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ORIGINAL ARTICLE

Pharmacokinetics of a HER2 tyrosine kinase inhibitor CP-724,714 in patients with advanced malignant HER2 positive solid tumors: correlations with clinical characteristics and safety

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Abstract
Purpose CP-724,714 is an orally available, small molecule, potent HER-2 tyrosine kinase inhibitor under development for the treatment of advanced HER2-overexpressing cancers. In this study, the influence of baseline clinical characteristics and pathophysiological variables on the pharmacokinetics (PK) of CP-724,714, and the correlation between PK exposure and safety were examined in patients treated in the First-in-Human trial. PK and safety were also simulated for a Phase 2 trial at the recommended Phase 2 dose (RP2D) to assess if the simulated PK exposures of CP-724,714 covered the preclinically predicted efficacious concentrations, and if the predicted incidence of hepatic toxicities (≥CTC grade 3) was acceptable.
Methods Patients ($n = 30$) with advanced malignant HER2 positive solid tumors were enrolled in this open label dose-escalation study, and treated with daily oral dosing of CP-724,714 in 21-day cycles at the following dose levels: 250 mg QD, 250 mg BID, 400 mg BID, and 250 mg TID. PK parameter values were estimated using noncompartmental techniques. PK exposure parameters were correlated with the baseline pathophysiological variables, clinical characteristics, and safety. The simulations of PK exposures and the incidence of ≥grade 3 liver toxicity at the recommended Phase 2 dose were performed by nonparametric bootstrap ($n = 1{,}000$).
Results C_{max} and AUC increased in an approximate dose proportional manner. The terminal $t_{1/2}$ was approximately 4.5 h, and was constant across the dose range from 250 to 400 mg. There was some accumulation with BID and TID dosing with a mean AUC accumulation ratio $\sim$1.2–1.5, consistent with the $t_{1/2}$. Inter-patient variability in PK parameters was 31–65%, resulting in a considerable overlap of systemic exposure parameters (C_{max} and AUC) at higher doses (i.e., 250 mg TID and 400 mg BID), as expected for the narrow dose range. Significant correlations were observed for body size and oral clearance (CL/F) ($r = 0.574$, $P = 0.001$) and oral steady-state volume of distribution (V_{dss}/F) ($r = 0.669$, $P = 0.0001$). The most frequently encountered toxicities were elevated ALT and AST, hyperbilirubinemia, rash, asthenia, and nausea/vomiting (N/V).

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The steady-state AUC0-24 h was significantly correlated with the elevation of total bilirubin ($r = 0.670$, $P = 0.001$), ALT ($r = 0.548$, $P = 0.002$), and AST ($r = 0.461$, $P = 0.010$). The simulation of the Phase 2 trial at 250 mg BID predicted that the 95% confidence interval of the simulated mean concentrations of CP-724,714 were above the preclinically predicted efficacious concentrations throughout the majority of the dosing interval. The probability for ≥33% incidence of grade 3 or greater elevations of liver function test (LFT) was low (1.1%).

Conclusions CP-724,714 demonstrates linear single-dose and multiple-dose PK. Both CL/*F* and V_{dss}/F correlate with body size. Elevations of ALT, AST, and total bilirubin positively correlate with the steady-state AUC0-24 h. The Phase 2 trial simulation suggests that CP-724,714 will be well tolerated and that PK exposures will exceed the preclinically predicted efficacious level at the recommended Phase 2 dose (250 mg BID), supporting further evaluation of CP-724,714 in the Phase 2 trial.

Keywords HER2 tyrosine kinase inhibitor · Pharmacokinetics · Pharmacodynamics

Introduction

A major milestone in breast cancer treatment has been targeting the erbB2 (HER2) receptor, a member of the epidermal growth factor family. 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]. Trastuzumab, a monoclonal antibody specific for HER2, has been approved to treat HER-2 overexpressing breast cancer [3]. In tumors overexpressing HER2, 35% of the patients responded when trastuzumab 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 trastuzumab was combined with current standard chemotherapy [5–7]. However, while adding trastuzumab to chemotherapy provides clinical benefit, the risks on the heart should be considered particularly in association with anthracyclines [8]. Unfortunately, in the metastatic setting, the majority of patients develop trastuzumab resistance.

One strategy for the treatment of trastuzumab-refractory HER2 overexpressing breast cancer is the use of ErbB-targeted tyrosine kinase inhibitors. Small molecule inhibitors of HER2 and/or EGFR (HER1), such as erlotinib, gefitinib, lapatinib, and the pan-erbB receptor tyrosine kinase inhibitor, CI-1033 have demonstrated preclinical and clinical activity against a variety of malignancies [9–14]. Lapatinib, a tyrosine kinase inhibitor targeting EGFR and HER2, has demonstrated improved time to progression when combined with capecitabine compared to capecitabine alone in trastuzumab-refractory HER2 overexpressing breast cancer [15]. Small-molecule orally administered kinase inhibitors provide the convenience of the oral administration over the intravenous trastuzumab, and may avoid toxicities associated with antibody-based treatment, particularly cardiotoxicity [8]. In addition, small molecule tyrosine kinase inhibitors are easier to manufacture than antibodies, and may be more cost effective than trastuzumab.

CP-724,714, 2-Methoxy-*N*-[(2E)-3-[4-[[3-methyl-4-[6-methyl-3-pyridinyl)oxy] phenyl]amino]-6-quinazolinyl]-2-propenyl]-acetamide, butanedioic acid ($C_{27}H_{27}N_5O_3 \cdot 1.5\ C_4H_6O_4$), 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 murine xenografts of human adenocarcinomas of breast (BT-474 and MDA-MB-453), pancreas (Panc-1), lung (Calu-3), and ovary (SK-OV-3) [16]. Predominant preclinical toxicities in rodents and dogs were gastrointestinal and hepatic toxicities. Hepatic toxicities included hepatocellular necrosis, Kupffer cell hypertrophy, increased serum ALT, AST, gammaglutamyl transpeptidase (GGT), and total bilirubin. No cardiomyopathy was observed in preclinical studies. In the rat, dog, and monkey, CP-724,714 appeared to be mainly eliminated by the CYP450-mediated metabolism, with less than 10% of unchanged drug excreted in the urine and bile. In vitro human hepatic microsomal studies suggested that CP-724,714 was mainly metabolized by CYP3A. The protein binding in the plasma of mouse, rat, dog, monkey, and humans ranged from 97 to 99% (Investigator's Brochure).

