

# Influence of mild and moderate hepatic impairment on axitinib pharmacokinetics

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**Summary Objective:** To evaluate the effects of hepatic impairment on the pharmacokinetics and safety of a single, oral axitinib dose in subjects with mild or moderate hepatic impairment. **Methods:** In this phase I, open-label, parallel-group study, a total of 24 subjects with either normal hepatic function ( $n=8$ ) or with mild ( $n=8$ ) or moderate ( $n=8$ ) hepatic impairment were administered a single, oral dose of axitinib (5 mg). Blood samples were collected at intervals up to 144 h following dosing, and plasma pharmacokinetics and safety were assessed. Changes in

axitinib plasma exposures in subjects with mild or moderate hepatic impairment were predicted using computer simulations and used to guide initial dosing in the clinical study. **Results:** Axitinib exposure was similar in subjects with normal hepatic function and those with mild hepatic impairment, but approximately twofold higher in subjects with moderate hepatic impairment. Axitinib exposure weakly correlated with measures of hepatic function but was not affected by smoking status. Axitinib protein binding was similar in the three treatment groups. No significant treatment-related adverse events were reported. **Conclusions:** Compared with subjects with normal hepatic function, moderate hepatic impairment increased axitinib exposure, suggesting that the oral clearance of axitinib is altered in these subjects. In addition, these data indicate a possible need for a dose reduction in subjects who develop moderate or worse hepatic impairment during axitinib treatment. A single 5-mg dose of axitinib was well tolerated in subjects with mild or moderate hepatic impairment.

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## Introduction

Axitinib is an oral, potent, and selective inhibitor of vascular endothelial growth factor (VEGF) receptors 1, 2, and 3 [1]. Early clinical trial experience has demonstrated that it has activity and is well tolerated as a single agent and in combination with chemotherapy for the treatment of a number of tumor types, including renal cell carcinoma [2, 3], thyroid cancer [4], and non-small cell lung cancer [5]. Axitinib is currently in phase III development for advanced renal cell carcinoma.

In the clinic, axitinib is administered at a 5-mg twice-daily, oral starting dose. The pharmacokinetics of axitinib are linear within the 5–10 mg clinical-dose range as assessed by single-dose administration in healthy volunteers and characterized by an oral absolute bioavailability of 58%, rapid oral absorption (with peak plasma concentrations occurring 3–6 h after dosing in the fed state), and a terminal phase plasma half-life ( $t_{1/2}$ ) of 3–5 h [6]. The plasma protein binding of axitinib is >99%; *in vitro* studies indicate that axitinib binds strongly to albumin and, to a lesser extent, to  $\alpha$ -1-acid glycoprotein (unpublished data).

Axitinib is metabolized primarily by oxidation via cytochrome P450 (CYP) 3A4/5, and to a lesser extent by CYP1A2, CYP2C19, and glucuronidation via the uridine diphosphate-glucuronosyltransferase (UGT) 1A1 pathway (unpublished data). In a human mass balance study, 30–60% of the administered radiolabeled axitinib oral dose was eliminated in feces, with renal elimination accounting for a further 23% (unpublished data). No unchanged axitinib was found in urine samples; all urinary radioactivity was related to axitinib metabolites. Metabolic profiling of fecal samples indicated that unchanged axitinib was the predominant radioactive component. Together, these data indicate that hepatobiliary excretion is the major elimination pathway for axitinib. Thus, a clinical study to characterize changes in axitinib exposure with hepatic impairment was warranted. To assist in the design of the clinical study and to select appropriate dosing, simulations were conducted using the Simcyp® Population-based Absorption, Distribution, Metabolism, and Excretion (ADME) Simulator (Simcyp® Ltd, Sheffield, UK) [7] to predict changes in axitinib plasma exposure in subjects with varying degrees of hepatic impairment.

The primary objectives of the present phase I study were to (1) evaluate the effect of hepatic impairment on axitinib pharmacokinetics and (2) assess safety and tolerability of a single oral 5-mg axitinib dose in subjects with mild or moderate hepatic impairment. Since VEGF is central to one of the target pathways for axitinib and plays a major role in wound healing, subjects with severe hepatic impairment were not included due to presumed mechanism-based risk of bleeding events and coagulopathy.

## Methods

### Prestudy Simcyp simulation of the effect of hepatic impairment on axitinib exposure

In order to assist in the development of the clinical study, predictions of the effects of hepatic impairment on the pharmacokinetics of axitinib were conducted using Simcyp Population-Based ADME Simulator version 8.2 SP2. The

model for hepatic impairment was developed using parameters obtained from preclinical assessments as well as built-in software assumptions and standard physiological parameter estimates. Plasma concentrations expected after administration of a single 5-mg oral dose of axitinib in subjects with normal hepatic function or mild or moderate hepatic impairment were simulated in ten clinical trials (with ten subjects per clinical trial) under fed conditions using a one-compartmental distribution and first-order absorption model.

Input parameters specific to axitinib estimated *in vitro* included the apparent intrinsic clearance for each recombinant CYP isoform: CYP3A4 (maximum velocity [ $V_{max}$ ]: 9.6 pmol/min/pmol of isoform; Michaelis-Menten constant [ $K_m$ ]: 4  $\mu$ M), CYP3A5 ( $V_{max}$ : 1.41 pmol/min/pmol of isoform;  $K_m$ : 1.9  $\mu$ M), CYP2C19 ( $V_{max}$ : 0.11 pmol/min/pmol of isoform;  $K_m$ : 5.9  $\mu$ M), and CYP1A2 (apparent intrinsic clearance: 0.17  $\mu$ L/min/pmol of isoform); the  $V_{max}$  and  $K_m$  for UGT1A1 (132.5 pmol/min/mg of microsomal protein and 44.2  $\mu$ M, respectively); fraction unbound in plasma (0.010); blood-to-plasma partition coefficient (0.79); fraction absorbed (1.0);  $-\text{Log}_{10}$  dissociation constant (4.2); absorption rate (1.98 L/h); and volume under steady state conditions (1 L/kg). Based on observations from a human mass balance study (unpublished data), the renal clearance for axitinib was assigned a value of zero. The Simcyp hepatic-impairment model assumes that the activity of CYP isoforms decreases with worsening hepatic function, specifically, that the activity of CYP3A4/5 is decreased by 42% and 62% in mild and moderate hepatic impairment, respectively, compared with normal hepatic function.

### Study design

This phase I, open-label, single-dose, parallel-group study assessed the pharmacokinetics, safety, and tolerability of a 5-mg single oral dose of axitinib in subjects with normal hepatic function or mild or moderate hepatic impairment according to the US Food and Drug Administration (FDA) guidelines on hepatic impairment studies [8]. The study was carried out at two centers (Orlando, FL, and Miami, FL) from May 16, 2008, to October 26, 2008.

