Cancer Chemother Pharmacol (2012) 69:15–24
DOI 10.1007/s00280-011-1644-6

ORIGINAL ARTICLE

Population pharmacokinetics of PM00104 (Zalypsis®) in cancer patients

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Received: 11 January 2011 / Accepted: 28 March 2011 / Published online: 18 May 2011

Abstract
Objective The aim of this study was to characterize the population pharmacokinetics of PM00104 (Zalypsis®) in cancer patients.
Methods A total of 135 patients included in four phase I clinical trials who receive intravenous PM00104 at doses ranging from 53 to 5,000 μg/m^2 and administered as 1-, 3-, or 24-h infusion every 3 weeks or as 1-h infusion on days 1, 8, and 15 of a 28-day cycle, or 1-h infusion daily during 5 consecutive days every 3 weeks were included in the analysis. Pharmacokinetic data were analyzed with non-linear mixed effect model using NONMEM VI software. The effect of selected patient covariates on PM00104 pharmacokinetics was investigated. Model evaluation was performed using predictive checks and non-parametric bootstrap.
Results An open four-compartment catenary linear model with first-order elimination was developed to best describe the data. Plasma clearance and its between-subject variability was 43.7 L/h (34%). Volume of distribution at steady state was 822 L (117%). Within the range of covariates studied, age, sex, body size variables, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, lactate dehydrogenase, creatinine clearance, albumin, total protein, hemoglobin, performance status, liver metastases, dose-limiting toxicity, and stable disease for 3 months were not statistically related to PM00104 pharmacokinetic parameters. Bootstrap and posterior predictive check evidenced the model was deemed appropriate to describe the time course of PM00104 plasma concentrations in cancer patients.
Conclusions The integration of phase I pharmacokinetic data demonstrated PM00104 linear elimination from plasma, dose proportionality up to 5,000 μg/m^2, and time-independent pharmacokinetics. No clinically relevant covariates were identified as predictors of PM00104 pharmacokinetics.

Keywords Cancer · PM00104 · Phase I · Clinical trial · Population pharmacokinetics · NONMEM

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Introduction

PM00104 (Zalypsis®) is a new synthetic cytotoxic agent related to jorumycins [1]. Although the exact mechanism of action of PM00104 has not been completely elucidated, preliminary insights suggest PM00104 exhibits effects on

the cell cycle and displays DNA binding properties as well as transcriptional inhibition [2]. PM00104 at high concentrations (150 nM) induced moderate apoptosis without previous cell cycle arrest, while at lower concentrations (<15 nM) induced a delay in the progression through the S phase of the cell cycle. Furthermore, PM00104 binds to DNA in vitro and these adducts induced the appearance of double-strand DNA breaks, which are a critical event in its cytotoxic action against tumor cells [3]. However, an additional non-DNA target may be required for PM00104 to elicit an optimal antitumor response. Actually, PM00104 displayed profound inhibition of the transcriptional response, with a similar pattern at all the concentrations studied. In addition, PM00104 strongly inhibited the activation of the transcription of other genes such as MDR1, a gene critically involved in resistance to many chemotherapeutic agents, without affecting constitutive transcription [4]. Thus, PM00104 appears to target selectively a set of cellular genes in a promoter-specific manner, besides binding to DNA.

PM00104 was chosen for clinical development as an antineoplastic agent because of its broad in vitro antiproliferative and antitumor activity against a number of human tumor cell lines and its significant in vivo cytotoxic response in several xenograft models, including breast, gastric, prostate, and renal tumors [1]. As xenograft studies hint at the possibility that selective administration schedules may be well suited for individual tumor settings, several Phase I dose-escalation studies in subjects with advanced tumors or lymphoma were conducted following different dosing schedule. Currently, the antitumor activity of PM00104 is under clinical investigation in ongoing Phase II studies in several cancer types.

