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ORIGINAL ARTICLE

Population pharmacokinetic model of PI-88, a heparanase inhibitor

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Abstract
Purpose The aim of this study was to investigate typical population pharmacokinetic (PK) parameters, potential covariates, and interindividual and residual variabilities of PI-88, a heparanase endoglycosidase enzyme inhibitor being developed for the treatment of cancer.
Methods A population PK model of PI-88 was developed and evaluated using nonlinear mixed effects modeling (NONMEM). Plasma concentration versus time data was obtained from a total of 76 subjects that participated in phase I trials of PI-88 delivered subcutaneously (SC) at doses ranging from 80 to 315 mg. Overall, the PK effects of 12 clinical covariates were evaluated, including weight, age, creatinine clearance, body surface area, body mass index, sex, cancer (vs. healthy subject), docetaxel coadministration, prior chemotherapy, prior investigational therapy, prior radiotherapy and prior surgery.
Results Population PK analysis of the data-set showed that apparent clearance (CL/F) and apparent volume of distribution (V/F) of PI-88 were positively correlated with body surface area and the absorption rate constant (KA) was positively correlated with body mass index. In addition, CL/F was found to be significantly lower in patients with malignancies versus healthy subjects. By incorporating these covariates into the PK parameter equations, the interindividual variability of CL/F was reduced from 30.6 to 20.2% (decrease of 34%), V/F was reduced from 31.4 to 20.7% (decrease of 34.1%) and KA was reduced from 52.6 to 46.2% (decrease of 12.2%).
Conclusions This population PK model indicates that the PK variability of PI-88 can be significantly reduced by taking BSA into account when dosing this drug SC.

Keywords Population pharmacokinetics · NONMEM · PI-88 · Heparanase

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Introduction

PI-88 (phosphomannopentaose sulfate) is an anticancer agent undergoing clinical trials in patients with advanced malignancies. Currently, there are three active trials in patients with metastatic melanoma (NCT00130442), stage IIIB or stage IV non-small cell lung cancer (NCT00103389) and an advanced malignancy or stage IV melanoma (NCT00073892) (www.clinicaltrials.gov). This drug is a sulfated oligosaccharide mixture prepared by the extensive sulfonation of the oligosaccharide phosphate fraction obtained from mild acid-catalyzed hydrolysis of the extracellular phosphomannan produced by *Pichia holstii* yeast [11, 31]. In vitro and in vivo studies have shown PI-88 to be a potent inhibitor of tumor growth, metastasis and angiogenesis by virtue of its ability to inhibit heparin sulfate cleavage by heparanase and antagonize the interaction between proangiogenic growth factors and heparin sulfate [17, 18, 21, 22]. In addition to antitumor activity, PI-88 has also demonstrated anticoagulant activity [30, 31].

Initially, PI-88 was administered as a continuous 2-h intravenous (IV) infusion in 24 normal volunteers; other than the anticipated biological effect of prolonged activated partial thromboplastin time (APTT), no other significant biological, clinical or hematological changes were seen (PI-88 Investigational Brochure, Brisbane: Progen Industries 1999). However, in patients with advanced malignancies, thrombocytopenia was the dose-limiting toxicity (DLT), occurring in three of 14 subjects (21%), and appeared to be immune-mediated with the development of anti-heparin platelet factor 4 complex (AHPF4) antibodies [26]. Because the DLT was observed at a dose at which there was limited evidence of biological activity as measured by the surrogate of APTT, alternate routes of administration were investigated.

The first study was undertaken in 18 healthy male subjects to establish the bioavailability of PI-88 given subcutaneously (SC) compared with IV dosing and to assess the local tolerability of PI-88 given SC. In this study, PI-88 was well tolerated by SC administration with approximately 100% bioavailability. A second study was conducted in 42 patients with advanced malignancies to evaluate the maximum tolerated dose (MTD) of PI-88 (80–315 mg/day) administered SC using two different dosing regimens and to assess the safety and tolerability of PI-88 administered SC in patients with advanced malignancies [2]. This study concluded that the recommended dose of PI-88 for both dosing regimens was 250 mg/day. At this dose, PI-88 was generally well tolerated and antitumor activity was seen in 10 (26%) patients. In a third phase I trial, PI-88 was combined with the cytotoxic agent docetaxel because clinical efficacy of docetaxel has been shown to improve when combined with anti-angiogenic agents [6]. This combination was well tolerated with evidence of disease stability in a variety of malignancies. In addition, docetaxel did not significantly interact with or change PI-88 pharmacokinetic (PK) parameters nor did PI-88 interact with or affect docetaxel PK.

When given by SC administration, the PK of PI-88 showed low intrapatient variability, but significant interpatient variability. In the cancer patients, the coefficient of variation (CV) of the area under the plasma concentration–time curve (AUC) from the MTD (250 mg/day) was approximately 40%. The aim of this study was to determine the sources that contribute to this PK variability. To do this, we employed population PK analysis, the goal of which is to estimate the mean and variance of the PK parameters in the patient population and the relationships between these parameters and specific patient covariates [19]. Thus, the objective of this work was to develop and validate a population PK model for PI-88 to investigate the typical population PK parameters, potential covariates, and interindividual and intraindividual variabilities of PI-88 disposition in healthy subjects and patients with advanced solid tumors. This population PK model should make it possible to predict the PK parameters of a particular subject from his or her covariates alone and allow for the design of alternative dosing regimens that will enhance both efficacy and safety.