### Subjects

Participants were male or female subjects aged  $\geq 18$  years with normal hepatic function or mild or moderate hepatic impairment, according to the Child-Pugh (CP) classification [9, 10]. Subjects were screened for participation within 21 days prior to the first dose of study treatment. Subjects with normal hepatic function were recruited after subjects with mild (CP class A [CP-A] score 5–6) and moderate (CP class B [CP-B] score 7–9) hepatic impairment had completed the study. In

all, eight subjects enrolled into each of the three groups. Control subjects with normal hepatic function were matched to subjects with hepatic impairment based first on weight, and then on age and gender. In order to match subjects based on body weight, four of the subjects with normal hepatic function were matched to four subjects in each of the mild and moderate hepatic-impairment groups. Individual subjects to match were those with the lowest ( $n=1$ ), highest ( $n=1$ ), and median ( $n=2$ ) weights ( $\pm 10$  kg) in each hepatic-impaired group. After matching subjects with normal hepatic function by body weight, they were then matched for age ( $\pm 10$  years) and gender to subjects with mild or moderate hepatic impairment.

Subjects with normal hepatic function were required to be healthy, defined as no clinically relevant abnormalities, which were identified by a detailed medical history and full physical examination, including blood pressure, pulse rate, 12-lead electrocardiogram (ECG), and clinical laboratory tests. Liver function tests, albumin, and prothrombin time were all required to be within normal range. Eligible subjects had a body mass index (BMI) of 18–32 kg/m<sup>2</sup> and a total body weight >50 kg.

Subjects with hepatic impairment were required to have hepatic dysfunction due to hepatocellular disease (not secondary to other diseases) as documented by medical history, physical examination, liver biopsy, hepatic ultrasound, computed tomography scan, or magnetic resonance imaging, and have adequate renal function, determined by a 24-h creatinine clearance >75 ml/min. Hepatic dysfunction was required to be stable, defined as no clinically significant change in disease status within the last 30 days, which was documented by recent medical history, including no worsening of clinical signs of hepatic impairment or no worsening of total bilirubin or prothrombin time >50%. Subjects with hepatic impairment also had to be on a stable treatment regimen or dose of medication, or a fluctuating treatment regimen if the underlying disease was under control, provided sponsor approval was granted prior to the first dose of study treatment. In addition to the general exclusion criteria for all groups outlined below, exclusion criteria specific to subjects with hepatic impairment included any other clinically significant diseases that contraindicated the use of axitinib or may have affected its pharmacokinetics; the use of food or prescription or non-prescription drugs that are potent inducers or inhibitors of CYP3A4/5 within 7 days or 5 half-lives (whichever was longer) prior to dosing; any clinically significant laboratory abnormality, except for those parameters influenced by hepatic impairment; or significant hepatic encephalopathy (grade >2 portal systemic encephalopathy score and severe ascites and/or pleural effusion).

Exclusion criteria for subjects in all groups included a history of febrile illness  $\leq 5$  days prior to dosing; any condition, such as gastrectomy, that could affect drug

absorption; 12-lead ECG demonstrating corrected QT interval >450 msec in healthy subjects (i.e., normal hepatic function) and >470 msec in subjects with mild or moderate hepatic impairment at screening; a history of regular alcohol consumption (>7 drinks/week for females or >14 drinks/week for males) within 6 months of screening; and the use of prescription or non-prescription drugs (except acetaminophen  $\leq 1$  g/day), vitamins, or dietary supplements within 7 days or 5 half-lives (whichever was longer) prior to dosing in the normal hepatic-function group. Herbal supplements and hormone replacement therapy were to have been discontinued 28 days prior to dosing. Female subjects of childbearing potential, including those with tubal ligation, were not included in the study.

All subjects were required to abstain from grapefruit-containing products for 7 days prior to and during the study until collection of the final blood sample for pharmacokinetic analysis, strenuous exercise 48 h prior to and during the study, and alcohol and caffeine consumption 24 h prior to and during the study. The use of tobacco- or nicotine-containing products equivalent to  $\leq 5$  cigarettes/day was permitted throughout the study period.

This study was performed in accordance with ethical principles originating in or derived from the Declaration of Helsinki and in compliance with the International Conference on Harmonization Good Clinical Practice guidelines and applicable local regulatory requirements and laws. An Institutional Review Board approved the protocol, and all participants gave written informed consent. This trial was registered on ClinicalTrials.gov (NCT00692341).

#### Axitinib administration

Subjects with hepatic impairment were admitted to the clinical research unit 2 days before dosing (day-1) in order to obtain a baseline 24-h urine collection for accurate assessment of renal function. Subjects with normal hepatic function were admitted the day before dosing (day 0).

Subjects fasted for  $\geq 10$  h prior to consumption of a standardized meal on day 1. The standardized meal consisted of 30% fat, 15% protein, and 55% carbohydrate, equivalent to 500–700 calories, and represented a typical meal consumed by an oncology patient. Subjects received a 5-mg single oral dose of axitinib administered as a film-coated immediate-release, dry-granulated tablet within 30 min of the start of the meal with 240 ml of water. All subjects were required to remain within the clinical research unit until day 3 and return for follow-up visits on days 5 and 7.

#### Evaluation of axitinib pharmacokinetics

Blood samples (5 ml) were collected in K<sub>3</sub>-EDTA anticoagulant tubes at 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 12, 16, 24, 36,

48, 96, and 144 h after the dose of axitinib was administered on day 1. Separate blood samples (10 ml) in  $K_3$ -EDTA anticoagulant were also collected on day 1 (0 and 4 h after dosing) for measurement of protein binding of axitinib in plasma. Blood samples for axitinib protein binding were initially scheduled to be collected 0 and 1–2 h after axitinib dosing (pre- and post-dosing, respectively). However, the observed time to maximum observed concentration of axitinib was delayed in patients with hepatic impairment (occurring 4 h after dosing). Thus, blood samples collected 0 and 4 h after dosing were used to determine protein binding of axitinib in plasma. To protect from photo-degradation, axitinib in all blood and plasma samples was protected from visible and ultraviolet light exposure during collection, storage, processing, and analysis. Plasma samples were analyzed for axitinib using a high-performance liquid chromatography with tandem mass spectrometric (LC/MS/MS) detection method (Charles River Laboratories, Shrewsbury, MA) validated for accuracy, precision, sensitivity, selectivity, matrix effects, recovery, and stability in compliance with Pfizer standard operating procedures, which conform to the requirements of the FDA bioanalytical method validation guidance [11], as well as a subsequent bioanalytical white paper generated by the FDA and industry representatives [12]. The standard curve was linear with weighted ( $1/\text{concentration}^2$ ) regression and included nine concentrations in duplicate (range 0.5–100 ng/ml). Each individual standard was within 12% of nominal concentration, and inter-assay precision at each standard level was within 4% coefficient of variation (CV). Duplicate quality control (QC) samples at four concentration levels spanning the assay range (1.5, 7.0, 40, and 80 ng/ml) were included in each analytical run to measure assay acceptance. At each QC level, inter-assay accuracy was within 6% of nominal concentration and inter-assay precision was within 6% CV.