Phase I dose-escalation studies in subjects with advanced tumors or lymphoma were conducted to determine the safety and tolerability and to identify the maximum tolerated dose (MTD) and the recommended dose (RD) of PM00104 for Phase II studies. In these studies, doses ranging from 53 to 5,000 μg/m^2 were intravenously administered as 1-, 3-, or 24-h infusion every 3 weeks or as 1-h infusion on days 1, 8, and 15 of a 28-day cycle or 1-h infusion daily during 5 consecutive days every 3 weeks. Under these dosage regimens, linear pharmacokinetics was demonstrated and no evidence of time-dependent pharmacokinetics was observed after several cycles of treatment. After intravenous administration, PM00104 plasma concentrations declined in a multi-exponential manner and, on average, the terminal half-life ($t_{1/2}$) was approximately 24 h. PM00104 was found to be widely distributed into the extravascular space, with apparent volumes of distribution at steady state (V_{ss}) around 800 L, and the binding to human plasma protein was estimated to be 99.37%. The PM00104 plasma clearance (*CL*) ranged from 30 to 50 L/h. The major routes of elimination have not been determined yet; however, preliminary results of metabolism studies indicated that PM00104 undergoes extensive microsomal-mediated metabolism in mouse, rat, dog, monkey, and human. In addition, PM00104 experienced moderate degradation in human plasma, with a $t_{1/2}$ of 2.77 h. Lastly, large interindividual variability was observed in plasma concentrations collected in Phase I studies [5].

In this analysis, the plasma pharmacokinetics of PM00104 following intravenous administration was characterized in cancer subjects using a population pharmacokinetic analysis after pooling the data obtained from four Phase I studies conducted up to date. In addition, the interindividual (between-subject) and interoccasion (within-subject) variabilities were quantified, and the effects of subjects' demographic characteristics and other covariates on pharmacokinetic parameters of PM00104 were evaluated.

Methods

Patient eligibility criteria and study design

Data from four Phase I studies conducted in patients with advanced solid tumor or lymphoma were pooled. All studies were conducted in accordance with principles for human experimentation as defined in the *Declaration of Helsinki* and were approved by the Human Investigational Review Board of each study center and by the Competent Authority of each country. Informed consent was obtained from each subject after being told about the potential risks and benefits, as well as the investigational nature of the study.

Patients were eligible for Phase I studies if they had histological or cytological confirmation of malignant tumor not amenable to established forms of effective therapy. Other eligibility criteria included an ECOG performance status <2, anticipated life expectancy of at least 3 months, and age >18 years. Previous anticancer radiation therapy and/or chemotherapy, if given, had to be discontinued for at least 4 weeks before entry into the study or 6 weeks in the case of pretreatment with nitrosoureas or mitomycin C. Patients had to have had a negative pregnancy test (only for female patients with reproductive potential) and normal hepatic and renal function, defined as bilirubin ≤1.5 times normal upper limit, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) ≤2.5 times normal upper limit (≤5 times normal upper limit in case of hepatic metastases), and serum creatinine ≤1.5 times normal upper limit. All patients had to have had acceptable bone marrow function, defined as white blood cells >3,500/μL, neutrophil count >1,500/μL, and platelets >100,000/μL. Patients

with one or more of the following criteria were not selected: prior extensive radiation therapy (>25% of bone marrow reserve); prior bone marrow transplantation or high-dose chemotherapy with marrow or stem cell rescue; concurrent radiation therapy, chemotherapy, hormonal therapy, or immunotherapy; participation in a clinical trial involving an investigational drug in the past 30 days or concurrent enrollment in another investigational trial; and any coexisting medical condition that was likely to interfere with study procedures and/or results.

In these four studies, patients received intravenous PM00104 as monotherapy at doses ranging from 53 to 5,000 μg/m^2 and given as 1-, 3-, or 24-h infusion every 3 weeks or as 1-h infusion on days 1, 8, and 15 of a 28-day cycle or 1-h infusion daily during 5 consecutive days every 3 weeks. Table 1 provides a summary of the study characteristics.

