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ORIGINAL ARTICLE

Population pharmacokinetics of a HER2 tyrosine kinase inhibitor CP-724,714 in patients with advanced malignant HER2 positive solid tumors

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Abstract
Purpose To develop a population pharmacokinetic (popPK) model for CP-724,714, a novel HER2 tyrosine kinase inhibitor is under development for the treatment of advanced HER2 positive cancers.
Methods Concentration-time data (n = 484) from 30 cancer patients receiving daily oral CP-724,714 at doses of 250 mg QD, 250 mg BID, 250 mg TID, and 400 mg BID in 21-day cycles in an open label First-in-Human dose-escalation study were evaluated. Serum concentrations of CP-724,714 were obtained after single dose and at steady state. Nonlinear mixed effect analysis in NONMEM using first order conditional estimation with interaction (FOCEI) was performed. The effect of covariates was assessed. Diagnostic plots, decrease of objective function value (>7.8), bootstrapping, and predictive check were used as model selection criteria.
Results A 2-compartment first-order absorption pharmacokinetic (PK) model with inter-subject variability (ISV) on the apparent oral elimination clearance (CL/F), apparent central volume of distribution (V1/F), apparent peripheral volume of distribution (V2/F), and first-order oral absorption rate constant (ka), interoccasion variability (IOV) on CL/F, and body weight (WT) as covariate on CL/F was developed. There was no evidence of dose-dependent and/or time-dependent PK. CL/F increased with increasing WT. The ISV of CL/F was reduced by approximately 20% with WT as a covariate. Age, race, and liver metastasis did not influence CP-724,714 disposition.
Conclusions A popPK model was developed that adequately described the pharmacokinetics of CP-724,714. WT was identified as a covariate on CL/F that reduced ISV and improved model performance. Future studies will further assess the importance of WT as a pharmacokinetic covariate. The proposed popPK model can be used to simulate CP-724,714 Phase 2/3 trials.

Keywords HER2 tyrosine kinase inhibitor · Population pharmacokinetics

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Introduction

Overexpression of erbB2 (HER2) receptor, a member of the epidermal growth factor family, is oncogenic and has been associated with a poor prognosis in various tumor types. In breast cancer, about 18–25% of breast tumors overexpress HER2, and these tumors are associated with a high recurrence rate and poor survival [1, 2]. Herceptin, a monoclonal antibody specific for HER2, has been approved to treat advanced HER-2 overexpressing breast cancer [3]. In tumors overexpressing HER2, 35% of the patients responded when

Herceptin was given as a single agent [4], and disease-free survival and overall survival in metastatic and in early stage breast cancer were improved when Herceptin was combined with current standard chemotherapy [5–7]. However, while adding Herceptin to chemotherapy provides clinical benefit, the risks on the heart should be considered particularly in association with anthracyclines [8]. Moreover, breast tumors in the majority of patients treated with Herceptin will recur.

Small-molecule orally administered kinase inhibitors provide the convenience of the oral administration over the intravenous Herceptin. Furthermore, a small molecule may avoid the cardiotoxicity associated with Herceptin [8].

CP-724,714 is a potent and selective small-molecule oral inhibitor of HER2. It inhibits HER2-chimera phosphorylation with an IC50 of 15 ng/ml (32 nM), and is >500-fold selective for HER2 relative to other kinases (e.g., EGFR, PDGFR, IGF-1R, VEGFR-2, abl, src). Antitumor activity of CP-724,714 was observed in the Fischer rat embryo (FRE) HER2 tumor as well as in murine xenografts of human adenocarcinomas of breast (BT-474), pancreas (Panc-1), lung (Calu-3), and ovary (SK-OV-3) [9]. Furthermore, the whole-body autoradioluminography (WBAL) study of CP-724,714 indicated a rapid distribution into more than 46 tissues within 0.5 h after dosing.

CP-724,714 was investigated first in human trial (FIH) as continuous oral daily dosing in 3-week cycles to determine its safety, tolerability, and pharmacokinetics in patients with advanced solid tumor malignancies that overexpress HER2. The overall safety, tolerability, and a traditional noncompartmental pharmacokinetic analysis (NCA) of this trial were reported previously [10, 11]. The major toxicity in humans was the elevation of hepatic transaminases and bilirubin. No cardiomyopathy as evaluated using either multigated nuclide analysis scan (MUGA) or echocardiogram (ECHO) was observed in both clinical and preclinical studies. The maximal tolerated dose (MTD) was determined to be 250 mg TID in the FIH trial, and the recommended Phase 2 dose (RP2D) was 250 mg BID. The mean steady state concentration of CP-724,714 at the RP2D exceeded the predicted efficacious concentration based on preclinical models. In the FIH study, the main serum concentration-time profiles of CP-724,714 were described as a rapid absorption followed by a bi-exponential decline, with a mean terminal half-life (t1/2) of approximately 4.5 h. Both Cmax and area-under-curve (AUC) of CP-724,714 increased in an approximate dose-proportional manner. Inter-patient and inter-occasion variability of oral elimination clearance (CL/F) as assessed from the NCA analysis were moderate (CV: 20–57% and 23%, respectively). CL/F appeared to increase with WT.