Plasma samples were fortified with deuterium-labeled (d7) axitinib internal standard (IS), followed by addition of 1 M sodium bicarbonate. The samples were extracted with ethyl acetate:hexanes (75:25, v/v), centrifuged, and the supernatants were evaporated to dryness under a gentle nitrogen stream in a TurboVap® (Caliper Life Sciences Inc, Waltham, MA) heated to 45°C. Sample residues were reconstituted in water/methanol/sodium bisulfite/ascorbic acid (75:25:0.5:0.5, v/v/v/v) and eluted from a pentafluorophenylpropyl (F5) column (Discovery HS F5, 50 × 2.1 mm, 5 μm [Supelco, Bellefonte, CA]) using a gradient of 20–80% B over 3 min (mobile phase A=10 mM ammonium acetate, mobile phase B=acetonitrile) at a 0.5 ml/min flow rate onto an API 3000® mass spectrometer (AB Sciex™, Concord, Ontario, Canada) with electrospray ionization source. Detection was in positive ion mode with multiple reaction monitoring (mass transitions  $m/z$  387 →

356 for axitinib and 394 → 360 for axitinib-d7). Peak areas were quantitated using Analyst® software (AB Sciex™, Concord, Ontario, Canada).

Standard axitinib pharmacokinetic parameters were estimated using non-compartmental methods with Pfizer proprietary software (eNCA). Pharmacokinetic parameters assessed were area under the plasma concentration-time curve (AUC) from time zero to infinity ( $AUC_{0-\infty}$ ), AUC from time zero to time of the last measurable concentration ( $AUC_{0-\text{last}}$ ), maximum observed plasma concentration ( $C_{\text{max}}$ ), time to  $C_{\text{max}}$ ,  $t_{1/2}$ , apparent oral clearance (CL/F), and apparent volume of distribution (Vz/F).

#### Evaluation of axitinib protein binding

Plasma samples were processed to determine axitinib protein binding using an equilibrium dialysis method, and concentrations of resulting unbound and bound axitinib were measured by high-performance LC/MS/MS detection methods (Charles River Laboratories, Shrewsbury, MA) validated in compliance with Pfizer standard operating procedures. Dialysis was performed in a 96-well Teflon dialysis plate (Equilibrium Dialysis Device HTD 96; HTDialysis LLC, Gales Ferry, CT) with wells divided by Spectra/Por®-regenerated cellulose membranes with a molecular weight cut-off of 12,000–14,000 Da (Spectrum® Laboratories, Rancho Dominguez, CA). Plasma samples were dialyzed in duplicate against equal volumes of phosphate-buffered saline (70 mM sodium chloride, 50 mM sodium phosphate, pH 7.4) for approximately 20 h at 37°C with orbital shaking at 80 rpm. Axitinib concentrations in plasma dialysate (representing total bound and unbound axitinib) were measured with the LC/MS/MS assay used for plasma pharmacokinetic samples. Each individual standard was within 10% of nominal concentration and intra-assay precision at each standard level was within 8% CV. At each QC level, intra-assay accuracy was within 9% of nominal concentration and intra-assay precision was within 6% CV.

Axitinib concentrations in buffer dialysate (representing unbound axitinib) were measured using a validated LC/MS/MS assay in  $K_3$ -EDTA plasma “water”, an aqueous layer of the supernatant generated by high-speed ultracentrifugation of plasma [13] ( $440,000 \times g$  for 2.5 h at 37°C) using a Beckman TL100 Micro UltraCentrifuge (Beckman Coulter Inc, Brea, CA). The plasma “water” assay had been developed previously and was considered fit-for-purpose for buffer dialysate study sample analysis due to matrix similarity. The standard curve was linear with weighted ( $1/\text{concentration}^2$ ) regression and included nine concentrations in duplicate (range 20–1,000 pg/ml). Each acceptable individual standard was within 13% of nominal concentration, and intra-assay precision at each standard level was



within 8% CV (17% CV at the lower limit of quantitation). Duplicate QC samples at three concentration levels spanning the assay range (60, 150, and 800 pg/ml) were included in each analytical run to measure assay acceptance. At each QC level, intra-assay accuracy was within 6% of nominal concentration and intra-assay precision was within 1% CV. In addition, QCs consisting of dialyzed plasma:dialyzed buffer (1:1, v/v) at concentrations corresponding to low and high areas of the standard curve ( $n=6$  at 60 and 800 pg/ml) were included to determine whether the matrix difference between standards and study samples affected quantitation accuracy. For the mixed plasma:buffer QCs, intra-assay accuracy was within 2% of nominal concentration and intra-assay precision was within 3% CV at each QC level.

Buffer dialysate samples were fortified with axitinib-d7 IS, followed by addition of 1 M sodium bicarbonate and 10 mM ammonium acetate aqueous solution (aq). The samples were extracted with ethyl acetate:hexanes (75:25, v/v), centrifuged, and the supernatants were evaporated to dryness under a gentle nitrogen stream in a TurboVap heated to 45°C. Sample residues were reconstituted in water/methanol/sodium bisulfite/ascorbic acid (75:25:0.5:0.5, v/v/v/v) and eluted isocratically with 10 mM ammonium acetate (aq):acetonitrile (60:40, v/v) mobile phase over 2 min from a pentafluorophenylpropyl (F5) column (Discovery HS F5, 50×2.1 mm, 5 μm) at a 0.5 ml/min flow rate onto an API 4000™ mass spectrometer (AB Sciex™, Concord, Ontario, Canada) with electrospray ionization source. Detection was in positive ion mode with multiple reaction monitoring (mass transitions  $m/z$  387→356 for axitinib and 394→360 for axitinib-d7). Peak areas were quantitated using Analyst software.

Fraction unbound ( $f_u$ ) was calculated as *axitinib in buffer dialysate (ng/ml) / (axitinib in plasma dialysate [ng/ml] + axitinib in buffer dialysate [ng/ml])*. Mean  $f_u$  for each sample was used to calculate pharmacokinetic parameters. Unbound axitinib pharmacokinetic parameters assessed included unbound  $AUC_{0-\infty}$  and  $AUC_{0-last}$  (calculated as  $AUC_{0-\infty} * f_u$  and  $AUC_{0-last} * f_u$ , respectively),  $C_{max}$  unbound (calculated as  $C_{max} * f_u$ ), unbound CL/F (calculated as  $CL/F / f_u$ ), and unbound Vz/F (calculated as  $Vz/F / f_u$ ).

### Safety evaluation

Safety assessments included recording of adverse events, ECGs, blood pressure, pulse rate, clinical laboratory tests (including liver and renal function tests, prothrombin time, and partial thromboplastin time), physical examinations, and vital signs. Blood pressure and pulse rate were measured at screening, pre-dose on day 1, and at 1, 2, 16, and 144 h post dose. A single standard 12-lead ECG was obtained at screening, post dose on day 1, and at 1.5, 48, and 144 h post dose.