Sample collection and bioanalytical method

At the scheduled times detailed in Table 1, blood samples were collected in heparinized tubes and centrifuged within 20 min for 15 min at 2,500*g* at 4°C to separate plasma, which was stored at −20°C or lower until analysis. From the 139 patients treated in the 4 clinical trials, a total of 2,245 blood samples from 135 patients were collected to be analyzed. The analytical procedure involved the extraction of PM00104 from human plasma by a liquid/liquid method using methyl tert-butyl ether. The human plasma samples were analyzed using a validated high-performance liquid chromatography with tandem spectrometric detection (HPLC–MS/MS) assay by ICON Development Solutions (ICON. Manchester, UK) [6]. [$^{13}C_2$,D_3]PM00104 was used as the internal standard. The transitions monitored were m/z 692.3 → 218.17 for PM00104 and m/z 697.3 → 218.15 for [$^{13}C_2$,D_3]PM00104. The lower and upper limits of quantification were established at 15 and 9,500 pg/mL, respectively. The interassay accuracy and precision were 1.07% and 7.44%, respectively.

Pharmacokinetic model development

Software

Non-linear mixed-effects modeling by extended least squares regression using the first-order conditional estimation (FOCE) method was implemented with the NONMEM VI level 2.0 software package (ICON, Hanover, MD, USA). Compilations were achieved using DIGITAL Visual Fortran Version 6.6C. Graphical and all other statistical analyses, including the evaluation of NONMEM outputs, were performed using S-Plus 6.2 Professional Edition for Windows (Insightful, Seattle, WA, USA).

Structural model

Based on the exploratory graphical analysis, an open three-compartment model with linear elimination and distribution from the central compartment was used to describe the time course of PM00104 plasma concentrations after intravenous dosing. This model was fit to plasma concentrations obtained from cancer patients included in Phase I studies and the typical value of *CL*, central volume of distribution (V_1), intercompartmental flow between central and shallow (Q_2) and deep (Q_3) peripheral compartments, and volume of distribution of the shallow (V_2) and deep (V_3) peripheral compartments were obtained. As the structural model selection was a data-driven process,

Table 1 Characteristics of the clinical studies included in the population pharmacokinetic analysis of PM00104 (Zalypsis®)

Study	Sample size	Dose range (μg/m^2)	IV infusion duration (h)	Dosing days	Cycle (days)	Sampling schedule
PM104-A-001-04	27	225–3,600	1	1	21	POI, at 1.5 h after SOI[b] and at 0.16, 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 24, 48, 72, 120, 168, and 240 h after EOI
	19	1,800–3,500	3			
PM104-A-002-05	11	53–475	1	1–5	21	POI and at 0.16, 0.25, 0.5, 1, 2, 3, 4, 6, 24 h after EOI on days 1 and 5. At 48, 72, 120, 168, 240, and 336 h after EOI on day 5
PM104-A-003-05	37[a]	133–5,000	24	1	21	POI, at 2, 12 h after SOI, at 0.16 h before EOI, and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 24, 48, 72, 120, 168, 240 and 336 h after EOI
PM104-A-004-05	45	75–3,037	1	1, 8, 15	28	POI, at 0.16 h before EOI and at 0.25, 0.5, 1, 1.5, 2, 3, 4, 5, 7, 24, 48, 72, 120, and 168 h after EOI

POI prior of infusion, *SOI* start of infusion, *EOI* end of infusion

[a] Four patients have been excluded because an erratic PK profile due to sample collection from central line

[b] Only if drug was administered as 3-h infusion

additionally, more complex disposition models were tested during the analysis in order to optimize the number of compartments (three vs four compartments), its structure (mamillary vs. catenary model) and the number of elimination routes (one vs. two elimination routes).