CP-724,714 was investigated in the first in human trial (FIH) as daily oral dosing in repeated 21-day cycles to determine its safety, tolerability, and pharmacokinetics (PK) in patients with advanced solid tumor malignancies that overexpress HER2. The overall safety, tolerability, and preliminary PK findings of this trial were reported previously [17, 18]. The most frequently encountered toxicities in humans were increased AST, increased ALT, hyperbilirubinemia, nausea, rash, and asthenia, which were reversible after discontinuation of the drug. No cardiomyopathy was detected using multigated nuclide analysis scans (MUGA) or echocardiography (ECHO). Similar adverse effects have been observed with other small molecule HER2 or EGFR inhibitors, such as erlotinib, gefitinib and even the geldanamycin-analogues that target HER2 by inhibiting heat shock proteins (HSP90) [19]. Hepatic toxicities are uncommon in patients treated with trastuzumab [4]. These findings collectively suggest that the safety profile of CP-724,714 is more similar to those seen with

other small molecule HER2 or EGFR kinase inhibitors, rather than with the monoclonal antibody trastuzumab.

The maximal tolerated dose (MTD) of CP-724,714 was 250 mg TID based on the cycle 1 safety data. However, at this dose, 33% of the patients developed grade 3 hepatotoxicities and 66% patients had at least grade 1 hepatic toxicity (change in liver aminotransferases) in subsequent cycles. Hence 250 mg BID was defined as the recommended Phase 2 dose (RP2D).

A primary objective of this analysis was to evaluate relationships between PK, clinical and pathophysiological characteristics of patients, and the severity of most frequently observed drug-related toxicities. The intent was to generate hypotheses regarding covariates influencing PK variability, the relationship between CP-724,714 exposure and safety, and clinical and pathological characteristics rendering patients susceptible to adverse events, which can be tested in future clinical trials. For this reason, P value <0.05 without adjustment for multiple comparisons was selected as the significance level for statistical analyses in this report, which was considered indicative of a potentially clinically relevant correlation. The second objective was, via a Phase 2 trial simulation, to assess whether the simulated PK exposures of CP-724,714 exceeded the preclinically predicted efficacious concentration, and the simulated incidence of grade 3 or greater hepatic toxicities was acceptable at the RP2D.

Methods

Patient and study design

The detailed patient eligibility and study design have been described elsewhere [18]. Patients with advanced solid tumor malignancies with HER2 overexpression 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 trastuzumab. Patients were required to have adequate bone marrow, renal, liver, cardiac function, and 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 were obtained before the beginning of the treatment.

This was an open-label, multi-center First-in-Humans (FIH) Phase I trial in which all eligible subjects received CP-724,714 as daily oral doses on empty stomach for repeated 21-day cycles for a pre-defined maximal duration of 17 cycles (51 weeks). The starting dose level was 250 mg QD. In subsequent dose cohorts, 250 mg BID, 400 mg BID, and 250 mg TID were evaluated. Patients received the same daily dose until disease progression, or un-acceptable toxicities. No intra-patient dose escalation was allowed. Patient compliance was assessed by patient diaries and tablet counts on returned CP-724,714 bottles.

Safety was evaluated at baseline and weekly in the first cycle and then every 3 weeks during the treatment, including a physical exam, vital signs, 12-lead ECG, complete blood cell count, hepatic (aminotransferases and bilirubin) and renal function assessment, coagulation profiles, and urine analysis. Cardiac function was assessed after every two cycles by either MUGA or ECHO. Laboratory results were reported as continuous variables. Adverse events (AE) and laboratory changes were graded according to National Cancer Institute's Common Terminology Criteria version 2.0 (CTC).

Pharmacokinetic sample collection and analysis

For each patient, serum pharmacokinetics of CP-724,714 was estimated following single oral dose of CP-724,714 on Day 1 of Cycle 1 (C1D1) (with 2-day washout) and after daily oral dosing for 3 weeks on Day 1 of Cycle 2 (C2D1). For the single dose PK assessment on C1D1, 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 multiple dose PK assessment on C2D1, 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 samples, which were then stored at temperature ≤–20°C until analysis.

The analytical assay of the total serum concentration of CP-724,714 has been described in detail previously [18]. Briefly, serum samples were analyzed by liquid chromatography (LC)-tandem mass spectrometry (MS/MS). The assay was linear from 1.0 to 500 ng/ml. Quality control (QC) samples were prepared at 3.00, 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 3.00 and 400 ng/mL by preparing QCs; the mean assayed value of high and low QC samples was within 15% of their nominal values.

Pharmacokinetic data analysis

Serum concentration-time data of CP-724,714 were analyzed by standard non-compartmental methods [20] using WinNonlin V.3.2 (Pharsight®, Mountain View, CA). The following PK parameters were estimated based on the C1D1 data: the maximum observed serum concentration ($C_{max_{SD}}$), the time of the first occurrence of C_{max} ($T_{max_{SD}}$), apparent terminal half-life ($t_{1/2}$), area under the serum concentration–time curve from time 0 to the last time with a quantifiable concentration ($AUC_{last_{SD}}$), area under the serum concentration–time curve over the dosing interval (tau) (AUC_{tauSD}), area under the serum concentration–time curve from time 0 to infinity (AUC_{inf}), oral clearance (CL/F), oral volume of distribution at steady state (V_{dss}/F). Also estimated were PK parameters based on the C2D1 data as follows: $C_{max_{MD}}$, $T_{max_{MD}}$, $AUC_{last_{MD}}$, $AUC_{tau_{MD}}$, area under the serum concentration–time curve from time 0 to 24 h (steady-state AUC0-24 h), and accumulation ratio (R_o). The subscripts (i.e., SD and MD) of aforementioned parameters denote C1D1 and C2D1, respectively. The cumulative area under the curve (AUC_{cum}) for each patient was calculated as the product of AUC_{inf} multiplied by the total number of doses ingested by the patient, based on the dosing history, from the start of treatment to the time of the highest-severity of the AE of interest. The cumulative dose ($DOSE_{cum}$) for each patient was calculated as the product of the single dose amount multiplied by the total number of doses ingested by the patient, based on the dosing history, from the start of treatment to the time of the highest-severity of the AE of interest. The AUC was normalized to 1 mg by calculating the dose-normalized AUC (AUC/dose) via dividing AUC_{inf} by the dose after the single dose administration on C1D1, and dividing AUC_{tau} by the dose after multiple dose administration on C2D1, respectively, for each patient.

As the initial assessment revealed a log-normal distribution of PK variables, parametric statistical tests of PK variables were performed after the logarithmic transformation of these data. Comparisons of AUC/dose grouped by the dose level(s) were assessed by analysis of variance (ANOVA). Comparisons of AUC/dose between C1D1 and C2D1 were evaluated by the paired t test. All tests were two-sided, and the significance level was set at P value <0.05.