The population pharmacokinetic (popPK) approach has been advocated as a useful tool in early development of new anticancer agents [12, 13]. The dense pharmacokinetic sampling in Phase 1 studies allows good characterization of the structural pharmacokinetic model, although the small number of subjects may not provide robust initial estimates of inter-individual and intra-individual variability [14,15]. In addition, it also offers the first opportunity of exploring the influence of individual patient characteristics on the pharmacokinetics of this agent. The preliminary popPK model could be used for trial simulations for subsequent Phase 2/3 studies. In this report, a popPK analysis was conducted for the CP-724,714 pharmacokinetic data from the FIH trial. The objectives were to develop a popPK model for CP-724,714 and explore the effect of covariates on CP-724,714 pharmacokinetics.

Methods

Patient eligibility

The detailed patient eligibility has been described elsewhere [11]. Patients with advanced solid tumor malignancies with HER2 overexpression, measured either by 3+ protein overexpression by immunohistochemistry or gene amplification measured by fluorescence in situ hybridization (FISH) amplification, were eligible for this trial. Patients had to have at least one prior treatment, and patients with breast cancer had to have failed prior therapy with Herceptin. Patients were required to have adequate bone marrow, renal, liver, and cardiac function. Other inclusion criteria included: age ≥18 years; Eastern Cooperative Oncology Group (ECOG) performance status ≥1. No concomitant medications that interfere the primary metabolism pathway of CP-724,714 via CYP3A were allowed, including grapefruit juice and St. John's Wort. Concurrent treatment with H-2 antagonists or proton pump inhibitors was prohibited. This study was conducted in accordance with the Declaration of Helsinki as amended in the 41st World Medical Assembly (Hong Kong 1989). Informed consent of patient and ethics committee approval was obtained from each patient before the beginning of the treatment.

Drug administration and pharmacokinetic sampling

This was an open-label, multicenter, dose-escalation Phase I trial using a modified Fibonacci schema. All

eligible subjects received CP-724,714 as continuous oral daily doses on 21-day cycles for a predefined maximal duration of 17 cycles (51 weeks). There was no washout between cycles. The starting dose level was 250 mg QD. In subsequent dose cohorts, 250 mg BID, 400 mg BID, and 250 mg TID were evaluated. The maximal tolerated dose was identified as 250 mg TID. For the same dose cohort, patients received the same daily dose until disease progression or unacceptable toxicities. No intra-patient dose escalation was allowed. CP-724,714 was supplied as 25 and 100 mg tablets.

Serum pharmacokinetics of CP-724,714 was estimated following single oral dose of CP-724,714 on Day 1 of Cycle 1 (with a 2 day washout) and at steady state on Day 1 of Cycle 2 after daily oral dosing for 3 weeks. For single dose pharmacokinetic assessment on Day 1 of Cycle 1, blood specimens were collected prior to dosing (time 0), 0.5, 1, 2, 3, 4, 6, 8, 10, 24, 32 and 48 h following the administration of CP-724,714. Timepoints of 8 and 10 h were replaced by 9 and 12 h for the BID dose cohort. For steady state pharmacokinetic assessment on Day 1 of Cycle 2, blood specimens were collected prior to dosing (time 0), 0.5, 1, 2, 3, 4, 6 and 8 h following the morning dose of CP-724,714 for QD, BID, and TID dose cohorts. Additional samples were taken at 10 and 24 h for the QD cohort, and at 12 h for the BID cohort. All blood specimens were centrifuged to obtain serum fractions, and serum samples were frozen and stored at temperature ≤–20°C within 1 h of collection.

Determination of CP-724,714 in serum samples by LC/MS/MS

Aliquots (150 μl) of human serum, 50 μl of the internal standard (IS) CE-245,346 and 200 μl of 0.1 N HCl were added, in sequence, to one well of a 96-well block. The drug and I.S. were extracted using a 96-well ANSYS MP1 15 mg Extraction Plate. The sample was washed with 0.1 N HCl and 100% methanol, and eluted with 200 μl 95/5 methanol/ammonium hydroxide. The eluent was isolated, evaporated to dryness, and reconstituted in 100 μl 82/18 acetonitrile/10 mM ammonium acetate (0.05% formic acid). Separation was performed by HPLC isocratic conditions. The mobile phase was a binary mixture (82/18) of acetonitrile and 10 mM ammonium acetate. The analytical column was a Phenomenex Luna 5 μ cyano 2.00×50 mm^2 HPLC column. The analysis was performed on a Perkin Elmer SCIEX API 3000 triple quadrupole mass spectrometer operated in the positive ion mode. The TurboIonSpray ion source was operated at 1,500 V, with a temperature of 350 C and 6 l/s nitrogen gas. Nitrogen nebulizer gas was set to 8 and curtain gas was set to 8. Collision gas was set to 6. Product ions at m/z 272.0 were monitored for both drug and IS. The assay was linear from 1.0 to 500 ng/ml. Quality control (QC) samples were prepared at 300, 200, and 400 ng/ml in control human serum. The bias and precision (%CV) of both intra- and inter-batch runs were within ±15 and ±8%, respectively. Stability of CP-724,714 to repeated freeze–thaw was determined at 300 and 400 ng/mL by preparing QCs; the mean assayed value of high and low QC samples was within 15% of their nominal values.