### Statistical methods

Sample size was based on recommendations from the FDA Guidance for Industry on hepatic impairment studies [8]. Pharmacokinetic parameters were estimated by using non-compartmental methods as described above, and summarized by descriptive statistics. Using one-way analysis of variance (ANOVA), natural log-transformed pharmacokinetic parameters ( $AUC_{0-\infty}$ ,  $AUC_{0-last}$ , and  $C_{max}$ ) were compared between the group of subjects with normal hepatic function and each group of subjects with hepatic impairment. Point estimates and 90% confidence intervals (CIs) for the differences between these groups on the log scale were constructed then back-transformed to provide estimates of the ratio of adjusted geometric means (i.e., moderate hepatic impairment/normal function and mild hepatic impairment/normal function) and 90% CIs for the ratios. The differences in pharmacokinetic parameters between subjects with hepatic impairment and with normal hepatic function were evaluated by examination of the ratios and 90% CIs. Relationships between axitinib exposure and laboratory components of CP score, including bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, and prothrombin time, were assessed graphically and by simple linear regression.

### Results

#### Predictions from Simcyp modeling

Using the hepatic impairment model in Simcyp, it was predicted that mild hepatic impairment would increase the axitinib  $AUC_{0-\infty}$ , and  $C_{max}$  by 1.31- and 1.05-fold, respectively, compared with normal hepatic function. When axitinib pharmacokinetics in moderate hepatic impairment subjects was simulated, Simcyp predicted a 1.52- and a 1.08-fold increase in  $AUC_{0-\infty}$  and  $C_{max}$ , respectively, compared with normal hepatic function. The axitinib CL/F predicted for subjects with normal hepatic function by Simcyp modeling using clearance parameters from *in vitro* experiments with recombinant CYPs was ~8 L/h, which is approximately threefold lower than what is observed clinically. The predicted 1.52-fold increase in AUC did not warrant the use of a lower axitinib dose in subjects with moderate hepatic impairment compared with those with normal hepatic function given the observed six-fold variability in a previously published healthy volunteers study [14].

#### Subjects

A total of 24 subjects ( $n=20$  male, 4 female) with a mean age of 51.3 years (range 39.0–60.0) and with normal

**Table 1** Subject baseline demographics

	Normal hepatic function ( <i>n</i> =8)	Mild hepatic impairment ( <i>n</i> =8)	Moderate hepatic impairment ( <i>n</i> =8)
Male/female, <i>n</i> (%)	7 (87.5)/1 (12.5)	7 (87.5)/1 (12.5)	6 (75.0)/2 (25.0)
Age, y			
Mean (SD)	47.4 (5.2)	52.5 (5.6)	54.3 (4.7)
Range	39–54	42–58	45–60
Ethnicity, <i>n</i> (%)			
Black	2 (25.0)	0	1 (12.5)
White	6 (75.0)	8 (100.0)	7 (87.5)
Weight, kg			
Mean (SD)	87.4 (14.4)	89.4 (8.3)	90.3 (13.9)
Range	60.0–105.9	76.3–100.0	68.2–115.4
Height, cm			
Mean (SD)	172.0 (8.0)	175.9 (6.3)	178.0 (9.1)
Range	156.0–184.0	162.6–183.0	159.0–188.0
Body mass index, kg/m <sup>2</sup>			
Mean (SD)	29.3 (2.8)	28.9 (2.4)	28.4 (3.0)
Range	24.7–32.6	24.1–30.8	23.9–32.7

Abbreviation: *SD* standard deviation

hepatic function (*n*=8) or mild (*n*=8) or moderate (*n*=8) hepatic impairment were enrolled in this study. Subject demographics are summarized in Table 1. The mean height, weight, and BMI of all subjects were 89.0 kg (range 60.0–115.4), 175.3 cm (range 156.0–188.0), and 28.9 kg/m<sup>2</sup> (range 23.9–32.7), respectively. The healthy-subject group was comparable to the two hepatic-impairment groups with respect to weight, age, and gender. All subjects received the 5-mg single oral dose of axitinib and were eligible for pharmacokinetic and safety analysis. There were no discontinuations or withdrawals from the study.

### Pharmacokinetics

The observed clinical plasma pharmacokinetic parameters of axitinib in patients with normal hepatic function or mild or moderate hepatic impairment are summarized in Table 2 and Fig. 1. The plasma concentration-time curves by treatment group are shown in Fig. 2. Overall, subjects with mild hepatic impairment had similar axitinib pharmacokinetics to those with normal hepatic function. However, axitinib exposure was higher among subjects with moderate hepatic impairment compared with subjects with normal hepatic function, as indicated by geometric mean  $AUC_{0-\infty}$  (304 ng h/ml [%CV 44] vs. 156 ng h/ml [%CV 63],

**Table 2** Pharmacokinetic parameters of axitinib following administration of a single 5-mg oral dose in subjects with normal hepatic function or mild or moderate hepatic impairment