Methods

Clinical studies and pharmacokinetic data

The clinical and PK data used for the population PK analysis were acquired from three phase I clinical trials conducted by Progen Pharmaceuticals Ltd. (Brisbane, Australia) to evaluate the SC administration of fixed PI-88 doses. These studies involved a total of 76 subjects, 18 without cancer (healthy subjects) and 58 with solid malignancies. The first study, aimed at assessing the local tolerability of PI-88 given SC, was a randomized, double-blind, placebo-controlled, parallel groups, dose-escalation study where 18 subjects received a single SC dose into the anterior abdominal wall of 80, 120 or 160 mg of PI-88, or placebo (0.9% w/v saline solution). Eligible subjects included healthy males between 18 and 45 years of age and within 15% of normal body weight. Each subject dosed with PI-88 provided a single set of 20 blood samples for analysis. On the day of treatment, samples were collected into sodium citrate monovettes before dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 2.25, 2.5, 2.75, 3, 4, 5, 6, 8, 10, 12, 14, 16 and 24 h after PI-88 administration. Within 30 min of collection, samples were centrifuged at 4°C at 1,500*g* for 10 min. The plasma was promptly removed and aliquots were stored at −70°C until analysis.

The second trial, aimed at evaluating the toxicity and PK of PI-88 administered SC by two different dosing regimens, was a dual phase study detailed in Basche et al. [2]. Part 1 was a single-center, single-arm, open-label, dose-escalation involving 33 patients with advanced malignancies. PI-88 was administered by SC injection into the stomach, thigh or arm once daily for 4 days (with a 10-day observation period) for two treatment periods. The dose was escalated until the MTD was reached, starting at 80 mg/day ($n = 3$), 106 mg/day ($n = 6$), 140 mg/day ($n = 3$), 190 mg/day ($n = 3$), 250 mg/day ($n = 12$), up to a maximum of 315 mg/day ($n = 6$). Part 2 was a single-center, single-arm, open-label study based on the MTD determined in Part 1. The nine patients enrolled in Part 2 were administered PI-88 at either 190 mg/day ($n = 3$) or 250 mg/day ($n = 6$) by SC injection for four treatment periods of once daily dosing for 4 days (with a 3-day observation period). Patients eligible for both of these studies included adults with pathologically confirmed solid

malignancies that were refractory to standard therapy or for which no standard therapy existed. On the first day of treatment, subjects provided blood samples taken before dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 h after PI-88 administration. Additional sampling was done throughout the studies, but only the initial day 1 samples were used for the population PK analysis.

The third trial was an open-label dose-escalation study designed to evaluate the safety, toxicity, pharmacological properties and biological activity of PI-88 with fixed weekly docetaxel in 16 patients with advanced solid malignancies [6]. Eligibility criteria included patients with histological or cytopathological malignancies that were refractory to standard therapy or for which no standard therapy existed. PI-88 was administered SC for four consecutive days per week for 3 out of 4 weeks. Doses were as follows: 106 mg/day ($n = 3$), 140 mg/day ($n = 3$), 190 mg/day ($n = 3$) and 250 mg/day ($n = 7$). Docetaxel was administered once weekly (immediately after PI-88 administration on day 1). Blood samples were collected prior to PI-88 dosing and at 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 24 h after dosing on day 1. Additional samples were collected but only the initial day 1 samples were used for the population PK analysis.

Concentrations of PI-88 were measured in the plasma samples at the Centre for Studies in Drug Disposition (CSDD) (University of Queensland at Royal Brisbane Hospital, Australia) using a fluorescence quenching assay developed and validated by Progen Pharmaceuticals Ltd. The lower limit of quantification for this assay was 0.05 μg/mL.

Pharmacokinetic model

PI-88 concentration versus time data were analyzed by nonlinear mixed effects modeling using NONMEM version VI level 2.0 (ICON Development Solutions, Ellicott City, MD, USA) and PDx POP Version 3.0 (ICON Development Solutions). For likelihood approximations, the first-order conditional estimation method (FOCE), which simultaneously produces estimates of the population parameters (θ) and estimates of the random interindividual variability (η), was used. Interaction was allowed between η and ε (residual variability).

The population PK model for PI-88 was developed in two steps: (1) covariate-free (base) model development and (2) covariate model development. Base model development involved the determination of an appropriate structural model as well as models to describe interindividual and residual variability. In accordance with previous studies [2, 6], the structural model (PK profile) was described by a one-compartment linear model with first-order absorption (implemented in NONMEM by the ADVAN2 TRANS2 subroutine). Estimates of the basic PK model were clearance (CL), volume of distribution (V), and absorption rate constant (KA). As all doses were administered SC, the parameters CL and V were interpreted as CL/F and V/F, respectively.

To account for the random interindividual variability (η), several error models were tried, including additive, proportional, and exponential. For the random residual variability (ε, including intrasubject variability, model misspecification and experimental and measurement error), additive (homoscedastic), proportional (heteroscedastic) and combined additive and proportional error models were evaluated. For selection between competing non-hierarchical models, we used the following criteria: improvement in Akaike information criterion (AIC), calculated as -2 log-likelihood + (2P), where -2 log-likelihood is the NONMEM objective function value (OFV) and P is the number of parameters, goodness-of-fit diagnostic plots (scatter plots of model-predicted concentrations versus observed concentrations, residuals and conditional weighted residuals), and precision (percent relative standard error, %RSE) of estimates.

For the random interindividual variability (η), an exponential interindividual variance model (which corresponds to a log-normal distribution of PK parameters) performed best:

$$P_i = P_{\mathrm{TV}} \cdot \exp\left(\eta_{P_i}\right) \quad (1)$$

where P_i is the individual value of model parameter P, P_{TV} is the typical population value (geometric mean) of parameter P in the population, and η_{P_i} is the individual-specific interindividual random effect for individual i and parameter P and is assumed to be independently and normally distributed with mean zero and variance ω^2.