Population pharmacokinetic modeling

Pharmacokinetic data were analyzed using the nonlinear mixed effect modeling software program NONMEM (version V, level 1.1, ICON, Ellicott City, MD, USA) with the G77 FORTRAN compiler [16]. The first-order conditional estimation with η–ε interaction (FOCEI) method was used. Data processing and diagnostic plots were performed using S-PLUS (version 6.2, Insightful Corporation, Seattle, WA, USA). Serum concentration of CP-724,714 from all treatment groups were simultaneously fitted using the NONMEM library PREDPP subroutines for various compartmental models. Development of the population model was performed in three distinct steps: (1) development of a base structural and statistical model; (2) evaluation of potential covariates; (3) evaluation of the model performance by bootstrap and visual predictive check.

First, various structural models (i.e., 1st order oral absorption, zero order oral absorption, and sequential/parallel 1st and zero order oral absorption with 1-, 2-, or 3-compartment distribution, and 1st order elimination from the central compartment, respectively) were assessed. Once the appropriate structural model was selected, the statistical models were assessed by evaluating inter-subject variability (ISV: η) and residual variability (ε) using alternate statistical models (i.e., additive, constant coefficient of variation, and exponential). Inter-occasion variability (IOV: κ) was also considered since patients underwent pharmacokinetic sampling on more than one treatment cycle, and it is obvious that pharmacokinetic parameters may vary randomly between study occasions [17]. The ISV, IOV, and residual variability were assumed to be normally distributed with zero mean and variance ω^2, π^2, and σ^2, respectively. Identification of the best base structural and statistical model was based on the change in the objective function value (ΔOFV) and/or the Akaike's information criterion (AIC), as appropriate, precision

of parameter estimates, and goodness-of-fit by visual inspection of various diagnostic plots [e.g., predicted versus observed (PRED versus OBS) concentrations].

The influence of covariates on pharmacokinetic parameters was systematically tested via a generalized modeling approach according to the following equation, using oral elimination clearance (CL/F) for example:

$$\mathrm{TVCL}/F = \theta 1 \times \left(\frac{\mathrm{WT}}{\mathrm{med(WT)}}\right)^{\theta_{\mathrm{WT}}} \times \theta 2^{\mathrm{MET}}$$

where TVCL/F denoted the typical value of CL/F in the population after adjusting for values of covariates of individual patients. θs were the regression coefficients to be estimated for continuous (e.g., weight [WT]) or dichotomous (e.g., liver metastasis [MET]) covariates. Continuous covariates included age, WT, height, body surface area (BSA), and total daily dose. Continuous covariates were centered on their median (e.g., med[WT]) values, allowing $\theta 1$ to represent the apparent oral CL/F estimate for a typical patient with median values of the covariates in this study. Dichotomous covariates included liver metastasis status and treatment period. These covariates were coded as 0 or 1 (e.g, MET = 1 if liver metastasis was present; otherwise, MET = 0). $\theta 2$ represented the change of the typical apparent oral CL/F by categorical covariates, e.g., liver metastasis. The treatment period was coded PERI = 1 for pharmacokinetic data collected on Cycle 2 Day 1, otherwise PERI = 0. Forward inclusion of covariates in the base model one at a time was conducted to assess influential covariates. Covariates were selected in the full model if (1) when compared to the base model, they produced a minimum reduction of 3.84 in the OFV (i.e., $P < 0.05$, χ^2, 1 *df*) and a reduction in the variability of the pharmacokinetic parameter (assessed by the associated ISV), (2) their effect was biologically plausible, and (3) the collinearity of covariates was avoided. Then backward elimination of covariates from the full model one at a time was conducted to obtain the final model. At this step, only covariates producing an increase in OFV > 7.8 (i.e., $P < 0.005$, χ^2, 1 *df*) were kept in the final model.

Evaluation of the final model

A nonparametric bootstrap procedure and simulations were used to evaluate the stability and performance of the final model [18–20]. For the bootstrap, 1,000 bootstrap data sets were created by randomly sampling the patient data (including concentration-time data, dosing history, and covariates) with replacement using each individual subject's data as the sampling unit, up to the total number of patients in the original dataset, using S-Plus (version 6.2, Insightful, Seattle, WA, USA). Then the final model was used to repeatedly fit the 1,000 bootstrap datasets, and the population pharmacokinetic parameters were estimated for each dataset. The mean, median, and 95% confidence interval (CI: 2.5–97.5 percentiles) of the 1,000 parameter estimates were calculated for each population pharmacokinetic parameter [21].

Predictive performance of the model was evaluated by simulating the 250 mg BID dose cohort (n = 15), as 250 mg BID was the recommended Phase 2 dosing regimen [14, 15, 22]. One thousand trials were simulated using the final model with the original patient dataset from the 250 mg BID cohort as the simulation template input. Then a visual predictive check was performed as described later: the 5th, 50th (median), and 95th percentiles from the resulting simulated concentrations at each time point were calculated and plotted over time, and overlaid with the observed concentrations (250 mg BID). Statistics of interest were also computed for each patient in each trial and for observed data. Statistics of interest were the steady state maximal concentration (Cmax) and area under the curve of the dosing interval (AUC_{0-12h}) of CP-724,714 on Cycle 2 Day 1. Percentiles (25, 50, 75, and 95%) of Cmax and AUC_{0-12h} were computed for the observed data and for each of the simulated studies. Then, simulated distributions of each percentile were compared with the observed values.

