenous recombinant humanized anti-p185HER2 monoclonal antibody in patients with HER2/neu-overexpressing metastatic breast cancer. J Clin Oncol 14:737–744
2. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga JM, Tripathy D et al (1998) Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment. J Clin Oncol 16:2659–2671
3. Cobleigh MA, Vogel CL, Tripathy D, Robert NJ, Scholl S, Fehrenbacher L et al (1999) Multinational study of the efficacy and safety of humanized monoclonal antibody in women who have HER2-overexpressing metastatic breast cancer that has progressed after chemotherapy for metastatic disease. J Clin Oncol 17:2639–2648
4. Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A et al (2001) Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 344:783–792
5. 2003 Herceptin Package Insert

6. 2003 Herceptin Biological License Application (BLA)
7. Payne RC, Allard JW, Anderson-Mauser L, Humphreys JD, Tenney DY, Morris DL (2000). Automated assay for HER-2/neu in serum. Clin Chem 42:175–182
8. Cockcroft DW, Gault MH (1976) Prediction of creatinine clearance from serum creatinine. Nephron 16:31–41
9. Beal SL, Boeckman A, Sheiner LB (1988–1992) NONMEM user's guides. University of California at San Francisco, San Francisco
10. Wählby U, Jonsson EN, Karlson MO (2001) Assessment of actual significance levels for covariate effects in NONMEM. J Pharmacokinet Pharmacodyn 28:231–252
11. Gobburu JVS, Lawrence J (2002) Application of resampling techniques to estimate exact significance levels for covariate selection during nonlinear mixed effects model building: some Inferences. Pharm Res 19:92–98
12. Ette EI (1997) Stability and performance of a population pharmacokinetic model. J Clin Pharmacol 37:486–495
13. Parke J, Holford NH, Charles BG (1999) A procedure for generating bootstrap samples for the validation of nonlinear mixed-effects population models. Comput Methods Programs Biomed 59:19–29
14. Gelman A, Meng XL, Stern H (1996) Posterior predictive assessment of model fitness via realized discrepancies. Statistica Sinica 6:733–807
15. Yano Y, Beal SL, Sheiner LB (2001) Evaluating pharmacokinetic/pharmacodynamic models using the posterior predictive check. J Pharmacokinet Pharmacodyn 28:171–192
16. Leyland-Jones B, Gelmon K, Ayoub JP, Arnold A, Verma S, Dias R et al (2003) Pharmacokinetics, safety, and efficacy of trastuzumab administered every three weeks in combination with paclitaxel. J Clin Oncol 21:3965–3971
17. Trang JM (1992) Pharmacokinetics and metabolism of therapeutic and diagnostic antibodies. In: Ferraiolo BL, Mohler MA, Gloff CA (eds) Protein pharmacokinetics and metabolism. Pharmaceutical biotechnology, vol 1. Plenum Press, New York, pp 223–270
18. Albanell J, Codony J, Lopez-Talaver J, Arribas J, Baselga J (1999) Inhibition of HER2 extracellular domain shedding by metalloprotease inhibitors and by Herceptin in breast cancer cells. Proc Am Soc Clin Oncol 18:2381
19. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L et al (2002) Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. J Clin Oncol 20:719–726
20. Leyland-Jones B, Marty M, Baselga J, Gatzemeier U, Barton C, Lennon S (2004) Effects of trastuzumab (H) and chemotherapy on circulating HER2 extracellular domain (ECD) in patients with metastatic breast cancer (MBC) and non-small cell lung cancer (NSCLC). Proc Am Soc Clin Oncol 23:19