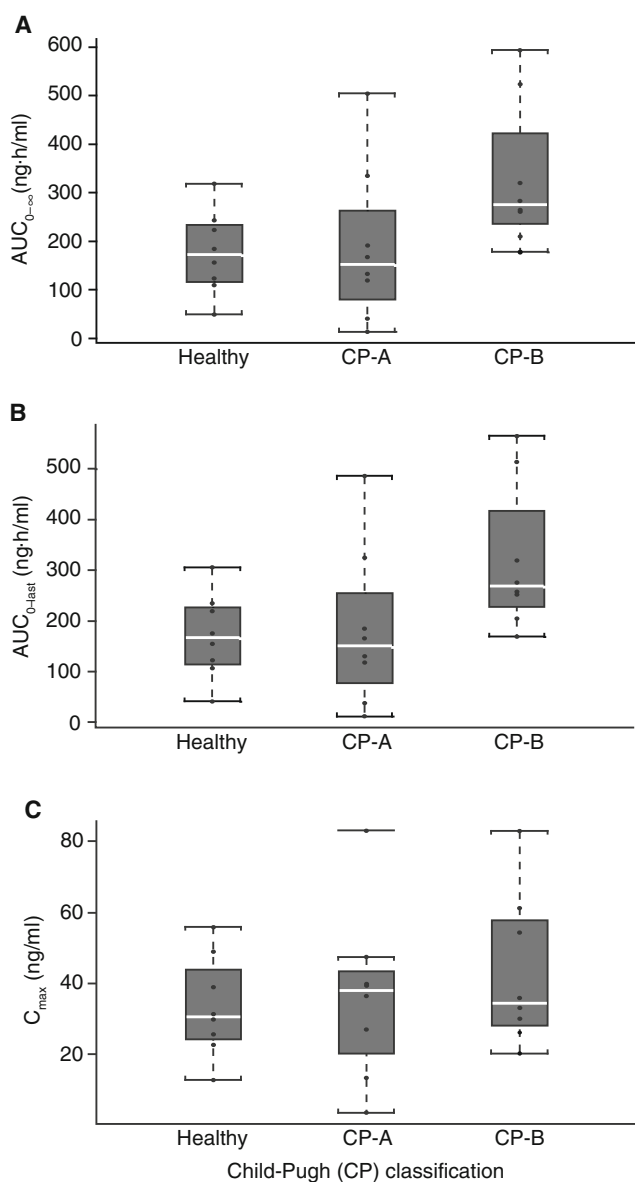
Pharmacokinetic parameter	Normal hepatic function ( <i>n</i> =8)	Mild hepatic impairment ( <i>n</i> =8)	Moderate hepatic impairment ( <i>n</i> =8)
$AUC_{0-\infty}$ (ng h/ml) <sup>a</sup>	156 (63)	122 (167)	304 (44)
$AUC_{0-last}$ (ng h/ml) <sup>a</sup>	148 (69)	116 (180)	295 (44)
$CL/F$ (L/hr) <sup>a</sup>	32.1 (63)	40.9 (167)	16.4 (44)
$C_{max}$ (ng/ml) <sup>a</sup>	30.4 (50)	27.0 (127)	38.8 (50)
$T_{max}$ (h) <sup>b</sup>	3.5 (2.0–4.0)	2.75 (1.0–4.0)	4.0 (1.5–4.0)
$Vz/F$ (L) <sup>a</sup>	166 (52)	163 (87)	128 (67)
$t_{1/2}$ (h) <sup>c</sup>	4.74 (80)	3.61 (84)	7.12 (91)
Fraction unbound <sup>a</sup>	0.004049 (25)	0.002987 (50)	0.004083 (134)
$AUC_{0-\infty, unbound}$ (ng h/ml) <sup>a</sup>	0.630 (56)	0.689 (82)	1.241 (109)
$AUC_{0-last, unbound}$ (ng h/ml) <sup>a</sup>	0.601 (61)	0.671 (81)	1.206 (106)
$C_{max, unbound}$ (ng/ml) <sup>a</sup>	0.123 (46)	0.132 (47)	0.159 (97)
$CL/F_{unbound}$ (L/hr) <sup>a</sup>	7933 (56)	7253 (82)	4029 (109)
$Vz/F_{unbound}$ (L) <sup>a</sup>	41,109 (57)	42,378 (40)	31,269 (121)

Abbreviations:  $AUC_{0-\infty}$  area under the concentration-time curve (AUC) from time zero to infinity,  $AUC_{0-last}$  AUC from time zero to time of the last measurable concentration,  $AUC_{0-\infty, unbound}$  AUC for unbound drug from time zero to infinity,  $AUC_{0-last, unbound}$  AUC for unbound drug from time zero to time of the last measurable concentration,  $CL/F$ , apparent oral clearance,  $CL/F_{unbound}$  apparent oral clearance of unbound drug,  $C_{max}$  maximum observed plasma concentration,  $C_{max, unbound}$  maximum observed plasma concentration for unbound drug,  $t_{1/2}$  half life,  $T_{max}$  time to maximum observed plasma concentration,  $Vz/F$  apparent volume of distribution,  $Vz/F_{unbound}$  apparent volume of distribution for unbound drug

<sup>a</sup> Geometric mean (% coefficient of variation [CV] around the geometric mean)

<sup>b</sup> Median (range)

<sup>c</sup> Arithmetic mean (% CV around the arithmetic mean)



**Fig. 1** Axitinib plasma exposure by treatment group for **a** area under the concentration-time curve (AUC) from time zero to infinity ( $AUC_{0-\infty}$ ), **b** AUC from time zero to time of the last measurable concentration ( $AUC_{0-last}$ ), and **c** maximum observed plasma concentration ( $C_{max}$ )

respectively) and  $C_{max}$  (38.8 ng/ml [%CV 50] vs. 30.4 ng/ml [%CV 50]), respectively.

Log-transformed pharmacokinetic parameters were compared using ANOVA. The adjusted geometric means ratio for  $AUC_{0-\infty}$  and  $C_{max}$  in subjects with mild hepatic impairment compared with normal hepatic function was 78.34% (90% CI 39.92–153.75) and 88.61% (90% CI 49.20–159.56), respectively. The adjusted geometric means ratio for  $AUC_{0-\infty}$  and  $C_{max}$  in subjects with moderate hepatic impairment compared with normal hepatic function was 195.25% (90% CI 99.49–383.18) and 127.67% (90% CI 70.90–229.91), respectively.

## Protein binding

Results of the protein-binding analysis are summarized in Table 2. Approximately 0.3% to 0.4% of axitinib was unbound in plasma in the subjects with varying hepatic function assessed in this study. The geometric means for the fraction unbound in plasma for axitinib were similar among treatment groups. The adjusted geometric mean ratio for fraction unbound in subjects with mild hepatic impairment compared with normal hepatic function was 73.78% (90% CI 37.40–145.56). The adjusted geometric mean ratio for fraction unbound in subjects with moderate hepatic impairment compared with normal hepatic function was 100.86% (90% CI 55.58–183.02).