Results

Patient characteristics

A total of 30 patients were treated in the study. Their characteristics are listed in Table 1. There were 15 patients treated at 250 mg BID, and the remaining patients split evenly in the other three dosing regimens. Individual patient's PK exposures were measured up to 48 h following the single dose on Cycle 1 Day 1. However, serum concentrations of CP-724,714 at 32 and 48 h postdosing were near the lower limit of quantitation (LLOQ) and highly variable. The extrapolated AUC from 24 h to infinity accounted for less than 5% of the extrapolated total AUC based on individual patients' concentration-time profiles. For these reasons, only serum concentrations of CP-724,714 up to 24 h postdosing on Cycle 1 Day 1 were included in the population pharmacokinetic analysis. For each patient, there are 10 and 8–10 concentration-time points after dosing on Cycle 1 Day

Table 1 Patient characteristics at baseline

Characteristics		Value
No. of patients studied		30
Gender	Female	30 (100)
Cancer type	Breast	28 (93)
	Nonbreast	2 (7)
Liver metastasis	Yes	13 (43)
	No	17 (57)
ECOG PS	0	10 (33)
	1	20 (67)
Age (years)		51.2 (37–71)
Weight (kg)		73.1 (39–121)
Height (cm)		162.7 (147–174)
BSA (m^2)		1.80 (1.31–2.35)

Continuous data are given as mean (range), and categorical data as number of patients (percentage of the total population)

1 and Cycle 2 Day 1, respectively. A total of 484 concentration-time points were available for pharmacokinetic modeling.

Population pharmacokinetic modeling

NONMEM analysis steps are summarized in Table 2. Development of the structural pharmacokinetic model indicated that the 2-compartment model with 1st order oral absorption and 1st order elimination from the central compartment was the most appropriate model identified (the OFV was decreased by 123 U relative to the 2-compartment with zero-order oral absorption), and was subsequently selected as the base structural model. For other structural models examined, the

Table 2 Representative NONMEM model building steps

	ΔOFV	$\omega^2_{CL/F}$	$\omega^2_{V1/F}$	$\kappa^2_{CL/F}$	σ^2	*P*
Structural model						
Zero order oral absorption[a]	–	0.192	0.619	–	0.255	–
1st order oral absorption	–123	0.142	0.693	–	0.246	–
Error model[b]						
$CL/F = \theta1 \times e^{\eta}$	–	0.142	–	–	0.246	–
$CL/F = \theta1 \times e^{(\eta+\kappa)}$	–11	0.0969	–	0.079	0.179	–
Forward inclusion[c]						
$TVCL/F = \theta1 \times (WT/70)^{\theta2}$	–10	0.0656	–	–	0.178	<0.005
$\theta1 \times (BSA/1.8)^{\theta2}$	–9	0.0661	–	–	0.178	<0.005
$\theta1 \times (age/50)^{\theta2}$	0	–	–	–	–	NS
$\theta1 \times (DOSE/250)^{\theta2}$	0	–	–	–	–	NS
$\theta1 \times \theta2^{PERI}$	0	–	–	–	–	NS
$\theta1 \times \theta2^{MET}$	0	–	–	–	–	NS
$TVV1/F = \theta2 \times (WT/70)^{\theta3}$	–4	–	0.516	–	0.247	<0.05
$\theta2 \times (BSA/1.8)^{\theta3}$	–4	–	0.517	–	0.247	<0.05
Full model[d]						
$CL/F = \theta1 \times (WT/70)^{\theta2} \times e^{(\eta1+\kappa)}$						
$V1/F = \theta3 \times (WT/70)^{\theta4} \times e^{\eta2}$	–14	0.0651	0.525	0.0753	0.179	–
Backward elimination						
WT on CL/F ($\theta2 = 0$)	+10	0.116	–	0.0720	0.174	<0.005
WT on V1/F ($\theta4 = 0$)	+4	–	0.516	–	0.247	NS
Final model						
$CL/F = \theta1 \times (WT/70)^{\theta2} \times e^{(\eta1+\kappa)}$	+4	0.0661	0.619	0.0817	0.178	NS
$V1/F = \theta3 \times e^{\eta2}$						

CL/F oral elimination clearance, *TVCL/F* population typical value of CL/F, *V1/F* apparent central volume of distribution, *TVV1/F* population typical value of V1/F, *WT* weight, *BSA* body surface area, *DOSE* total daily dose, *PERI* period, *MET* liver metastasis status, θs fixed effect values of typical CL/F and V1/F, and regression coefficients for continuous and dichotomous covariates; ('s random effects for CL/F and V1/F; ω^2's inter-patient variability; κ^2 inter-occasion variability; σ^2 residual variability; – not appropriate; *NS* not significant; *ΔOFV* difference in objective function values

$P < 0.05$ at the forward inclusion step and $P < 0.005$ at the backward elimination step

[a] Rejected model; reference fit for evaluation of the 1st order oral absorption model

[b] For the error model building and rest of NONMEM analysis, the 1st order oral absorption model is used as the structural model

[c] From the forward inclusion step on, the base model is the 1st order oral absorption model with both inter-patient and inter-occasion variability on CL/F, and inter-patient variability on V1/F, V2/F (apparent peripheral volume of distribution), and ka (1st-order oral absorption rate constant)

[d] Reference fit for the backward elimination step, and evaluation of the final model

minimization steps were unsuccessful. The NONMEM subroutine ADVAN4 TRANS4 was used in all subsequent analyses with the selected base structural model. In the statistical model building step, based on visual inspection of diagnostic plots, the ISV and residual error were adequately described by the exponential error model and proportional error model, respectively. Estimates of ISV of CL/F, apparent central distribution volume (V1/F), peripheral distribution volume (V2/F), and 1st-order oral absorption rate constant (ka) were greater than zero, and exclusion of any of these parameters resulted in increased OFV and larger variability of other parameters, and adversely affected the fit based on visual inspection of diagnostic plots. In contrast, the ISV on the oral intercompartment clearance (Q/F) approached zero, and exclusion of the ISV of Q/F had no effect on the OFV and did not alter the fit and the pharmacokinetic parameter and associated variability estimates. It was also revealed that inclusion of IOV in CL/F resulted in 17% lower ISV in CL/F, and an improved fit. Thus, the base statistical model included both ISV and IOV on CL/F, and ISV on V1/F, V2/F, and ka.