PK correlations with patient characteristics

Correlations of PK parameters (i.e., CL/F and V_{dss}/F) with baseline patient characteristics were assessed. The effect of categorical baseline patient variables on PK parameters was assessed by the two-tailed independent t test. The categorical variables evaluated were: presence of liver metastasis, presence of elevated liver-associated biochemical lab results (ALT, AST, and total bilirubin), ECOG performance status, and extensiveness of prior chemotherapies (>5 prior chemotherapies). Pearson correlation coefficients (r) were calculated to evaluate the relationship(s) between PK parameters and continuous baseline variables. The continuous variables evaluated were age, height, weight, and body surface area (BSA).

Parametric statistical tests of correlations were performed after logarithmic transformation of the data. To be considered indicative of a potential clinically relevant correlation with PK parameters, a P value <0.05 was required for categorical baseline patient variables, while a P value <0.05 and $|r| \geq 0.4$ were required for continuous baseline patient variables.

Given the intent of the analysis was to generate hypotheses, P value <0.05 without adjustment for multiple comparisons was selected as the significance level for all statistical analyses, allowing for conservative screening of potential clinically relevant correlations that can be tested further.

PK correlations with safety

Relationships between PK parameters and safety endpoints were assessed to evaluate whether the severity of a given adverse effect (AE) was associated with PK exposures after the single dose or multiple dose administration. Safety endpoints examined were: increased AST, increased ALT, hyperbilirubinemia, nausea, rash, and asthenia. PK parameters included in this analysis were $C_{max_{SD}}$ and AUC_{inf} of C1D1, $C_{max_{MD}}$ and steady-state AUC0-24 h of C2D1, and cumulative AUC (AUC_{cum}) and dose ($DOSE_{cum}$).

There were eight patients who had PK samples from C1D1 but not C2D1, among whom three patients had grade 3 ALT and/or AST elevations. As the previous population PK analysis of the same data suggested a low intra-patient variability (CV%: 18%) [21], $C_{max_{MD}}$ and $AUC_{tau_{MD}}$ of C2D1 were imputed for these eight patients by multiplying $C_{max_{SD}}$ and $AUC_{tau_{SD}}$ of C1D1 by the mean accumulation ratio (R_o) at the respective dose level. Then steady-state AUC0-24 h of C2D1 were imputed from the predicted $AUC_{tau_{MD}}$.

For nausea, rash, and asthenia, correlations between the highest severity of each of these AEs (in CTCAE grades) and PK parameters were performed using the Spearman correlation test. A P value <0.05 without adjustment for multiple comparisons was considered indicative of a potentially clinically relevant correlation.

For ALT, AST, and total bilirubin, their correlations with PK parameters were evaluated in two steps, as described below. First, the maximal elevation of ALT, AST, and total bilirubin above the respective upper limit of the normal (ULN) was calculated as:

$$\text{Multiple}_{\text{LFT}} = \frac{\text{LFT}_{\text{Max}}}{\text{LFT}_{\text{ULN}}}, \quad (1)$$

where LFT_{Max} represented the measured maximum value of ALT, AST, and total bilirubin from each patient during the study; LFT_{ULN} represented the ULN of ALT, AST, or total bilirubin; $\text{Multiple}_{\text{LFT}}$ represented the maximum fold of elevation of ALT, AST, or total bilirubin above the respective ULN. Pearson correlations between PK parameters and each of aforementioned $\text{Multiple}_{\text{LFT}}$'s were calculated after logarithmic transformation of the data.

Then at the 2nd step, the $\text{Multiple}_{\text{LFT}}$ was scaled according to the following equations:

$$\text{Multiple}_{\text{LFT\_scaled}} = \frac{\text{Multiple}_{\text{LFT}}}{5} \quad (2)$$

for ALT and AST, and

$$\text{Multiple}_{\text{LFT\_scaled}} = \frac{\text{Multiple}_{\text{LFT}}}{3} \quad (3)$$

for total bilirubin. A value of $\text{Multiple}_{\text{LFT\_scaled}}$ greater than 1 represented a grade 3 or greater elevation of ALT, AST, or total bilirubin. A scatter plot of steady-state AUC0-24 h and the highest score of $\text{Multiple}_{\text{LFT\_scaled}}$ from each patient was plotted. The highest score of $\text{Multiple}_{\text{LFT\_scaled}}$ among ALT, AST, and total bilirubin from a patient represented the most severe elevated liver function test (LFT) for that patient. The Pearson correlation between the steady-state AUC0-24 h and the highest score of $\text{Multiple}_{\text{LFT\_scaled}}$ from individual patients was also calculated after logarithmic transformation of the data to assess the relationship(s) between the most severe LFT elevation and steady-state AUC0-24 h.

For the assessment of relationships between LFT elevations and PK parameters, the suggestion of a potentially clinically relevant correlation was indicated by $|r| \geq 0.4$, and the P value of <0.05 without adjustment for multiple comparisons.

Correlation of most severe LFT elevations with patient characteristics

Relationships between the most severe LFT elevation (or the highest $\text{Multiple}_{\text{LFT\_scaled}}$ score) of each patient and corresponding patient characteristics were evaluated by Chi-square test or Fisher's exact test as appropriate. The most severe LFT elevation was categorized as the presence or absence of ≥grade 3 elevation of ALT, AST, or bilirbuin, and patient characteristics included the presence of baseline liver metastasis, abnormal baseline LFTs (≥grade 1 elevation of ALT, AST, and/or total bilirubin), an increase of liver metastasis during treatment, and concomitant administration of acetaminophen. $P < 0.05$ was considered indicative of a potentially clinically relevant correlation.

Prediction of PK and safety of CP-724,714 at the recommended Phase 2 dose

Based on safety and PK findings from the Phase 1 trial, the recommended Phase 2 dose (RP2D) was selected as 250 mg BID [18]. A limited Phase 2 trial was planned to assess the efficacy of CP-724,714 orally administered as 250 mg BID in thirty patients with HER-2 over-expressing metastatic breast cancer previously untreated with a HER2 inhibitor. A Phase 2 trial simulation for PK exposures and the incidence of ≥grade 3 LFT elevations at the RP2D was performed by nonparametric bootstrap to assess if simulated mean PK exposures were above the preclinically predicted efficacious concentrations, and the simulated incidence of ≥grade 3 LFT elevations were acceptable (<33.3%).