To account for the residual variability (ε), a combined additive and proportional variance model performed best:

$$C_{\mathrm{obs},ij} = C_{\mathrm{pred},ij} \cdot \left(1 + \varepsilon_{\mathrm{p}ij}\right) + \varepsilon_{\mathrm{a}ij} \quad (2)$$

in which $C_{\mathrm{obs},ij}$ is the jth observed concentration in the ith individual, $C_{\mathrm{pred},ij}$ is the jth predicted concentration from the ith individual, and $\varepsilon_{\mathrm{p}ij}$ and $\varepsilon_{\mathrm{a}ij}$ are the proportional and additive residual errors, respectively, for individual i and measurement j and are assumed to be independently and normally distributed with mean zero and variance σ^2.

Several criteria were used to evaluate the model performance in the progression from the base model to the final model. In addition to goodness-of-fit diagnostics plots and %RSE of the estimates mentioned previously, the likelihood ratio test was used for comparing rival hierarchical models; the difference between the NONMEM OFV between two hierarchial models (a full and a reduced model) is approximately distributed χ^2 with one degree of freedom IF exactly one parameter in the full model is fixed

in the reduced model. Accordingly, decreases in the OFV of $\geq$3.84, $\geq$6.63 and $\geq$10.83 are significant at $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively. Finally, the biologically feasibility of the fixed-effect and random-effect parameter estimates was assessed.

The initial screening for covariates was done by adding each individual covariate, with continuous covariates centered on the covariate mean (covariate minus covariate mean), to each PK parameter independently using a linear additive covariate-parameter model. A covariate was retained for further analysis if the model with the covariate (full model) decreased the OFV by $\geq$3.84 from the model without the covariate (reduced model). Each candidate covariate identified in the independent correlation analyses was evaluated for forward inclusion in the PK parameter model in a stepwise (one at a time) fashion. Using linear additive covariate-parameter models, covariates were added in the order of highest to lowest significance. Covariates decreasing the OFV by $\geq$6.63 from the previous hierarchial model were retained. Finally, a stepwise backward deletion was done for the full covariate model. Covariates were deleted in the order of lowest to highest significance and remained in the final population PK model if the deletion of the covariate resulted in a $\geq$10.828 increase in the OFV.

To evaluate the reliability and precision of the parameter estimates from the final model, a non-parametric bootstrap analysis was performed [9]. For this analysis, the original data-set was re-sampled with replacement to create 1,000 new data sets with the same number of patients as the original data-set. The new data sets were fitted individually to the final model and all model parameters, including 95% confidence intervals (CIs), were estimated.

To evaluate the predictive performance of the final model versus the base model, internal validation for both models was performed by cross-validation because it was not practical to collect new data for use as a validation set. For the cross-validation of each model, data splitting was repeated 10 times, creating 10 index sets comprised of 80% of the subjects and 10 corresponding validation sets comprised of the remaining 20% of the subjects. The parameter estimates were obtained for each index set and then fixed in the corresponding validation set and prediction errors were determined for PI-88 concentrations and AUCs as well as for the PK parameters CL, *V* and KA.

The population-predicted PI-88 concentration for each individual was taken directly from the NONMEM results file and compared with the respective observed PI-88 concentration for that individual. The AUC_{0-t} for each individual was then determined by non-compartmental analysis using the predicted and observed concentrations. The predicted PK parameters were calculated using the equations derived from the models and the individual-specific empirical Bayesian estimates of the PK parameters were used as the observed PK parameters. The prediction error (PE) was calculated as follows:

$$\text{PE} = \text{prediction} - \text{observation} \quad (3)$$

The mean prediction error (MPE) was used as a measure of bias and the mean absolute prediction error (MAPE) was used as a measure of precision [28].

Statistical analyses

Statistical analysis was performed using Prism v4.02 (GraphPad Software, San Diego, CA, USA). For the comparison of means, two-tailed paired Student's *t* tests were used with data that could be described by a parametric (normal) distribution. For data that was non-parametrically distributed, two-tailed non-parametric Wilcoxon's signed rank test for paired differences was used. To determine correlation coefficients, Pearson's correlation coefficient was used for parametric distributions and Spearman's rank correlation coefficient was used for non-parametric distributions.

Results

Base model development

A total of 940 plasma concentrations from 76 subjects enrolled in three phase I clinical trials were used for population PK analysis. The concentration–time profiles of these subjects are shown in Fig. 1, where considerable interindividual variability is evident. For example, in the PI-88 trials conducted in patients with advanced malignancies [2] and with docetaxel in patients with advanced malignancies [6], the CVs of the AUCs from the MTD (250 mg/day) were 39.8 and 38.6%, respectively. Much of this variability may be attributable to differences in individual patient characteristics which affect drug PK. The clinical covariates of the patients from all three trials combined are provided in Table 1.

In accordance with previous studies [2, 6], the structural model (PK profile) was described by a one-compartment linear model with first-order absorption. Therefore, the PK parameters investigated were clearance (CL), volume of distribution (*V*), and absorption rate constant (KA). As all doses were administered SC, the parameters CL and *V* were interpreted as CL/F and V/F, respectively. For the base model, the OFV was 460.694 and the interindividual variability (CV%) of CL, *V* and KA were 30.6, 31.4 and 52.6%, respectively.