Among the seven covariates tested at the forward inclusion step, two (WT and BSA) provided significant improvements in the fit when individually entered in the models of CL/F (ΔOFV $\leq$–9; $P < 0.005$) and V1/F (ΔOFV = –4; $P < 0.05$), and reduced the ISV of CL/F and V1/F by approximately 20 and 14%, respectively. As illustrated in Fig. 1, the inclusion of WT as a covariate for CL/F reduced the trend between the weight and the post hoc Bayesian-estimated individual patient's variability of CL/F, and decreased the magnitude of ISV in CL/F. The age, height, total daily dose, treatment period, and the presence of liver metastases did not appear to have a significant effect on the fit. Since WT and BSA were highly correlated ($r = 0.98$, $P < 0.0001$), WT was chosen in the full models of CL/F and V1/F as it provided a slightly greater reduction in ISV for both parameters, as follows:

$$\mathrm{CL/F} = \theta 1 \times (\mathrm{WT}/73)^{\theta 2} \times \exp(\eta 1 + \kappa)$$

$$\mathrm{V1/F} = \theta 3 \times (\mathrm{WT}/73)^{\theta 4} \times \exp(\eta 12)$$

where $\theta 1$ and $\theta 3$ were the typical values of CL/F and V1/F for patients with the median weight, $\theta 2$ and $\theta 4$ were power coefficients for the effect of weight on CL/F and V1/F, respectively; the ηs represented the inter-subject random effect of CL/F and V1, and κ was the inter-occasion random effect of CL/F.

At the backward elimination step, the full model was tested against a reduced model by removing the weight covariate from either the CL/F or the V1/F model one at a time. Only the elimination of weight from CL/F resulted in a significant degradation of the model fit (ΔOFV = +10, $P < 0.005$). Thus, weight was kept as the covariate only for CL/F in the final model. The final model adequately described the majority of the observed serum concentration, as demonstrated by the PRED-DV and IPRE-DV plots (Fig. 2). There was no identifiable major pattern in the distribution of weighted residuals (the PRED-WRES plot, Fig. 3). Table 3 summarizes the final population pharmacokinetic parameter estimates and their precision. For a patient with a median body weight (73 kg), CL/F and Vss/F (V1/F + V2/F) were estimated to be 34.9 l/h and 50.7 l, respectively. All CVs of ISV were low to moderate (19.0–24.9%) for pharmacokinetic parameters except V1/F (81.4%). The IOV of CL/F and the residual variability were 28.6 and 42.2%, respectively. The fixed-effect parameters, IOV, and residual error variance parameters were precisely estimated with standard errors (%SE) ranging from 8 to 31%. Standard errors (%SE) of ISV parameters were estimated with lower precision (29–76%), but these standard error terms are generally not well estimated so should be interpreted with caution.

Model evaluation

The final model obtained with the original dataset was subjected to a nonparametric bootstrap analysis. As shown in Table 3, the mean parameter estimates obtained from the bootstrap process, 1,000 runs, were consistent with the final model estimates previously obtained with the original dataset. Moreover, the bootstrap procedure provided estimates of accuracy of the population parameters as well as of nonparametric statistics of the estimates. Employing the visual predictive check method, as shown in Fig. 4, more than 90% observed concentrations fell within the 90% predicted interval, and there was a good agreement between distribution of observed and simulated steady state CP-724,714 concentrations at 250 mg BID. There was a trend for the model to under-predict the median and 75th percentile of the observed steady state Cmax and AUC by ~11–17%, as shown in Fig. 5.

Discussions

The population analysis approach is being applied more frequently in early drug development [13, 23–25], which permits observational data obtained from patients during clinical trials to be used to assess the