For the PK simulation, one thousand trials were simulated by nonparametrically bootstrapping one thousand times the patient PK data (including serum concentration-time data and dosing history) from 250 mg BID (n = 15); in each bootstrap, patient PK data were randomly sampled with replacement using each individual patient's data as the sampling unit, up to a total number of 30 patients. Then the mean of the resulting simulated serum concentrations at each time point was calculated for each trial, and a visual inspection of the distribution of the mean concentration–time profiles from the 1,000 trials was performed as described below: the 5th, 50th (median), and 95th percentiles of the mean serum concentrations at each time point were calculated and plotted over time, and overlaid with the preclinically projected efficacious serum concentrations. The preclinically projected efficacious serum concentrations were predicted from the mean serum concentrations in murine xenograft models of HER-2 over-expressing human breast adenocarcinomas (BT-474 and MDA-MB-453) when >50% tumor growth inhibition (TGI) was observed [Investigator's Brochure].

For the simulation of the incidence of ≥grade 3 LFT elevations, one thousand trials were simulated by nonparametrically bootstrapping one thousand times the most severe LFT elevation data from patients (n = 28) whose steady-state AUC0-24 h on Day 1 of Cycle 2 was equal to or less than the upper end of steady-state AUC0-24 h (i.e., 38,160 h ng/ml) on Day 1 of Cycle 2 observed in patients treated at 250 mg BID. In each bootstrap, patient LFT data

were randomly sampled with replacement using each individual patient's data as the sampling unit, up to a total number of 30 patients. Then the number of ≥grade 3 LFT elevations was counted for each simulated trial, and the probability of having varying incidence of ≥grade 3 LFT elevations was calculated based on the simulated 1,000 trials.

In all aforementioned methods, S-Plus (version 6.2, Insightful, Seattle, WA) was used to generate graphs, and perform all statistical tests including ANOVA, *t* tests, correlation tests, and nonparametric bootstrap.

Results

Patient characteristics

A total of 30 patients were treated in the study. There were 4 patients treated at 250 mg QD, 15 patients at 250 mg BID, 6 patients at 250 mg TID, and 5 patients at 400 mg BID. Their characteristics are listed in Table 1. A typical individual was a 51-year old and 73 kg female breast cancer patient. Prior to the treatment, there were 13 patients (43%) with liver metastasis, 22 patients (74%) with normal LFT values (<1 × ULN), 16 patients (53%) receiving ≥6 prior chemotherapy regimens, and all patients with a ECOG score of 0 or 1. Fourteen patients (47%) received daily acetaminophen prior to and during the trial.

Table 1 Patient characteristics at baseline. Continuous data are given as mean (range), and categorical data as number of patients (percentage of the total population)

Characteristics		Value
Number of patients studied		30
Gender	Female	30 (100)
Cancer type	Breast	28 (93)
	Non-breast	2 (7)
ECOG PS	0	10 (33)
	1	20 (67)
Liver metastasis	Yes	13 (43)
	No	17 (57)
Liver function tests	Normal	22 (74)
	Grade 1	8 (26)
Number of prior chemotherapy regimens	1–5	14 (47)
	≥6	16 (53)
Number of concomitant acetaminophen treatment	Yes	14 (47)
	No	16 (53)
Age (years)		51.2 (37–71)
Weight (kg)		73.1 (39–121)
Height (cm)		162.7 (147–174)
BSA (m2)		1.80 (1.31–2.35)

Pharmacokinetics

The serum concentration–time profile of CP-724,714 was described by rapid oral absorption with a median T_{max} of approximately 1–2 h, followed by a bi-exponential decline post absorption with the terminal $t_{1/2}$ ranging from 4.31 to 4.80 h across the dose range from 250 to 400 mg, as illustrated in Fig. 1. Mean C_{max} and AUC of CP-724,714 increased with dose in an approximate dose proportional manner after single and multiple oral dosing; there was some accumulation of CP-724,714 in the BID and TID dosing regimens with a mean accumulation ratio ranging from 1.2 to 1.5 (Table 2); intra- and inter-patient variability in the PK exposure parameters was ∼20 and 31–65%, respectively. AUC/dose and C_{max}/dose were constant across dose levels following both single dose and multiple dose administration, as illustrated in Fig. 2. C_{max}/dose and AUC/dose were not different between dose levels following single dose (P value = 0.751 for C_{max}/dose, and 0.845 for AUC/dose) and multiple dose administration (P value = 0.412 for C_{max}/dose, and 0.460 for AUC/dose), as assessed by ANOVA. AUC_{inf} following single dosing on C1D1 was not different from AUC_{tau} following multiple dosing on C2D1 (P value = 0.141). Both steady-state AUC0-24 h and C_{max} after multiple dosing on C2D1 increased with dose from 250 mg QD to 250 mg TID, but were overlapped considerably with similar median values at 250 mg TID and 400 mg TID, as illustrated in Fig. 3.

PK correlations with patient characteristics

The relationships of baseline clinical, pathophysiological, and biochemical characteristics of patients to PK parameters (i.e., CL/F and V_{dss}/F) are shown in Table 3. Since

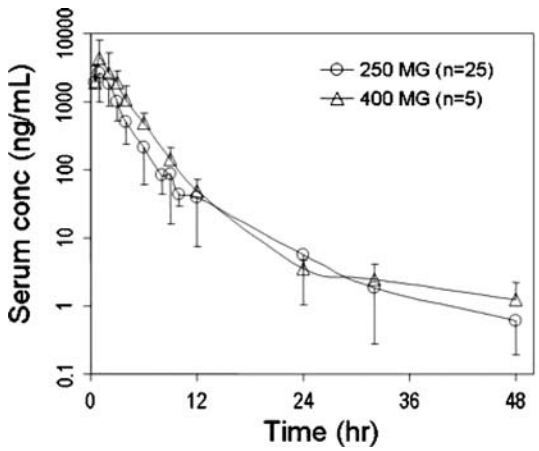

Fig. 1 Mean (±SE) CP-724,714 serum concentration–time profiles after single dose administration on Day 1 of Cycle 1 at 250 and 400 mg

Table 2 Mean (SD)[a] pharmacokinetic (PK) parameters of CP-724,714 in cancer patients following a single oral dose (Day 1 of Cycle 1) and continuous daily oral administration of CP-724,714 (Day 1 of Cycle 2)

Doses	Single dose PK parameters (Day 1 of Cycle 1)					Multiple dose PK parameters (Day 1 of Cycle 2)				
	No. Patients	$T_{max_{SD}}$[a] h	$C_{max_{SD}}$ ng/ml	$AUC_{last_{SD}}$ h ng/ml	$t_{1/2}$ h	Number of patients	$T_{max_{SD}}$[a] h	$C_{max_{MD}}$ ng/ml	$AUC_{last_{MD}}$ h ng/ml	R_o^b
250 mg QD	5	1.00 (0.5, 3.0)	3,990 (2,290)	7,720 (3,560)	4.80 (0.981)	4	1.25 (0.5, 3.0)	2,110 (646)	6,600 (2,520)	1.02 (0.46)
250 mg BID	15	1.00 (0.5, 3.0)	2,740 (1,360)	7,360 (3,140)	4.80 (1.42)	10	1.00 (1.0, 2.0)	3,660 (1,930)	9,300 (4,770)	1.18 (0.69)
250 mg TID	5	1.00 (0.5, 1.0)	3,110 (1,810)	6,460 (2,800)	4.31 (1.11)	5	1.00 (1.0, 2.0)	4,180 (2,250)	9,250 (5,270)	1.51 (0.71)
400 mg BID	5	1.00 (1.0, 3.0)	4,900 (3,170)	11,900 (6,520)	4.34 (1.27)	3	2.00 (1.0, 4.0)	4,940 (1,570)	14,900 (4,470)	1.47 (0.60)