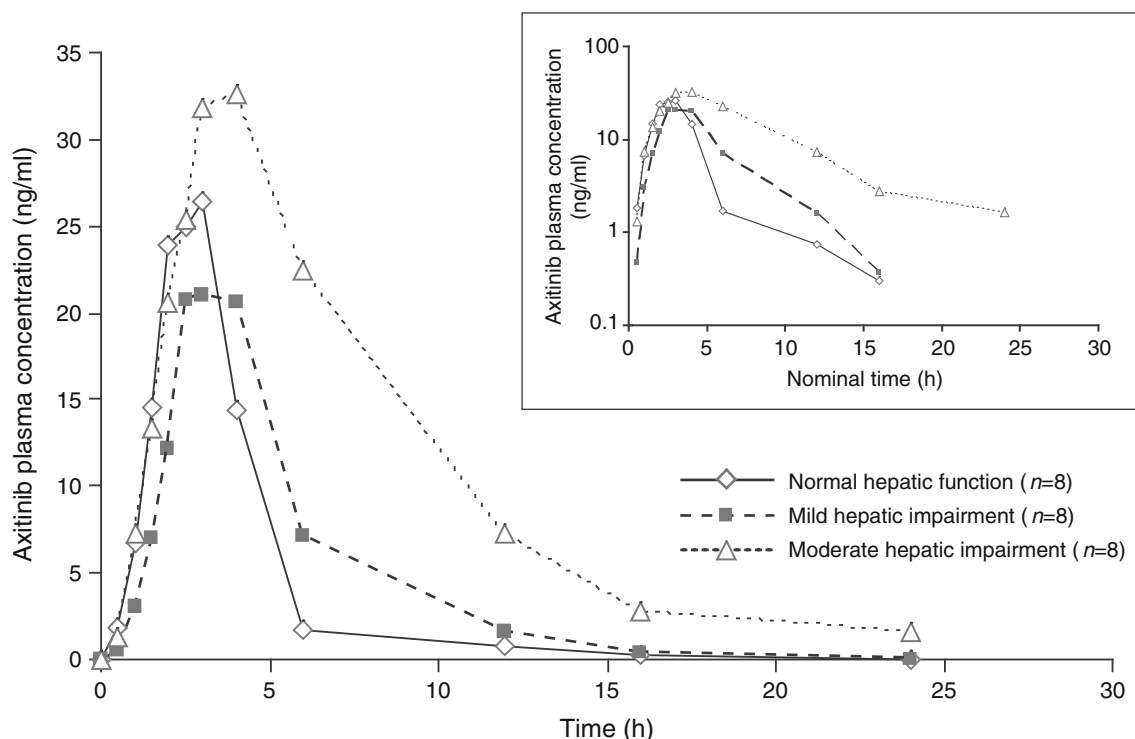
Overall, the results for the unbound pharmacokinetic parameters mimicked those for total pharmacokinetic parameters (Table 2). The axitinib geometric mean (%CV for geometric mean) plasma  $C_{max, unbound}$  values were similar in subjects with normal hepatic function and mild hepatic impairment. Likewise, axitinib geometric mean plasma  $AUC_{0-\infty, unbound}$  values and geometric mean  $AUC_{0-last, unbound}$  parameter estimates were similar in subjects with normal hepatic function and mild hepatic impairment, but were higher in those with moderate hepatic impairment (Table 2).

## Clinical correlations of laboratory parameters

The relationship between axitinib exposure and laboratory parameters that are components of the CP classification system, such as albumin, AST, ALT, bilirubin, and prothrombin time, were explored. When axitinib plasma exposure (indicated by plasma AUC) was assessed in relation to measures of liver function, there were marginal trends for axitinib AUC to increase with lower serum albumin ( $r^2=0.14$ ;  $p=0.0644$ ), higher AST ( $r^2=0.10$ ;  $p=0.1028$ ), higher ALT ( $r^2=0.02$ ;  $p=0.4969$ ), and higher bilirubin ( $r^2=0.13$ ;  $p=0.0710$ ) (Fig. 3). Increased axitinib exposure was also associated with higher prothrombin time ( $r^2=0.11$ ;  $p=0.1067$ ). These trends did not reach statistical significance. When axitinib AUC was evaluated according to smoking status, the median exposures were 200, 236, and 263 ng h/ml, respectively, for non-smokers, active smokers, and ex-smokers (i.e., not actively smoking at the time of screening who would refrain from smoking throughout the study period). Weak correlations between the fraction unbound in plasma of axitinib and both plasma albumin and bilirubin concentrations were observed (data not shown).

## Safety

**Adverse events** The 5-mg single dose of axitinib was well tolerated in subjects with mild or moderate hepatic



**Fig. 2** Median plasma concentration-time plot for axitinib by treatment group. Inset shows data on log-scale

impairment or normal hepatic function. There were no deaths, serious adverse events, or discontinuations due to study treatment. A total of four adverse events were reported in three subjects in the group with normal hepatic function, including headache ( $n=1$ ) and lethargy ( $n=3$ ). The three adverse events of lethargy were considered to be treatment-related, were mild or moderate in nature, and resolved without treatment. No adverse events were reported in the mild or moderate hepatic-impairment groups.

**Laboratory parameters** The incidence of laboratory abnormalities was low, and no clinically important trends were observed. No laboratory abnormalities were reported as adverse events. There were a total of 13 reports of laboratory abnormalities, 8 of which were also reported at baseline (2 with normal hepatic function, 3 with mild hepatic impairment, and 8 with moderate hepatic impairment). In the healthy-subject group, there was one report each of urine white blood cell count  $\geq 6$ /high power field (HPF) and urine hyaline casts  $>1$ /low power field (LPF). In the mild hepatic-impairment group, there was one report each of urine protein  $\geq 1$ , urine protein/hemoglobin  $\geq 1$ , and urine hyaline casts  $>1$ /LPF. In the moderate hepatic-impairment group, there was one report each of eosinophil %  $>1.2$  times upper limit of normal (ULN), absolute monocyte count 1.2 times ULN, urine white blood cell count  $\geq 6$ /HPF, urine glucose  $\geq 1$ , urine hyaline casts  $>1$ /LPF,

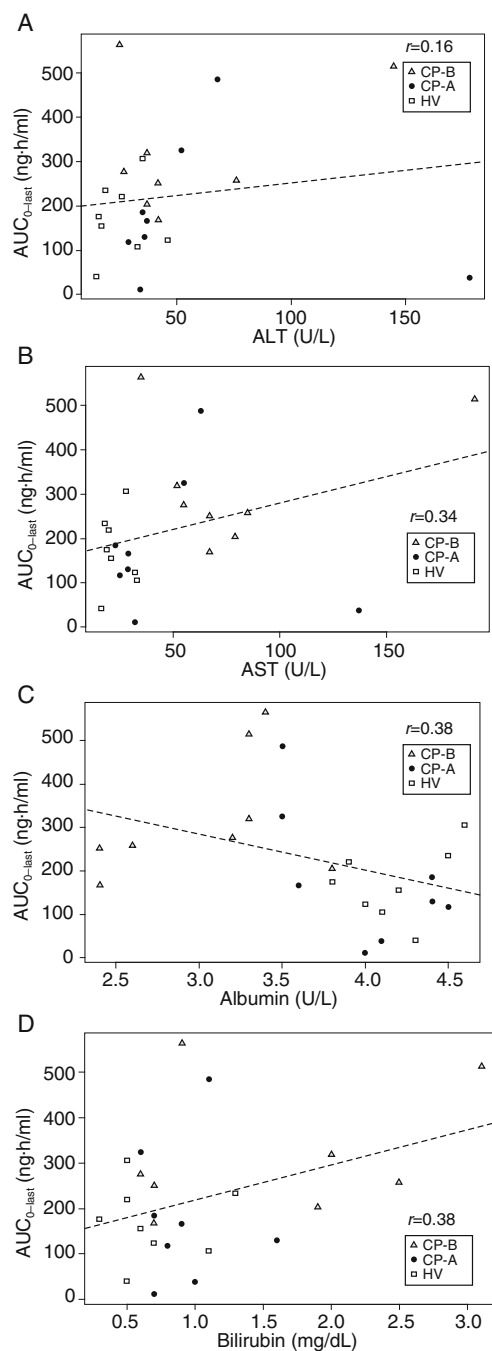
and urine granular casts  $>1$ /LPF, and two reports of urine protein  $\geq 1$ .