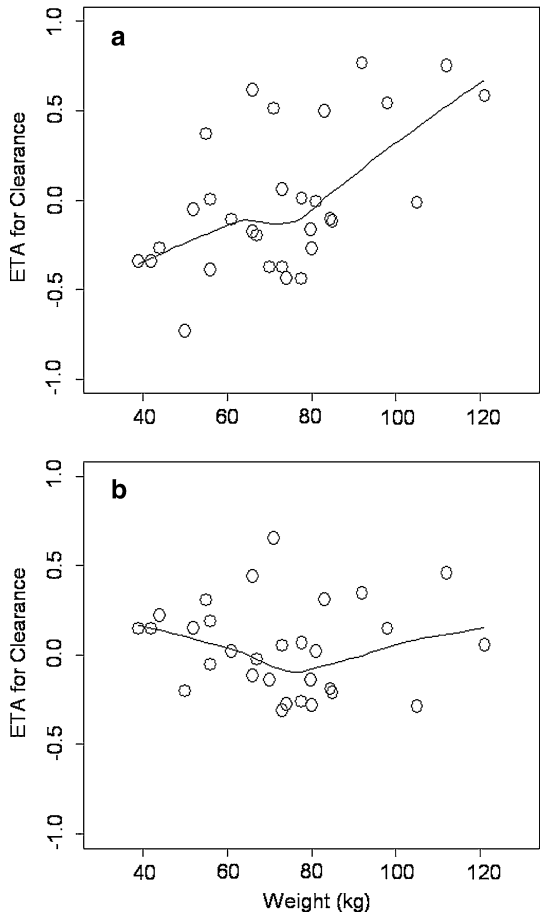

Fig. 1 Random effect (ETA) for apparent oral clearance (CL/F) versus body weight (WT) obtained from the fitting before (**a**) and after (**b**) the inclusion of WT as a covariate of clearance. *Solid lines* indicate the loess fit

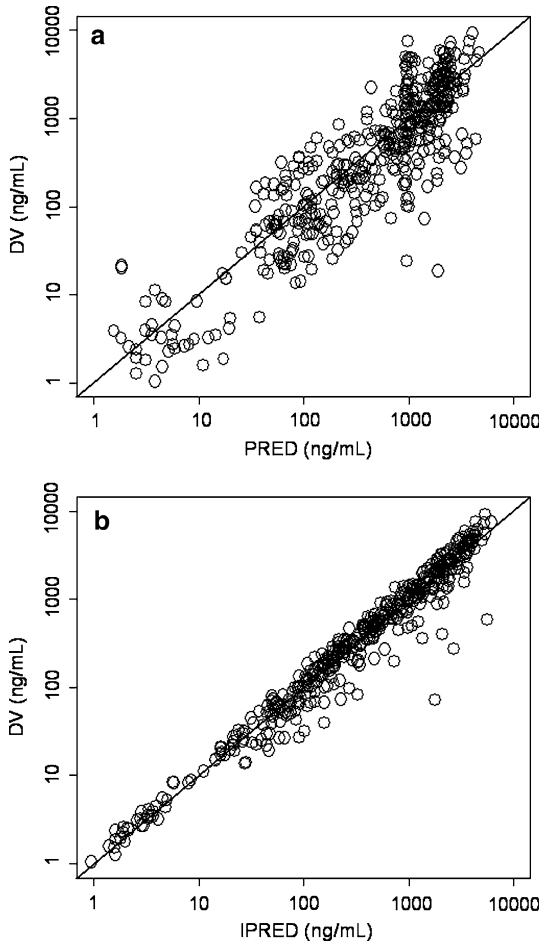

Fig. 2 Observed serum concentrations (DV) versus the population prediction (PRED: **a**) and individual predictions (IPRED: **b**) of CP-724,714. The *solid lines* indicate the lines of identity. The data are plotted on a log scale for ease of visualization

pharmacokinetic/pharmacodynamic (PK/PD) variability in patient populations, and find those covariates associated with significant changes in PK, and relate the PK parameters to clinical outcome [26]. This approach has been integrated into the clinical development plan of CP-724,714, an anti-tumor agent under investigation. To design and implement limited sampling strategies for future trials, it was necessary to characterize CP-724,714 pharmacokinetics through population pharmacokinetic modeling, e.g., nonlinear mixed-effect modeling (NONMEM) and iterative two-stage approach (IT2S) [27]. To this end, a population analysis was conducted using the Phase 1 trial data, and covariates predictive of the inter-patient variability of CP-724,714 CL/F were preliminarily examined. We conducted this analysis using nonlinear mixed-effect modeling, and our analysis revealed that the phase 1 pharmacokinetic data were best described by the 2-compartment distribution model with 1st order oral

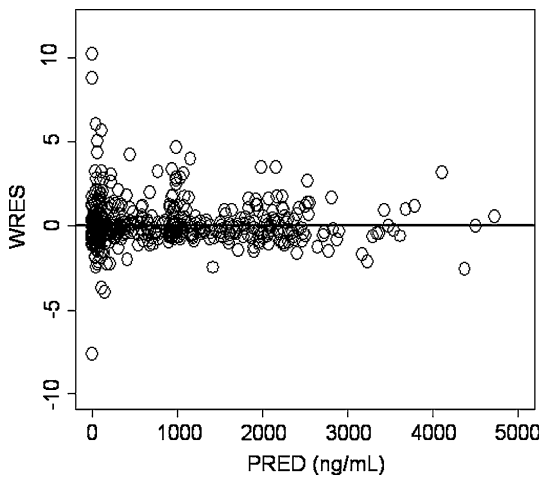

Fig. 3 Weighted residuals (WRES) versus the population predicted serum concentrations (PRED) of CP-724,714. The *dotted line* indicates the loess fit

Table 3 Population pharmacokinetic parameters of CP–724,714 and bootstrap validation