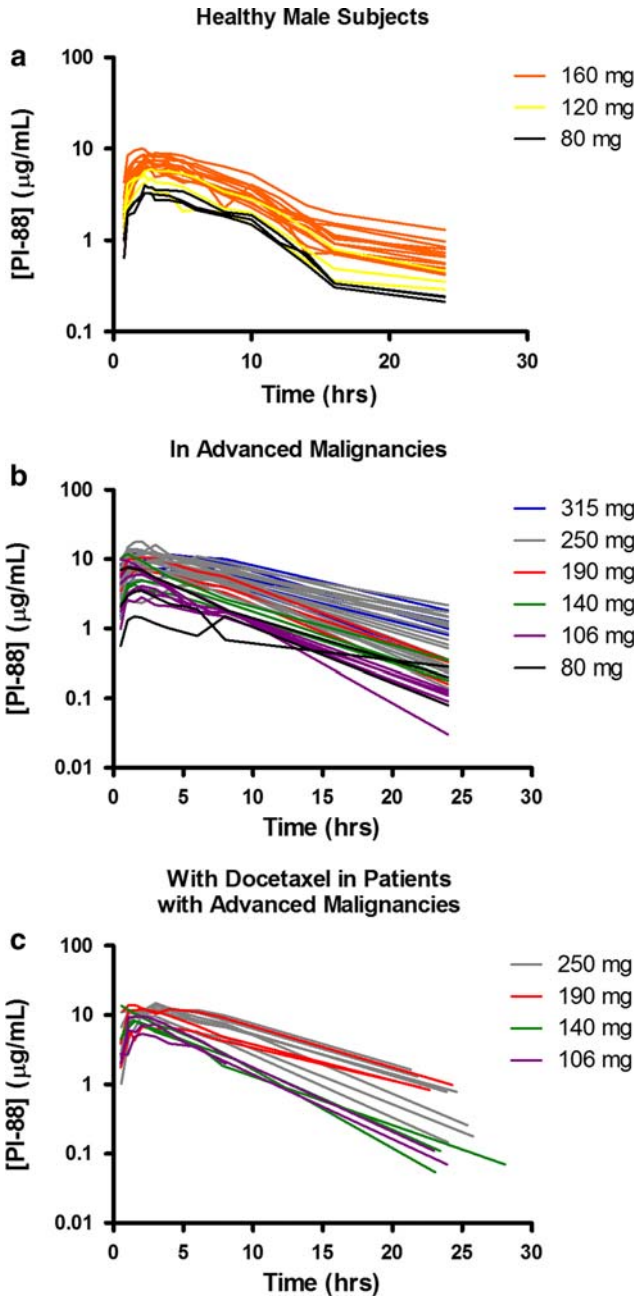

Fig. 1 Concentration–time profiles of subjects administered different doses of PI-88 SC in three phase I clinical trials. **a** 18 healthy male subjects, **b** 42 patients with advanced malignancies and **c** 16 cancer patients given PI-88 in combination with docetaxel. All data are from study day 1

Covariate model development

Individual covariate analyses using linear additive covariate-parameter models were performed for each PK parameter. A total of 12 covariates were analyzed, including weight (WT), age (AGE), creatinine clearance (CRCL), body surface area (BSA, calculated using the Dubois formula [8]), body mass index (BMI), sex (SEX), cancer (CANC) (vs. healthy subject), concurrent docetaxel administration (DOC), prior chemotherapy (CHEM), prior investigational therapy (INVT), prior radiotherapy (RADT) and prior surgery (SURG). All continuous covariates were centered on the covariate mean (covariate minus covariate mean). To discriminate between hierarchial models, a drop in the OFV by ≥ 3.84 ($P < 0.05$) from the base model was considered reason for inclusion as a potentially significant covariate in the forward full covariate model building. The independent correlation of each covariate revealed 10 of 12 covariates to be retained for CL, 6 of 12 covariates to be retained for *V*, and 3 of 12 covariates to be retained for KA. In order of most significant to least significant, for CL, these covariates were BSA, WT, SEX, CRCL, CANC, SURG, AGE, CHEM, DOC, and BMI; for *V*, these covariates were BSA, WT, BMI, SEX, DOC, and CRCL; and for KA, these covariates were BMI, WT and BSA.

Each candidate covariate identified in the independent correlation analyses was evaluated for forward inclusion in the PK parameter model in a stepwise (one at a time) fashion. Using linear additive covariate-parameter models, covariates were added in the order of highest to lowest significance. Covariates decreasing the OFV by ≥ 6.63 ($P < 0.01$) from the previous hierarchial model were retained. BSA, CRCL and CANC were found to significantly improve the model for CL, BSA and DOC for *V*, and BMI for KA.

Next, a stepwise backward deletion was done for the full covariate model. Covariates were deleted in the order of lowest to highest significance and remained in the final population PK model if the deletion of the covariate resulted in a ≥ 10.828 increase in the OFV ($P < 0.001$). The final models for the typical population values (TV) of each parameter are presented in Eqs. 4–6:

Table 1 Characteristics of patients administered PI-88

	Mean	Range
Continuous covariates		
Weight (kg)	76.8	44–82.3
Age (years)	48.5	19–78
Creatinine clearance (mL/h)	6,541.4	2,250–14,700
Body surface area (m^2)	1.90	1.39–2.55
Body mass index ($kg/m^{2)}$)	25.7	17.5–41.6
Categorical covariates		
Sex	48 males	28 females
Docetaxel	16 with	60 without
Cancer	58 with	18 without
Prior chemotherapy	45 with	31 without
Prior investigational therapy	16 with	60 without
Prior radiotherapy	30 with	46 without
Prior surgery	48 with	28 without

$$\text{TVCL} = 3,440 + [1,800 \times (\text{BSA} - 1.9)] - [(867 \times \text{CANC})] \quad (4)$$

$$\text{TVV} = 16,900 + [14,400 \times (\text{BSA} - 1.9)] \quad (5)$$

$$\text{TVKA} = 1.07 - [0.0401 \times (\text{BMI} - 25.7)]. \quad (6)$$

For the final model, the OFV was 335.167, a decrease of 125.527 from the base model. The goodness-of-fit plots of observed and final model-predicted PI-88 concentrations are shown in Fig. 2. The interindividual variability of CL was reduced from 30.6% in the base model to 20.2% in the final model, showing that BSA and CANC account for 34.0% of the intersubject variability (Fig. 3a). The interindividual variability of *V* was reduced from 31.4% in the base model to 20.7% in the final model, showing that BSA accounts for 34.1% of the intersubject variability (Fig. 3b). The interindividual variability of KA was reduced from 52.6% in the base model to 46.2% in the final model, showing that BMI accounts for 12.2% of the intersubject variability (Fig. 3c). The final value of the PK parameter CL for a healthy subject with the study average BSA of 1.9 and the study average BMI of 25.7 was estimated to be 3,440 mL/h, whereas for a cancer patient with the same BSA and BMI, the CL was estimated to be 2,573 mL/h. The final values of *V* and KA for subjects, regardless of cancer, with the study average BSA and BMI were 16,900 mL and 1.07 h^{-1}, respectively.