**Vital signs** There were no clinically significant changes in vital signs or ECGs. An increase in post-baseline systolic and diastolic blood pressure was observed for some subjects in the mild and moderate hepatic-impairment groups (mean increases of 24.9 mm Hg and 14.8 mm Hg, systolic and diastolic blood pressure, respectively, 16 h post dosing), but blood pressures returned to near baseline levels at the next collection time point on day 7. Transient and reversible increases in blood pressure following axitinib single-dose administration have been noted in previous studies of healthy volunteers.

## Discussion

The results from this study demonstrate three important findings. First, moderate hepatic impairment increased the exposure ( $AUC_{0-\infty}$  and  $C_{max}$ ) of axitinib compared with normal hepatic function; however, mild hepatic impairment did not change these pharmacokinetic parameters. Second, there were no overt changes in plasma protein binding of axitinib among the three subject groups. Third, a 5-mg single oral dose of axitinib was well tolerated in subjects





**Fig. 3** Correlation between measures of hepatic function and axitinib exposure determined by area under the concentration-time curve from time zero to time of the last measurable concentration ( $AUC_{0-last}$ ) for **a** alanine aminotransferase (ALT), **b** aspartate aminotransferase (AST), **c** serum albumin, and **d** bilirubin. Abbreviations: *CP* Child-Pugh class, *HV* healthy volunteers

with mild or moderate hepatic impairment and in those with normal hepatic function.

The impact of hepatic impairment on the pharmacokinetics and disposition of drugs as well as on their efficacy and tolerability is well known, and is of particular

importance in identifying patient populations that may be at increased risk for toxicity. Both the FDA and the European Medicines Agency have issued guidelines for the evaluation of the pharmacokinetics of drugs in clinical development in patients with impaired hepatic function [8, 15]. In this study, following administration of a 5-mg single oral dose of axitinib, the pharmacokinetics was not affected by mild hepatic impairment (CP-A). However, axitinib plasma exposure was increased approximately two-fold by moderate hepatic impairment (CP-B) compared with normal hepatic function.

The primary route of elimination for axitinib is via hepatobiliary excretion. Axitinib has an estimated low hepatic extraction ratio in humans ( $\sim 0.3$ , computed from observed systemic clearance after an intravenous dose to humans, experimentally observed blood-to-plasma ratio for axitinib, and theoretical hepatic blood flow in humans) and a high degree of plasma protein binding ( $>99\%$  bound). Based on the well-stirred model of hepatic clearance, the hepatic clearance of a low-extraction drug would be assumed to be the product of the intrinsic clearance and fraction unbound in plasma [16–18]. For low-extraction drugs with high plasma protein binding ( $\geq 90\%$ ), the free fraction of drug is typically increased in patients with liver cirrhosis, which is associated with an increase in plasma clearance [19, 20]. No overt changes in protein binding of axitinib in subjects with mild or moderate hepatic impairment were observed in this study and, therefore, the changes in exposure are likely due to a change in the intrinsic clearance. This is probably associated with lowered metabolic capacity in subjects with moderate impairment or shunting of hepatic blood flow resulting in drug bypassing the hepatic parenchymal cells (with metabolic enzymes).

Mechanisms for decreases in binding of drugs to plasma proteins with hepatic impairment include (1) changes in albumin synthesis, leading to lower levels of plasma albumin, and (2) accumulation of endogenous compounds such as bilirubin [21]. Expectedly, subjects with moderate impairment in this study had higher baseline bilirubin values compared with subjects in the mild-impairment group. Also, subjects with moderate hepatic impairment had lower serum baseline albumin levels compared with subjects with mild impairment. However, this did not translate into a meaningful change in plasma protein binding for axitinib; there were no overt changes in fraction unbound in plasma among the three groups assessed in the study. Presumably the altered lab parameters for albumin and bilirubin with hepatic impairment were not sufficient to elicit a change in fraction unbound for a drug (e.g., axitinib) that is highly protein bound ( $>99\%$ ). Further, the detection of minor changes in protein binding may be limited by the current bioanalytical methodology as well as the variability seen in protein binding between subjects. In addition, weak

correlations were found between axitinib exposure and clinical laboratory parameters that are typical measures of hepatic function, including AST, ALT, albumin, bilirubin, and prothrombin time. Since axitinib is metabolized by CYP1A2, albeit to a minor extent, relationships with smoking status were also evaluated since smoking has been shown to induce CYP1A2 [22]. Although only a small number of patients were evaluated, the study results indicated that axitinib exposure was similar in non-smokers, active smokers, and ex-smokers. Smoking status is being evaluated as a covariate for axitinib exposure in ongoing population pharmacokinetic analyses from multiple axitinib trials.

While the observed changes in axitinib pharmacokinetics with hepatic impairment did not precisely match those predicted by Simcyp, they were in general agreement, particularly for the moderate-impairment group. It should be noted that the Simcyp predictions for axitinib pharmacokinetics in subjects with normal hepatic function resulted in overprediction of the plasma exposures compared with what is observed clinically. The reasons for the differences between Simcyp-predicted and clinically observed data could be ascribed to the selected input parameters in the hepatic-impairment model used by the software and built-in standard physiological parameters used for the simulations. While the existing input parameters based on *in vitro* data underestimated the clinical plasma clearance for axitinib (Simcyp CL/F estimation of 8 L/h vs. observed human CL/F ~24 L/h), it was still able to reasonably predict the expected fold change in exposures with hepatic impairment. Overall, Simcyp-predicted 1.3- to 1.5-fold higher exposures expected with mild and moderate impairment and provided confidence in the selection of a 5-mg single dose for this clinical study when it was being designed, which was based on the previous safe administration of up to six-fold higher (i.e., 30 mg) single doses of axitinib in patients [6]. These results support the use of *in silico* modeling tools such as Simcyp to aid in the design of clinical studies.

This study also demonstrated that a single dose of axitinib was well tolerated in subjects with hepatic impairment, with no adverse events reported in either the mild- or moderate-impairment groups. Three treatment-related adverse events were reported during this study, all of which occurred in the group with normal hepatic function. These were mild or moderate events and resolved without further treatment. This study does not address the safety and tolerability of axitinib in cancer patients with hepatic impairment, but the data reported here may be used to guide dosing in these patients.

While the exact change in axitinib plasma exposure anticipated in subjects with severe hepatic impairment (CP-C) was not evaluated in this study, it can be reasonably expected

to exceed or equal changes observed in subjects with moderate hepatic impairment (CP-B). In ongoing studies of axitinib in patients with solid tumors, subjects are required to have adequate hepatic function at the time of study entry. However, for patients who might develop hepatic impairment during axitinib treatment, the results of this study indicate the need for appropriate dose reduction, particularly when the extent of hepatic impairment approaches moderate impairment as defined by CP criteria. Ongoing studies in cancer patients will provide guidance on appropriate dose reductions for axitinib based on observed safety profiles of individual patients. These study results indicate the need for particular attention to dose adjustments in patients who develop moderate (or worse) hepatic impairment.