[a] Median (range) for T_{max}

[b] $R_o = AUC_{tau_{MD}}/AUC_{tau_{SD}}$

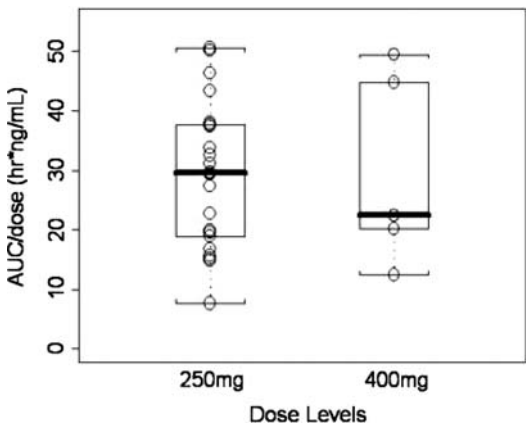

Fig. 2 Box-and-whisker plots of the dose-normalized AUC (AUC/dose) after single dose administration on Day 1 of Cycle 1 at 250 mg ($n = 25$) and 400 mg ($n = 5$). The AUC/dose on *Y* axis is normalized to 1 mg. Individual patients' dose-normalized AUCs are shown as *circles*. The *horizontal lines* inside the *boxes* represent the median, the *box edges* show the lower and upper quartiles, and the whiskers show the 5th and 95th percentiles

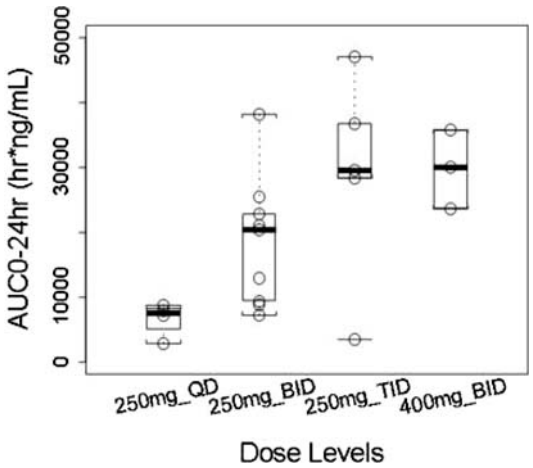

Fig. 3 Box-and-whisker plot of steady-state AUC0-24 h on Day 1 of Cycle 2 grouped by dose levels. The *horizontal lines* inside the *boxes* represent the median, the *box edges show* the lower and upper quartiles, and the whiskers show the 5th and 95th percentiles. Individual patients' observed steady-state AUC0-24 h's are given as *circles*

weight and BSA were highly correlated ($r = 0.98$, $P < 0.0001$), only results of weight were presented in the table. There were no significant differences in the mean CL/*F* and V_{dss}/F of patients when categorized according to liver function, the presence of hepatic metastasis, the number of prior chemotherapies, and the performance status. In addition, age was not correlated to CL/*F* or V_{dss}/F. However, body size (WT and BSA) was associated with CL/*F* ($r = 0.574$, $P = 0.001$) and V_{dss}/F ($r = 0.669$, $P = 0.0001$), as illustrated in Fig. 4. Height did not appear to be associated with CL/*F* ($r = 0.344$, $P = 0.068$), but seemed to be weakly correlated to V_{dss}/F ($r = 0.42$, $P = 0.023$).

Table 3 Statistical comparisons or Pearson's correlation of oral clearance (CL/*F*) and oral volume of distribution at the steady state (V_{dss}/F) between subpopulations or individual patients according to baseline clinical, demographic, pathological, or treatment-related factors

Variable	Number of patients	CL/*F*	*P* value[a]	V_{dss}/F	*P* value[a]
ECOG performance status					
0	10	36.7 ± 18.8	0.308	120 ± 80.8	0.243
1	20	40.4 ± 16.1		139 ± 78.6	
Serum liver function tests					
Normal	22	40.3 ± 17.7	0.276	128 ± 80.7	0.370
Grade 1	8	36.8 ± 15.3		143 ± 77.1	
Hepatic metastases					
+	13	39.4 ± 15.7	0.884	146 ± 78.2	0.103
–	17	38.9 ± 18.3		122 ± 79.4	
Number of prior chemotherapies					
1–5	14	36.6 ± 13.5	0.289	130 ± 77.1	0.289
>5	16	41.2 ± 19.2		146 ± 86.0	
Age		–0.009	0.965	–0.059	0.761
Height		0.344	0.068	0.421	0.023
Total body weight		0.574	0.001	0.669	0.0001

For variables in the upper four rows, the geometric mean ± SD are presented for CL/*F* (l/h) and V_{dss} (l); while in the bottom three rows, Pearson's correlation coefficients are presented; Pearson's correlation was calculated after the logarithmic transformation of variables from individual patients

[a] Two-tailed *t* test of the logarithmic-transformed data

PK correlations with safety

Relationships between PK parameters and most frequently encountered drug-related AEs are shown in Table 4. Elevated total bilirubin was correlated with steady-state AUC0-24 h ($r = 0.670$, $P = 0.0001$) and $C_{max_{MD}}$ ($r = 0.533$, $P = 0.002$) of C2D1. The increase of ALT was associated with steady-state AUC0-24 h ($r = 0.548$, $P = 0.002$) and $C_{max_{MD}}$ ($r = 0.42$, $P = 0.0481$) of C2D1. Also correlated were increased AST and steady-state AUC0-24 h ($r = 0.461$, $P = 0.010$), but not increased AST and $C_{max_{MD}}$ ($r = 0.288$, $P = 0.121$) of C2D1. The correlation between steady-state AUC0-24 h and elevated total bilirubin was illustrated graphically in Fig. 5. Other PK parameters, such as $C_{max_{SD}}$ and AUC_{inf} after the single dose administration and cumulative AUC (AUC_{cum}) or dose ($DOSE_{cum}$), did not seem to correlate to elevated ALT, AST, or total bilirubin.