Model evaluation based on parameter estimates

To evaluate the stability and precision of the parameter estimates from the final model, a bootstrap analysis consisting of 1,000 runs was performed. The mean parameter estimates obtained from bootstrap analyses with successful runs in which both the estimation and covariance steps successfully converged (89.7%) were compared with those obtained from the final model and found to be highly consistent and within ±10% of each other, indicating that the final model estimates were precise. In addition, all of the parameter estimates of the final model fell within the

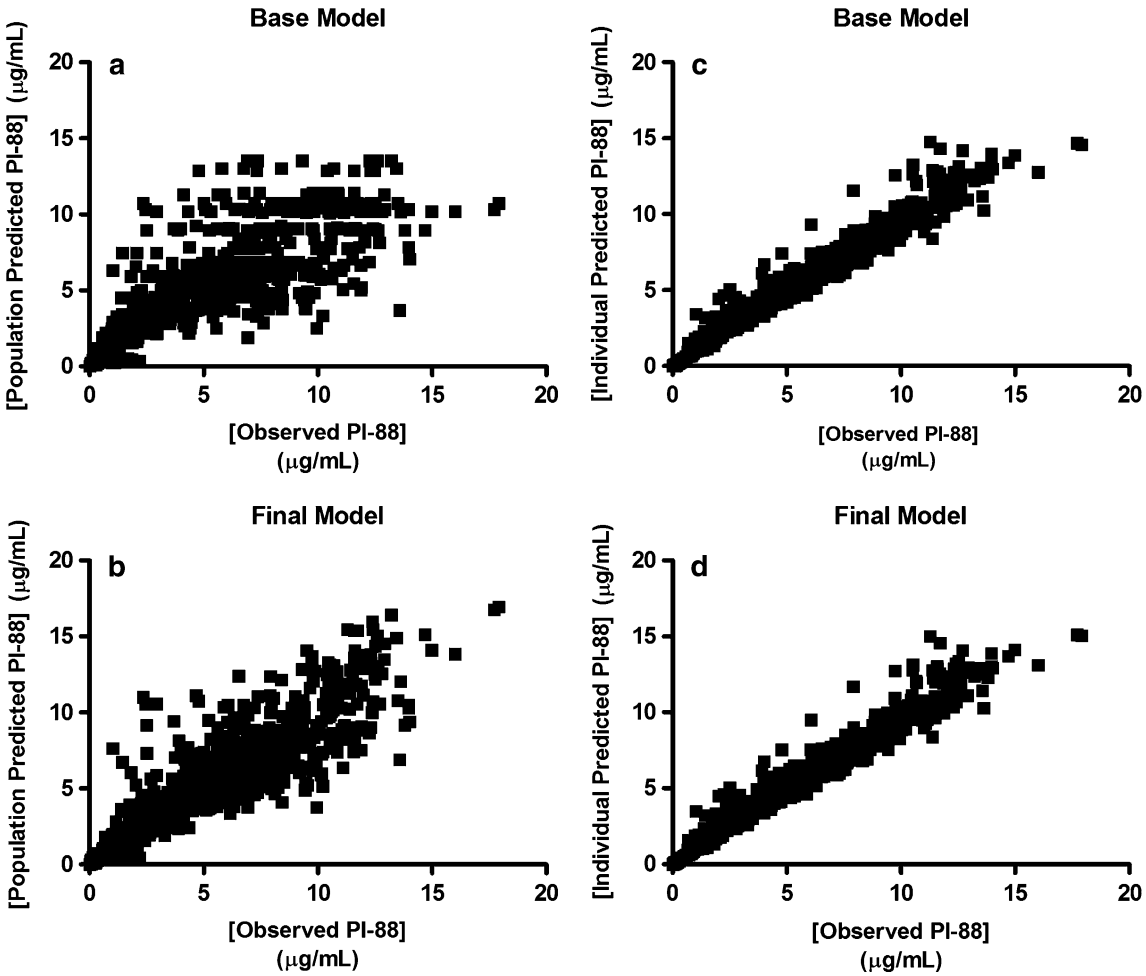

Fig. 2 Goodness-of-fit plots of observed and model-predicted PI-88 concentrations. **a** Base model-predicted population values, **b** final model-predicted population values, **c** base model-predicted individual values and **d** final model-predicted individual values