Parameter	Final model original dataset Mean (% SE)	Bootstrap[a] Mean (95% CI)
Structure model		
CL/F (l/h)	34.9 (8)	35.0 (29.8, 41.2)
θ_{WT}	0.742 (31)	0.773 (0.352, 1.26)
V1/F (l)	27.1 (23)	31.3 (18.6, 50.4)
V2/F (l)	23.6 (14)	23.4 (16.3, 30.3)
Q/F (l/h)	5.32 (20)	5.33 (3.34, 7.21)
ka (/h)	0.724 (12)	0.803 (0.611, 1.34)
Statistical model		
ω (CL/F, %)	24.9 (55)	23.9 (7.30, 39.0)
κ (CL/F, %)	28.6 (29)	27.6 (20.0, 35.5)
ω (V1/F, %)	81.4 (42)	67.7 (16.1, 108)
ω (V2/F, %)	21.7 (63)	21.4 (4.70, 36.5)
ω (ka, %)	19.0 (76)	15.6 (0.000, 40.2)
σ (%)	17.8 (12)	17.9 (0.140, 0.221)

SE standard error; 95% CI 2.5–97.5 percentiles; CL/F, Q/F oral elimination and intercompartmental clearance; V1/F, V2/F apparent central and peripheral distribution volumes; ka 1st-order oral absorption rate constant; θ_{WT} weight covariate for CL/F; ω interpatient variability; κ interoccasion variability; σ residual variability

[a] Data from 1,000 bootstrap analyses

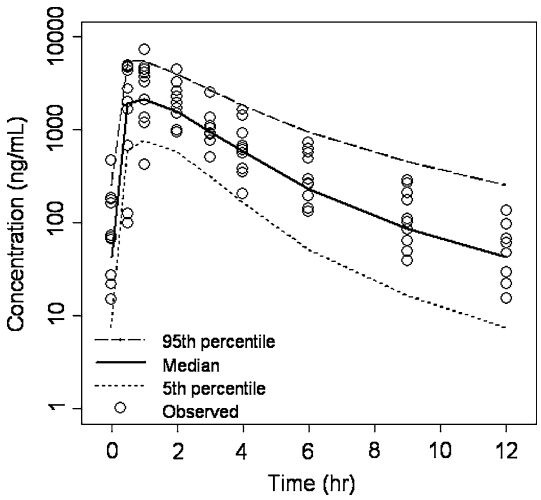

Fig. 4 Simulated (median, 5th, and 95th percentiles: n = 1,000 simulations for 15 patients) steady state serum concentrations of CP-724,714 versus observed values (n = 15 patients) at 250 mg BID

absorption and 1st order elimination from the central compartment, as demonstrated by goodness-of-fit diagnostic plots, OFV values, success of the covariance step, and acceptable precision in the parameter estimates.

In this population analysis, the effect of covariates on the pharmacokinetic parameters of CP-724,714 was assessed using the step-wise forward inclusion and backward elimination method. It was revealed that the inclusion of the treatment period and/or total daily dose as fixed effects of CL/F did not improve the fit; nor did they substantially affect the OFV or estimates of ISV, or IOV in CL/F. These findings agreed with what was previously understood from the traditional NCA analysis, i.e., there was no evidence of dose-dependent and/or time-dependent pharmacokinetics of CP-724,714 in the Phase 1 trial [10].

It was also revealed (Fig. 1) that, when WT was not included as a covariate of CL/F, the post hoc Bayesian-estimated ISV in CL/F appeared to increase with WT; however, after the inclusion of WT as a covariate of CL, it diminished the correlation between the ISV in CL/F and WT, and reduced the ISV in CL/F. The WT covariate for CL/F (θ_{WT}) was precisely estimated (Table 3) from the bootstrap (n = 1000); the typical value (95% CI) of θ_{WT} was 0.773 (0.352–1.26). CL/F increased with increasing WT by 3.4% for each kilogram of body weight. Table 4 summarizes the effects of changes in WT. Varying WT within the extreme values as seen in the Phase 1 trial (39–121 kg) would result in a –37 to +45% variation of CL/F around the median (34.9 l/h), and a +59 to –31% variation of the steady state AUC0-12 h around the median (7200 h*ng/ml). Thus, based on the popPK analysis, WT appeared to be a covariate of CL/F. This agreed with the previous finding from a correlation analysis of the NCA-estimated CL/F and weight, i.e., a greater CL/F appeared to be correlated with a larger WT [10]. However, due to the small sample size of the Phase 1 trial (n = 30), and the potential covariate selection bias of the step-wise method used in the covariate model building [28], the clinical importance of weight as a covariate of CL/F needs to be further assessed in future trials.

In this analysis, the effect of age and liver metastasis on CL/F was also assessed. The failure to observe an effect of these covariates might be related to the characteristics of patients enrolled in the trial. For example, in the study patient population, the age of the majority of patients (between 10 and 90% percentiles) were between 39 and 61 years; only two patients were older than 65 years. The narrow range of age plus few patients older than 65 years may have confounded the effect of age on CL. Regarding the status of liver metastasis, although 13 out of 30 patients had liver metastasis in the study, only patients with adequate liver function (i.e., bilirubin < 1.5 mg/dl, and liver transaminases and alkaline phosphatase not greater than grade 2 by CTC version 2.0) were enrolled in this study. Patients with liver metastasis and abnormal liver function were excluded from this study. Since CP-724,714 appeared to be mainly metabolized by CYP3A (suggested by in vitro drug metabolism data), it