The most severe LFT elevation from each patient, expressed as the highest score of $Multiple_{LFT\_scaled}$ among ALT, AST and total bilirubin, was also plotted versus the patient's observed or imputed (if missing) steady-state AUC0-24 h, as shown in Fig. 6. It was revealed that the majority of patients (26 out of 30 patients) followed a trend of increasing severity of elevated LFT with increasing steady-state AUC0-24 h, except for four patients. When excluding these four apparent outliers, the most severe LFT elevations appeared to be associated with steady-state AUC0-24 h ($r = 0.810$, $P < 0.0001$) and C_{maxMD} ($r = 0.572$, $P = 0.002$) of C2D1, but not with other PK exposure parameters.

The Spearman rank correlation test revealed no evidence of correlations between any PK exposure parameters and the severity of nausea, rash, or asthenia ($P > 0.1$).

Correlation of most severe LFT elevations with patient characteristics

Also evaluated were the correlation(s) between the most severe elevated LFT and patient characteristics, such as the baseline abnormality of liver functions and presence of liver metastasis, and the concomitant treatment of acetaminophen and an increase of liver metastasis during the trial. However, none of these patient characteristics appeared to correlate with the most severe elevated LFT according to the Fisher's exact test ($P > 0.1$).

Prediction of PK and hepatotoxicity of CP-724,714 at the recommended Phase 2 dose

The median and 97.5th and 2.5th percentiles of the simulated mean human serum concentration at each time point from the 1,000 trials were plotted against the time (Fig. 7), and overlaid with the preclinically projected mean

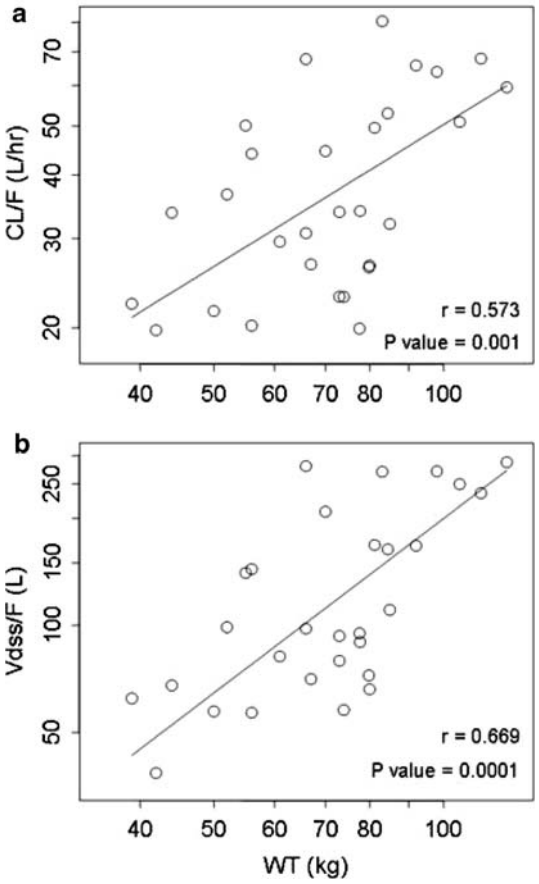

Fig. 4 Correlation between weight (WT) and CL/*F* (plot **a**), and between weight (WT) and V_{dss}/F (plot **b**). Each *symbol* represents data from an individual patient. *Sold lines* represent fitted lines calculated by the regression analysis. Also shown are correlation coefficients (*r*) and *P* values. Both axes are shown in the natural logarithmic scale

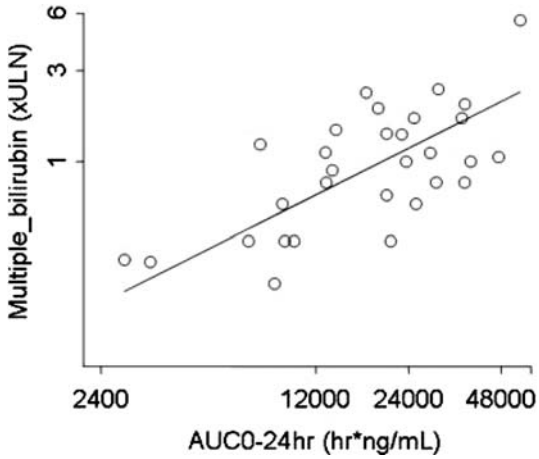

Fig. 5 Correlation between steady-state AUC0-24 h and the maximal fold elevation of total bilirubin (Multiple_bilirubin). Steady-state AUC0-24 h was from Day 1 of Cycle 2; the maximal fold of elevation was relative to the upper limit of the normal (*ULN*) of total bilirubin. Each *symbol* represents data from an individual patient. *Sold lines* represent fitted lines calculated by the regression analysis. Both axes are shown in the natural logarithmic scale

efficacious serum concentration–time profile based on xenograft mouse models of HER-2 over-expressing human breast adenocarcinomas. As indicated in Fig. 7, the 95% confidence interval of the simulated mean concentration of CP-724,714 at the RP2D are at or above the preclinically predicted efficacious concentrations over most of the dosing interval.

The Phase 2 trial simulation of hepatotoxicity at the RP2D is shown in Table 5, predicting that the probability of observing less than 26.7% of ≥grade 3 elevated LFT is 97.5%, and it would be rare (only a probability of 1.1%) to observe ≥33.3% of grade 3 or greater LFT elevations.

Discussions

This report describes the first comprehensive analysis of PK and correlation with clinical characteristics and safety of CP-724,714 based on the FIH trial results. The current non-compartmental analysis revealed that there was no evidence of either dose or time dependent PK of CP-724,714 and the PK intra-patient variability was low

Table 4 Pearson correlation (*P* value) of various pharmacokinetic (PK) parameters of CP-724,714 and the maximal elevation of liver function tests (*LFT*s) relative to the upper limit of the normal (*ULN*)

	r (P-value)					
	$C_{max_{SD}}$ ng/mL	AUC_{inf} h ng/ml	$C_{max_{MD}}$ ng/ml	AUC0-24 h h ng/ml	AUC_{cum} h ng/ml	$DOSE_{cum}$ mg
ALT	0.214 (0.235)	0.299 (0.197)	**0.420 (0. 0481)**	**0.548 (0.002)**	0.327 (0.078)	0.208 (0.269)
AST	0.282 (0.135)	0.264 (0.169)	0.288 (0.121)	**0.461 (0.010)**	0.266 (0.156)	0.107 (0.572)
Total bilirubin	0.275 (0.057)	0.278 (0.059)	**0.533 (0.002)**	**0.670 (0.0001)**	0.059 (0.757)	0.177 (0.349)