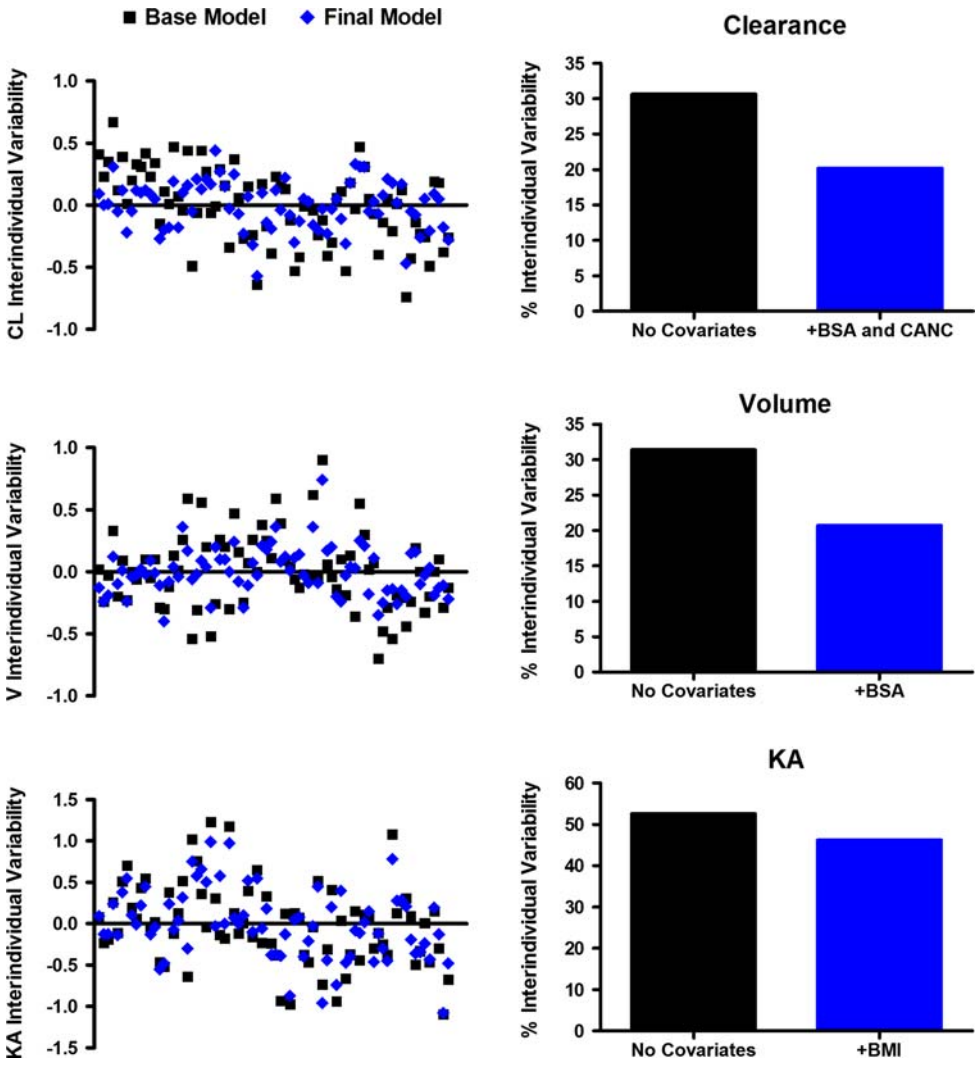

Fig. 3 Interindividual variability (η) reduction from base model to final model, **a** interindividual variability and percentage of interindividual variability in CL, **b** interindividual variability and percentage of interindividual variability in *V* and **c** interindividual variability and percentage interindividual variability in KA

Table 2 Cross-validation mean prediction errors (MPE) and mean absolute prediction errors (MAPE) of base model versus final model-predicted concentrations, AUCs, and PK parameters CL, *V* and KA

	Base model	Final model	*P* value
Predicted concentration			
MPE (μg/mL) (95% CI)	0.0181 (−0.1263–0.1624)	−0.0150 (−0.1374–0.1075)	<0.0001
MAPE (μg/mL) (95% CI)	1.459 (1.353–1.565)	1.201 (1.109–1.293)	<0.0001
Predicted AUC			
MPE (μg/mL per h) (95% CI)	−4.916 (−10.72–0.8905)	−2.744 (−7.230–1.741)	0.7097
MAPE (μg/mL per·h) (95% CI)	18.76 (14.74–22.78)	13.66 (10.41–16.90)	0.0005
Predicted CL			
MPE (mL/h) (95% CI)	−142.7 (−334.2–48.82)	−59.10 (−186.5–68.28)	0.2263
MAPE (mL/h) (95% CI)	682.5 (568–797)	461.2 (389.4–533)	0.0002
Predicted V			
MPE (mL) (95% CI)	−697.5 (−2,021–626.6)	−393.3 (−1,343–556.8)	0.5052
MAPE (mL) (95% CI)	4,003 (3,038–4,968)	2,763 (2,051–3,475)	0.0019
Predicted KA			
MPE (h^{-1}) (95% CI)	−0.1349 (−0.2947–0.0248)	−0.1467 (−0.3022–0.0087)	0.9814
MAPE (h^{-1}) (95% CI)	0.4458 (0.3195–0.5722)	0.4289 (0.3041–0.5536)	0.2449