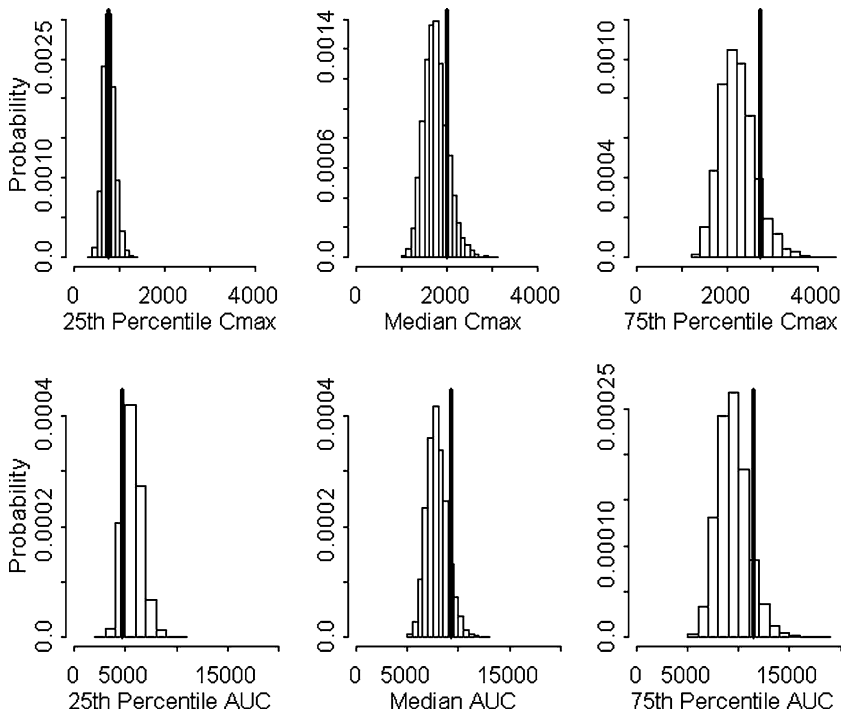

Fig. 5 Steady state Cmax and AUC (0–12 h) at 250 mg BID: observed percentiles (*vertical lines*) and distributions of percentiles from 1,000 simulated trials (*histograms*)

Table 4 Final population pharmacokinetic model

Covariate	Median	Change in weight (observed value)		Effect on CL/F		Effect on AUC_{0-12h}	
		Min.	Max.	Min.	Max.	Min.	Max.
Weight	73 kg	–47% (39 kg)	+66% (121 kg)	–37%	+45%	+59%	–31%

Weight effects (minimal and maximal observed weight in this population) on CL/F and steady-state systemic exposure (AUC_{0-12}) of CP–724,714 when administered at 250 mg BID

remains unknown if the metabolism and CL/F of CP-724,714 is compromised in these patients. In future trials, the effect of age and the presence of liver metastasis need to be further assessed when patients with a broad age range (especially age >65 years) and less restricted liver function requirement are allowed to participate in the trials.

The popPK analysis also revealed that there existed inter-occasion variability (IOV) in the apparent oral CL/F, and inclusion of IOV reduced OFV and more importantly the inter-subject variability in CL/F, as shown in Table 2. However, this finding was not unexpected. CP-724,714 was a basic compound with pH-dependent aqueous solubility that varied from >7.9 mg/ml at pH 1.0 to 0.003 mg/ml at pH 7.0. In the Phase 1 trial, CP-724,714 was supplied as 25 and 100 mg tablets, and administered orally on an empty stomach, i.e., 1 h before or 2 h after food intake (~500 calories). From day to day, the acidity in the GI tract may vary, as a result of various factors, e.g., potential differences in the type and quantity of food/drink consumed, and the time elapse since the last meal or before the next meal. Consequently, the aqueous solubility and fraction of absorption of CP-724,714 may vary from day to day. The IOV estimated from the population pharmacokinetic analysis was moderate (28.6%). However, due to the small number of patients with each patient's PK data from only two occasions in this Phase 1 trial, the IOV in CL/F and its clinical relevance remains to be examined when more clinical data are available.

The final population PK model was evaluated by nonparametric bootstrap and simulation. Bootstrap results (Table 3) suggested that model structural parameters were well estimated based on the 95% confidence interval (CI) associated with these parameters. The 95% CI of variability estimates was wider, which were not unexpected, given that there were only data from 30 patients in the analysis. The simulation suggested that the model in general adequately predicted the variability (90% CI) of the observed steady-state concentration-time data at 250 mg BID, the recommended

Phase 2 dose (RP2D). The model prediction of the 5th, 50th, and 95th percentiles of the steady state Cmax and AUC_{0-12h} were within 20% error from observed values at the RP2D. The bias in the simulation may be due to biased estimation of variance parameters and/or limited data during the absorption phase. However, the clinical relevance of the <20% prediction error relative to the large variability of Cmax (CV: 53%) and AUC_{0-12h} (CV: 51%) from the Phase 1 study at 250 mg BID is still unknown and needs to be further understood after more clinical data become available.

In summary, a 1st order oral absorption 2-compartment distribution population PK model was developed for CP-724,714. The modeling results revealed that CL/F varied randomly between occasions (IOV: 28.6% [standard error = 29%]), and weight appeared to be a covariate that reduced the ISV in CL/F by approximately 20% and improved model performance. These covariate findings need to be further investigated in future trials. The model evaluation suggested that model parameters were estimated with adequate precision, and there was a good agreement between simulated and observed concentrations of CP-724,714 at 250 mg BID, the RP2D. The proposed popPK model may be used to evaluate PK sampling strategies for future Phase 2/3 trials.

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