ALT alanine aminotranserase, *AST* aspartate aminotransferase, $C_{max_{SD}}$ the peak concentration after the single dose on Day 1 of Cycle 1, AUC_{inf} area under the curve from time zero to infinity after the single dose on Day 1 of Cycle 1, AUC0-24 h the steady-state AUC0-24 h on Day 1 of Cycle 2 after the multiple dose administration, $C_{max_{MD}}$ the steady-state peak concentration on Day 1 of Cycle 2 after the multiple dose administration, AUC_{cum} cumulative area under the curve based on individual patient's dosing history, $DOSE_{cum}$ the cumulative amount of drug received by each patient based on the dosing history. All PK and LFTs parameters were logarithmic-transformed before the Pearson correlation test

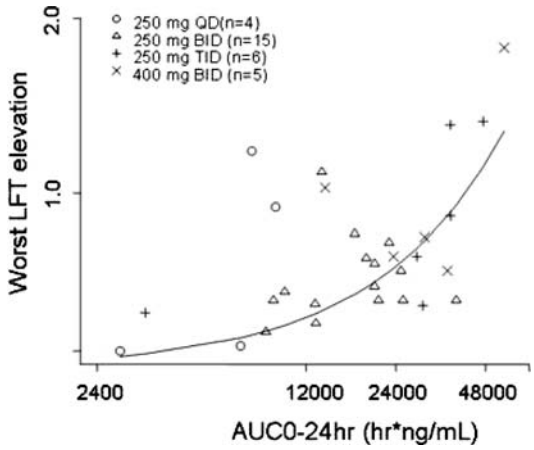

Fig. 6 Relationship between steady-state AUC0-24 h of Day 1 of Cycle 2 and the most severe elevation of the liver function test (*LFT*). Both observed and imputed (if missing) steady-state AUC0-24 h of Day 1 of Cycle 2 are included. The most severe elevation of LFT is the highest score of $\text{Multiple}_{\text{LFT\_scaled}}$ in a patient. Each *symbol* represents data from an individual patient. Different symbols are used for different dose levels, while the same symbol is used for patients at the same dose level. The *sold line* represents the fitted line calculated by the regression analysis after excluding the four patients who lie far outside the regression line. The *x* axis is shown in the natural logarithmic scale

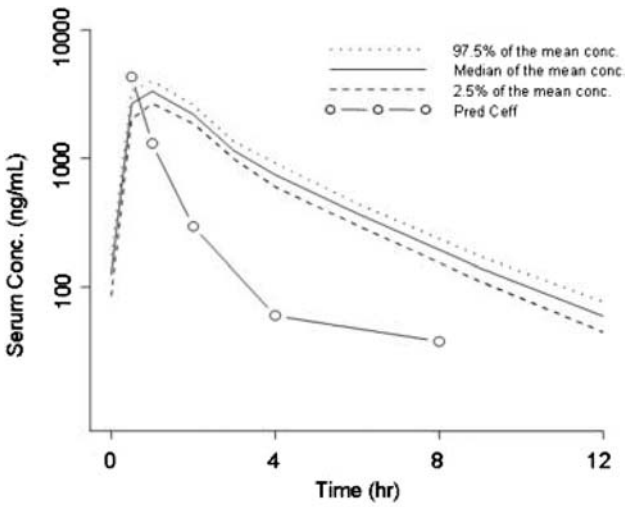

Fig. 7 The distribution of the simulated mean serum concentration–time curves of CP-724,714 at the recommended Phase 2 dose (250 mg BID) from one thousand simulated trials by nonparametric bootstrap. The *solid* and *dotted lines* represent the median and the 95% confidence interval of the simulated mean concentration–time profiles. Also shown is the preclinically predicted efficacious concentration (Pred Ceff)—time profile in joined cycles

(~20%), consistent with previous findings from a population PK modeling of the same dataset [21], which justifies to extrapolate the single dose data to approximate the missing steady-state PK data. There were substantial overlaps in both C_{maxMD} and steady-state AUC0-24 h at 250 mg TID and 400 mg BID (Fig. 3), which was not unexpected given the small daily dose difference and considerable PK variability (31–65%). This might explain why safety and tolerability were not improved when the dose was lowered from 400 mg BID to 250 mg TID. On the other hand, most steady-state AUC0-24 h and C_{maxMD} at 250 mg QD and 250 mg BID were lower than those at 250 mg TID and 400 mg BID, consistent with the improved safety and tolerability at these lower daily doses.

Both CL/*F* and V_{dss}/F, when estimated from the single dose data, were associated with body size, as shown in Table 3. A significant correlation (P value <0.05) was also seen between body size and CL/*F*, when CL/*F* was either estimated from the observed steady-state $\text{AUC}_{\text{tau}_{\text{MD}}}$, or estimated from the observed plus imputed steady-state $\text{AUC}_{\text{tau}_{\text{MD}}}$ (data not shown), although the correlation was slightly weaker when only the observed steady-state AUC_{tau} data were included in the analysis, which probably was due to the smaller sample size. The PK association with body size agrees with the previous finding from the population PK modeling of the same dataset, i.e., body size was indicated as a covariate of CL/*F* [21]. Varying WT within the extreme values as seen in the phase 1 trial (39–121 kg) resulted in a –37 to +45% variation of CL/*F* around the median (34.9 l/h). The correlation between body size and PK parameters may lie in the drug metabolism characteristics of the compound. CP-724,714 appears to be mainly metabolized by CYP3A based on in vitro data, and its CL/*F* is likely to be higher when there is a greater amount of CYP3A available as a result of a bigger intestine and liver size because of a larger body size [22].

The effect of other baseline variables on PK was also assessed, including age, liver metastasis, liver function, performance status, and number of prior chemotherapies. However, as revealed by the analysis, none of them appeared to be associated with PK parameters. As to the number of prior chemotherapies, this finding was consistent with the fact that no strong inducers or mechanism-based inhibitors of CYP3A were present in the list of prior chemotherapies. For some of the other variables, the failure to observe an effect 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 2 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/*F* and V_{dss}/F. With regards to the liver function and the presence of liver metastasis, only patients with adequate liver function (i.e., total bilirubin <1.5 mg/dl, and liver aminotransferases and alkaline phosphatase not greater than grade 2 by CTC version 2.0) entered the trial. Patients with liver metastasis and grade 2 or greater liver abnormality were excluded from the study. Since CP-724,714 appears to be mainly

Table 5 Nonparametric bootstrap-predicted probabilities of observing varying incidences of ≥grade 3 elevation of liver function test (LFT) in the simulated Phase 2 trial

Incidences of ≥grade 3 LFT elevation (%)	≤3.33	≤13.3	≤26.7	<33.3	≥33.3
Probability (%)	2.5	50	97.5	98.9	1.1

The incidence was calculated as the number of patients experiencing any ≥grade 3 LFT elevations divided by the total number of 30 patients in the simulated Phase 2 trial. One thousand nonparametric bootstraps with resampling were conducted

metabolized by CYP3A (suggested by in vitro drug metabolism data), it remains unknown if the metabolism and CL/*F* of CP-724,714 is compromised in patients with liver metastasis and >grade 1 liver abnormality. In future trials, the effect of age, liver function, and the presence of liver metastasis needs to be further assessed when an adequate number of patients with a broad age range (especially age >65 years) and less restricted liver function requirement are allowed to participate in the trials.