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## References

- Hu-Lowe DD, Zou HY, Grazzini ML, Hallin ME, Wickman GR, Amundson K, Chen JH, Rewolinski DA, Yamazaki S, Wu EY, McTigue MA, Murray BW, Kania RS, O'Connor P, Shalinsky DR, Bender SL (2008) Nonclinical antiangiogenesis and anti-tumor activities of axitinib (AG-013736), an oral, potent, and selective inhibitor of vascular endothelial growth factor receptor tyrosine kinases 1, 2, 3. *Clin Cancer Res* 14:7272–7283. doi:10.1158/1078-0432.CCR-08-0652
- Rixe O, Bukowski RM, Michaelson MD, Wilding G, Hudes GR, Bolte O, Motzer RJ, Bycott P, Liau KF, Freddo J, Trask PC, Kim S, Rini BI (2007) Axitinib treatment in patients with cytokine-refractory metastatic renal-cell cancer: a phase II study. *Lancet Oncol* 8:975–984. doi:10.1016/S1470-2045(07)70285-1
- Rini BI, Wilding G, Hudes G, Stadler WM, Kim S, Tarazi J, Rosbrook B, Trask PC, Wood L, Dutcher JP (2009) Phase II study of axitinib in sorafenib-refractory metastatic renal cell carcinoma. *J Clin Oncol* 27:4462–4468. doi:10.1200/JCO.2008.21.7034
- Cohen EE, Rosen LS, Vokes EE, Kies MS, Forastiere AA, Worden FP, Kane MA, Sherman E, Kim S, Bycott P, Tortorici M, Shalinsky DR, Liau KF, Cohen RB (2008) Axitinib is an active treatment for all histologic subtypes of advanced thyroid cancer: results from a phase II study. *J Clin Oncol* 26:4708–4713. doi:10.1200/JCO.2007.15.9566
- Schiller JH, Larson T, Ou SH, Limentani S, Sandler A, Vokes E, Kim S, Liau K, Bycott P, Olszanski AJ, von Pawel J (2009) Efficacy and safety of axitinib in patients with advanced non-small-cell lung cancer: results from a phase II study. *J Clin Oncol* 27:3836–3841. doi:10.1200/JCO.2008.20.8355
- Rugo HS, Herbst RS, Liu G, Park JW, Kies MS, Steinfeldt HM, Pithavala YK, Reich SD, Freddo JL, Wilding G (2005) Phase I trial of the oral antiangiogenesis agent AG-013736 in patients with advanced solid tumors: pharmacokinetic and clinical results. *J Clin Oncol* 23:5474–5483. doi:10.1200/JCO.2005.04.192

7. Jamei M, Marciniak S, Feng K, Barnett A, Tucker G, Rostami-Hodjegan A (2009) The Simcyp(R) Population-based ADME Simulator. *Expert Opin Drug Metab Toxicol* Feb 6 [Epub ahead of print] doi:10.1517/17425250802691074
8. US Department of Health and Human Services Food and Drug Administration (2003) Guidance for industry pharmacokinetics in patients with impaired hepatic function: study design, data analysis, and impact on dosing and labeling. US Food and Drug Administration Center for Drug Evaluation and Research. <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm072123.pdf>. Accessed 2 November 2009
9. Child CG, Turcotte JG (1964) Surgery and portal hypertension. In: Child CG (ed) *The liver in portal hypertension*. WB Saunders, Philadelphia, pp 50–52
10. Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R (1973) Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 60:646–649
11. U.S. Department of Health and Human Services Food and Drug Administration CfDEaRC, Center for Veterinary Medicine (CVM) (2001) Guidance for industry bioanalytical method validation. US Food and Drug Administration Center for Drug Evaluation and Research. <http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070107.pdf>. Accessed 24 May 2010
12. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, Shah VP, Skelly JP, Swann PG, Weiner R (2007) Quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. *AAPS J* 9:E30–42. doi:10.1208/aapsj0901004
13. Nakai D, Kumamoto K, Sakikawa C, Kosaka T, Tokui T (2004) Evaluation of the protein binding ratio of drugs by a micro-scale ultracentrifugation method. *J Pharm Sci* 93:847–854. doi:10.1002/jps.20012
14. Pithavala YK, Tortorici M, Toh M, Garrett M, Hee B, Kuruganti U, Ni G, Klamerus KJ (2010) Effect of rifampin on the pharmacokinetics of Axitinib (AG-013736) in Japanese and Caucasian healthy volunteers. *Cancer Chemother Pharmacol* 65:563–570. doi:10.1007/s00280-009-1065-y
15. European Medicines Agency (2005) Guideline on the evaluation of the pharmacokinetics of medicinal products in patients with impaired hepatic function. EMEA (European Medicines Agency) Web site. <http://www.emea.europa.eu/pdfs/human/ewp/23390en.pdf>. Accessed 2 November 2009
16. Pang KS, Rowland M (1977) Hepatic clearance of drugs. I. Theoretical considerations of a “well-stirred” model and a “parallel tube” model. Influence of hepatic blood flow, plasma and blood cell binding, and the hepatocellular enzymatic activity on hepatic drug clearance. *J Pharmacokinet Biopharm* 5:625–653
17. Pang KS, Rowland M (1977) Hepatic clearance of drugs. II. Experimental evidence for acceptance of the “well-stirred” model over the “parallel tube” model using lidocaine in the perfused rat liver in situ preparation. *J Pharmacokinet Biopharm* 5:655–680
18. Pang KS, Rowland M (1977) Hepatic clearance of drugs. III. Additional experimental evidence supporting the “well-stirred” model, using metabolite (MEGX) generated from lidocaine under varying hepatic blood flow rates and linear conditions in the perfused rat liver in situ preparation. *J Pharmacokinet Biopharm* 5:681–699
19. MacKichan JJ (2006) Influence of protein binding and use of unbound (free) drug concentrations. In: Burton ME, Shaw LM, Schentag JJ, Evans WE (eds) *Applied pharmacokinetics & pharmacodynamics—principles of therapeutic drug monitoring*. Lippincott Williams & Wilkins, Philadelphia, pp 82–120
20. Blaschke TF (1977) Protein binding and kinetics of drugs in liver diseases. *Clin Pharmacokinet* 2:32–44
21. Verbeeck RK (2008) Pharmacokinetics and dosage adjustment in patients with hepatic dysfunction. *Eur J Clin Pharmacol* 64:1147–1161. doi:10.1007/s00228-008-0553-z
22. Sesardic D, Boobis AR, Edwards RJ, Davies DS (1988) A form of cytochrome P450 in man, orthologous to form d in the rat, catalyses the O-deethylation of phenacetin and is inducible by cigarette smoking. *Br J Clin Pharmacol* 26:363–372