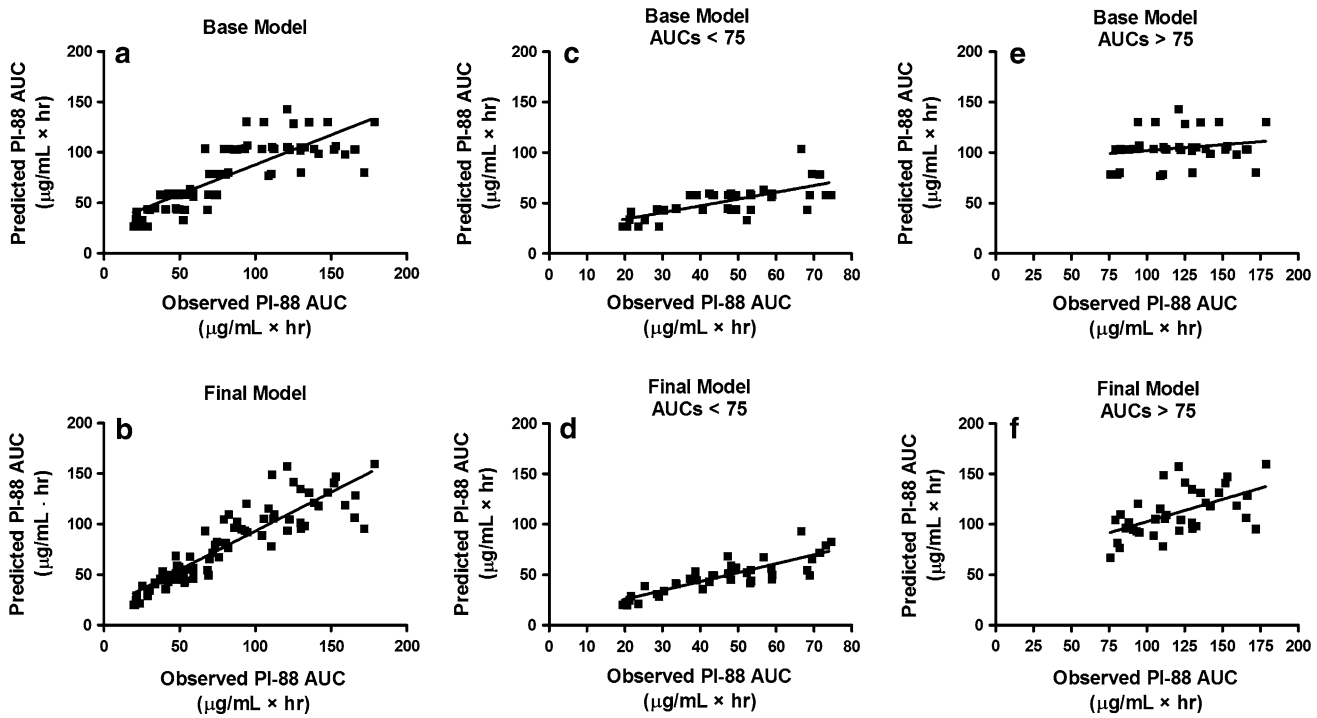

Fig. 4 Goodness-of-fit plots of observed and model-predicted PI-88 AUCs. **a** Base model-predicted AUCs, **b** final model-predicted AUCs, **c** base model-predicted AUCs <75 μg/mL per h, **d** final model-predicted AUCs <75 μg/Ml per h, **e** base model-predicted AUCs >75 μg/mL per h and **f** final model-predicted AUCs >75 μg/mL per h

95% CIs of the corresponding parameters obtained from the 1,000 bootstrap runs, indicating that the final model was stable.

Model evaluation based on predictive performance

To evaluate the predictive performance of the final model versus the base model, internal validation for both models was performed by cross-validation of each model. Data splitting was repeated 10 times, creating 10 index sets comprised of 80% of the subjects and 10 corresponding validation sets comprised of the remaining 20% of the subjects. The parameter estimates were obtained for each index set and then fixed in the corresponding validation set and prediction errors were determined. For PI-88 concentrations and AUCs as well as for the PK parameters CL and *V*, the final model showed superior predictive capabilities versus the base model in terms of both bias (MPE) and precision (MAPE) (Table 2). Moreover, the MAPEs were significantly better ($P < 0.002$) in all final model predictions except for KA. The observed and model-predicted AUCs from the base model and the full model are shown in Fig. 4. Spearman's rank correlation coefficients (non-parametric distribution) for the observed versus base model-predicted- and final model-predicted AUCs were 0.8346 and 0.9210, respectively. Both the base model and the final model were better at predicting the lower AUCs (<75 μg/mL per h), with Pearson's correlation coefficients (parametric distribution) of 0.6926 and 0.8440, respectively, and worse at predicting the higher AUCs (>75 μg/mL per h), with Pearson's correlation coefficients (parametric distribution) of 0.2034 and 0.5574, respectively.

Discussion

This is the first study describing a population PK NONMEM analysis of PI-88 and the influence of clinical covariates on PI-88 disposition. The results of this investigation are important because PI-88 shows significant interpatient variability when administered as a fixed dose. Currently, the PK/pharmacodynamic (PD) relationship between PI-88 exposure and efficacy and toxicity is unknown; however, it is likely that better control of drug exposure will lead to improved patient outcomes. Based on our population PK model, BSA and BMI should be taken into account when dosing PI-88. In addition, we have shown that healthy subjects and cancer patients clear this drug at different rates, which should be considered when extrapolating PK data from the former to the latter.

Regarding CL, our population PK model determined that BSA significantly influenced this PK parameter; larger BSA values resulted in greater PI-88 clearance rates and thus, different drug exposures. Previously, a two-step

population PK analysis was done looking at the effects of age, creatinine clearance and weight on PI-88 CL [2]; although none of these covariates were found to significantly correlate with CL, the *P* value for weight and CL was 0.0153. Therefore, it is not surprising that BSA, which incorporates weight, was found to significantly impact CL in our NONMEM population PK analysis.

The substantial effect of BSA on CL is exemplified by considering the study cancer patient with the smallest BSA value (1.39 m^2, patient 108) and the largest BSA value (2.55 m^2, patient 105). Both were administered a fixed dose of 106 mg of PI-88 that resulted in drug exposures (determined non-compartmentally) of 47.21 μg/mL per h for patient 108 and 28.53 μg/mL per h for patient 105. Thus, the patient with the smaller BSA value was exposed to nearly twice as much drug as the patient with the larger BSA value when PI-88 was given as a fixed dose. If we use Eq. 4 to compute the CL for each patient based on BSA, patients 108 and 105 clear PI-88 at rates of 1,655 and 3,743 mL/h, respectively. With this information, we can calculate the dose for each patient that would result in equivalent exposure using the following relationship:

$$\text{Dose} = \text{CL} \times \text{AUC} \quad (7)$$

Choosing the 106 mg cohort average exposure of 34 μg/mL per h as the target AUC, patients 108 and 105 should receive PI-88 doses of 56 and 127 mg, respectively. Clearly, this is more than a twofold difference in dose to achieve the same PI-88 exposure in cancer patients with differing BSA values, demonstrating the importance of taking BSA into account when dosing this drug.