Patients with missing steady-state PK data were included in the exposure-LFT elevation correlation analysis after imputing their steady-state PK from single dose data. The main rationale for including these patients in the correlation analysis is as follows. When including all patients with observed and imputed steady-state PK data, the pearson correlation between steady-state PK and ALT or AST was weaker, compared to those when including only patients with observed steady-state PK data (data not shown). This appeared to be due to that three of the eight patients with missing steady-state PK data had grade 3 ALT and/or AST elevations, and two of these three patients happened to be outliers in Fig. 6. In order to get a more conservative estimate of the correlation, patients with imputed steady-state PK data were included in these analyses.

Even when patients with imputed steady-state PK data were included, the exposure-safety analysis revealed that elevated ALT, AST, and total bilirubin were still significantly correlated to the steady-state AUC0-24 h, and at a less degree to the steady-state$C_{\max_{MD}}$, as shown in Table 4, but were not correlated to $C_{\max_{SD}}$ and AUC_{inf} after the single dose administration and the cumulative dose ($DOSE_{cum}$) and AUC (AUC_{cum}) after multiple dose administration. These findings agreed with other information from the trial. For example, the median onset of elevated ALT, AST, and total bilirubin was 15 days (data not shown), suggesting that LFT elevations require multiple dose administration of CP-724,714 in the trial setting. On the other hand, some patients, who were on treatment for a longer period but had a lower steady-state AUC0-24 h when compared to the rest of patients, experienced little or no LFT elevations, indicating that steady-state AUC0-24 h be an important determinant of hepatotoxcities during the multiple dose administration. The threshold steady-state AUC0-24 h associated with ≥grade 3 elevated LFT was suggested to be approximately 34,000 h ng/ml, according to the trend curve of increasing severity of elevated LFT with increasing steady-state AUC0-24 h for the majority of patients (26 out of a total 30 patients) (Fig. 6).

These four patients who departed from the trend (Fig. 6) experienced ∼grade 3 elevated ALT and/or AST, but not total bilirubin. They took concomitant acetaminophen during the study, which was known to cause hepatotoxicities when overdosed [23–25]. One patient also experienced disease progression due to increased liver metastasis, although the other three patients did not. When the relationship(s) between the severity of ALT/AST elevations and the concomitant use of acetaminophen and/or increased liver metastasis were examined, no correlations were detected. Since the number of patients was small in this trial (n = 30), the cause for the apparently greater susceptibility to grade 3 elevations of ALT/AST in some patients needs to be further evaluated in future trials.

Besides elevated ALT, AST, and total bilirubin, other most frequently encountered side effects were rash, asthenia, and nausea/vomiting (N/V). Although no correlations were detected of these side effects with PK parameters of CP-724,714 or baseline clinical characteristics (such as rash, asthenia, and N/V at baseline) (data not shown), it remains possible that these toxic manifestations of CP-724,714 are not directly mediated by the parent drug, but rather metabolite(s), the pharmacological effects of which are not closely associated with PK parameters of CP-724,714. Furthermore, anticipatory N/V as the cause for observed N/V cannot be excluded, since patients in this trial received extensive prior chemotherapies that were emetogenic (the median number of prior chemotherapy = 6), such as anthracyclines (doxorubicin and epirubicin), cyclophosphamide, 5-FU, and paclitaxel and/or docetaxel [26, 27].

Based on findings from the Phase 1 trial, the 250 mg BID dosing was considered as the recommended Phase 2 dose (RP2D). Simulation of the steady-state serum concentration–time profile for a limited Phase 2 trial in patients with HER-2 over-expressing metastatic breast cancer (n = 30) by nonparametric bootstrap (n = 1,000) suggested that the 95% confidence interval of the simulated mean concentration at the RP2D were at or above the preclinically predicted efficacious concentrations over the entire dosing interval. However, it still remains to be

determined how well the preclinically predicted efficacious exposures from murine xenograft models translate to patients with HER-2 over-expressing metastatic breast cancer.

At the RP2D (250 mg BID), only one out of fifteen patients (6.7%) developed grade 3 LFT elevation, which was much lower than the incidences of grade 3 LFT elevations observed at other dose levels. At 250 mg QD, 250 mg TID, and 400 mg BID, there were one of four patients (25%), two of six patients (33%), and two of five patients (40%) who developed grade 3 LFT elevations, respectively. However, individual patients' observed steady-state AUC0-24 h at the RP2D (250 mg BID) spaned a wide range from 7,240 to 38,160 h ng/ml, with the upper limit of the range greater than the steady-state AUC0-24 h of the majority of patients at 250 mg TID and 400 mg BID, as shown in Fig. 3. As the exposure-safety analysis had suggested that the severity of LFT elevations was associated with the steady-state AUC0-24 h, the worst LFT elevation data of patients at all dose levels, whose observed or imputed (if missing) steady-state AUC0-24 h were ≤38,160 h ng/ml, were pooled together to project the incidence of ≥grade 3 elevated LFT at the RP2D via nonparametric boostrap.

A total of 28 patients met the inclusion criteria (the steady-state AUC0-24 h were ≤38,160 h ng/ml) for the nonparametric bootstrap simulation. The result from the simulation of the incidence of ≥grade 3 elevated LFT at the RP2D suggested that the 250 mg BID oral dosing would be well tolerated, and it would be rare (only 1.1% probability) to observe ≥33% grade 3 LFT elevations. The underlying assumption of this simulation was that the hepatotoxicity susceptibility of the Phase 2 patients was similar to that observed in the Phase 1 trial. However, this remains to be examined in future trials.

In summary, CP-724,714 demonstrates linear single-dose and multiple-dose PK. Both CL/F and V_{dss}/F correlate with body size. However, given the small number of patients (n = 30) in this trial, the influence of body size on PK warrants further evaluation in future trials. The elevation of ALT, AST, and total bilirubin positively correlate with the steady-state AUC0-24 h. The Phase 2 trial simulation suggests that CP-724,714 be well tolerated and PK exposures exceed the preclinically predicted efficacious level at the recommended Phase 2 dose (250 mg BID), supporting further evaluation of CP-724,714 in the Phase 2 trial.

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