The dosing of most anticancer agents has traditionally been adjusted according to BSA [15] based on the idea that physiologic parameters relevant to drug CL (metabolism and elimination), such as basal metabolic rate, renal and hepatic function and cardiac output, scaled between individuals according to BSA [7, 27]. However, there is weak evidence that BSA correlates with any measure of organ function except for cardiac output; accordingly, the routine use of BSA in dosing chemotherapeutics has been questioned [10, 14, 24]. In a retrospective assessment of 33 anticancer drugs (involving 21 classes of agents), only five (15%) of the chemotherapeutics were significantly associated with a reduction in interindividual PK variability when BSA-based dosing was employed [1]. Nevertheless, for the drugs that showed a significant relationship between BSA and CL, including eniluracil/5-FU (oral fluoropyrimidine), temozolomide (alkylating agent), docetaxel (antimicrotubule agent), paclitaxel (antimicrotubule agent) and troxacitabine (L-nucleoside analog), the relative reduction in the interindividual variability of CL was between 15 and 35% [1]. In our model, the incorporation of BSA into the equation for PI-88 CL decreased the interindividual variability by 22.6%, thus suggesting that dosing this drug based on BSA is warranted.

Although drug efficacy and toxicity are often correlated with plasma drug concentrations, these effects are ultimately the result of drug concentrations in tissues. The PK parameter *V* relates the amount of drug in the plasma to the amount of drug distributed in the tissues, which is a function of drug structure, tissue perfusion, cell membrane permeability and drug affinity for plasma and tissue molecules. In our population PK analysis of PI-88, *V* was found to be positively correlated with BSA and the incorporation of this covariate into Eq. 5 for this PK parameter reduced the interindividual variability by 34.1%. This dependence of *V* on BSA is consistent with the notion that blood volume is related to BSA [13, 25]. As *V* is also positively correlated with dose such that a subject with a greater *V* must receive a higher dose than a patient with a lesser *V* to achieve the same drug exposure in the plasma as well as in the target tissue(s), this further supports our conclusion that our study cancer patient with the largest BSA value (2.55 m^2, patient 105) should be administered more PI-88 than our study cancer patient with the smallest BSA value (1.39 m^2, patient 108), as their corresponding *V*s based on Eq. 5 are 26,260 and 9,556 mL, respectively.

The third PK parameter that was assessed in our population PK analysis was the KA of PI-88 when administered SC. We found that KA was significantly affected by BMI and including this term in Eq. 6 for KA decreased the interindividual variability from 52.6 to 46.2%, a 12.2% reduction. BMI is likely important for the KA of PI-88, as this drug was administered as a SC injection primarily into the abdominal fat on day 1 of the study (only data from day 1 was used in our analysis). Our Eq. 6 for KA indicates that a larger BMI value results in a smaller the KA. In other words, more adipose tissue leads to slower drug absorption, presumably due to differences in vascularization and thus, adipose tissue blood flow.

Another factor that could affect the KA of PI-88 is the injection site. Overall, 67% of subjects were injected into the abdominal fat, 4% into the thigh, and for the other 29% of subjects, the injection site was not documented. Because the data-set was incomplete, we were not able to incorporate injection site as a covariate in our population PK analysis of PI-88; however, it is possible that the initial anatomical location of the drug does have an effect on KA. This has been shown for human growth hormone [3, 20] and insulin [4, 29], both of which are absorbed faster from the abdomen than from the thigh. In addition, intraregional differences in the absorption of insulin from the abdominal wall have been demonstrated [12]. We suspect that both the regional and intraregional differences in the PI-88 injection site can partly account for the remaining (46.2%)

interindividual variability of KA. However, in this study, neither AUC nor C_{max} correlate with KA; so, although the interindividual variability is high for this PK parameter, it does not appear to be clinically relevant to PI-88 exposure or maximum concentration.

In addition to BSA and BMI, we also found that the presence of cancer (vs. healthy subject) affected the PK of PI-88. For two subjects with the study average BMI (1.9 m^2), the cancer patient and healthy subject would clear PI-88 at rates of 2,573 and 3,440 mL/h, respectively. Subsequently, if both subjects were administered a fixed dose of 250 mg of PI-88, the cancer patient's exposure would be 97.16 μg/mL per h and the healthy subject's exposure would be 72.67 μg/mL per h. This means that the cancer patient would have a 34% higher PI-88 exposure than a healthy subject with the same BSA. Thus, extrapolating PI-88 PK from a healthy subject to a cancer patient could be problematic and of clinical relevance if the toxic AUC is reached.

Slower clearance rates in cancer patients versus healthy subjects have been reported in studies involving Apomine™ (SR-45023A) [5] and tipifarnib [23]; healthy subjects were found to clear drug 300 and 21% faster than cancer patients, respectively. As mentioned in the work by Bonate et al. and Perez-Ruixo et al., possible reasons for these observations include a difference in protein binding, specifically α_1-acid glycoprotein, between these two subject populations. Concentrations of this protein are reported to be higher in cancer patients than in healthy subjects [16]. Thus, cancer patients will have less free drug in their plasma available for elimination, therefore, CL will occur more slowly.

In conclusion, a population PK model has been developed for PI-88 using NONMEM. This analysis identified BMI, BSA and the presence of cancer (vs. healthy subject) as significant covariates that affect the PK parameters of PI-88. Although BMI correlated with KA, KA did not affect drug exposure (AUC) or C_{max} and thus, is not likely to be of clinical relevance. In contrast, our data indicate that BSA should be considered when dosing PI-88. This will result in a decrease in PK variability and a greater likelihood of achieving the target AUC, which will enhance both efficacy and safety. Lastly, we show that healthy subjects and cancer patients clear PI-88 at different rates; thus, caution should be taken when extrapolating data from one population to another, as this may result in drug dosing that leads to unanticipated PI-88 exposure.

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