Statistical model

Visual inspection of individual concentration–time profile suggested that each pharmacokinetic parameter varied between subjects. PM00104 full pharmacokinetic profile was available from two occasions in several subjects and evidenced random within-subject variability. As a limited number of subjects had full PK profiles in two occasions, the through levels available in these subjects were arbitrarily assigned to the second occasion. Between-subject (interindividual, IIV) and within-subject (interoccasion, IOV) variabilities in a pharmacokinetic parameter, P, were included in the model and assumed to be log-normally distributed, according to Eq. 1:

$$P_{j,k} = P^* \cdot e^{(\eta_j + \tau_k)} \quad (1)$$

where P_{jk} is an individual pharmacokinetic parameter for the jth individual and kth occasion, P^* is the typical value of the pharmacokinetic parameter, η_{pj} and τ_{pk} are independent and normally distributed between- and within-subject random variable with zero mean and variance ω_P^2 and π_P^2, respectively. The magnitudes of IIV and IOV were expressed as coefficients of variations (CVs). Residual variability was evaluated using an additive error model after natural logarithmic transformation of the measured concentrations and model predictions, according to the Eq. 2:

$$\ln C_{\text{obs}} = \ln C_{\text{pred}} + \varepsilon \quad (2)$$

where C_{obs} was the observed plasma PM00104 concentration; C_{pred} was the corresponding model predicted concentration; and ε was the residual departure of the natural logarithm of the observed concentration from the natural logarithm of predicted concentration in plasma, which was assumed to follow an independent random Gaussian distribution with mean zero and variance, σ^2.

Model selection criteria

The improvement in the fit obtained for each model was assessed in several ways. First, the resulting NONMEM generated minimum value of the objective function (MFOV) after fitting the models evaluated was used to perform the likelihood ratio test. This test is based on the change in the minimum value of the objective function (ΔMVOF), which is equal (up to a constant) to minus twice the log-likelihood of the data and is asymptotically distributed like χ^2 with the degrees of freedom equal to the number of parameters added to the model. For hierarchical models, a ΔMVOF of 7.88 was required to reach statistical significance ($P = 0.005$) for the addition of one fixed effect. In addition, the improvement in the model fit by including covariates into the population pharmacokinetics model was assessed by the reduction in the IIV, IOV, and residual variability, the reduction of the standard errors and correlations between parameters, and the examination of diagnostic plots.

Covariate analyses

Covariates listed in Table 2 were explored as possible sources of IIV in PM00104 pharmacokinetics. However, height was not tested as independent covariate because of its correlation with body weight. Height was only used to calculate the body surface area, lean body mass, and ideal body weight, which were explored graphically but not formally tested for significance. If after the covariates analysis, body weight was identified as a significant covariate, then body surface area, lean body mass, and ideal body weight would be evaluated to investigate whether any of these covariates improve the fit relative to the body weight. Missing values for the quantitative covariates were imputed using the median value in each dataset, and missing values for categorical covariates were analyzed as an independent category.

Once the structural model was identified, empirical Bayes estimates (EBE) of interindividual random effects were computed and the shrinkage was evaluated. The covariate screening was guided by graphical assessment and stepwise linear regression of the relationships between the EBE and the covariates. After the univariate analysis in NONMEM, the covariates with statistically significant effects on pharmacokinetic parameters (χ^2: 7.88, *df*: 1, $P < 0.005$) were incorporated simultaneously into the population model to obtain the intermediate population pharmacokinetic model. A full model was determined when no additional improvement seems possible by including additional covariates. Then, the relative contribution of each covariate to the goodness of fit was re-evaluated by deleting the covariates from the full model. If the exclusion of a fixed effect resulted in an increase in MVOF of less than 10.83 (χ^2 test, *df*: 1, $P < 0.001$), the covariate was deleted from the model. An apparently conservative P value was selected to avoid the inclusion of weak and clinically non-relevant effects and to control for multiple testing. With this methodology, only covariates showing significant contributions were kept in the pre-final population model.

Table 2 Summary of subjects' characteristics at baseline

Subject characteristics	Analysis dataset	PM104-A-001-04	PM104-A-002-05	PM104-A-003-05	PM104-A-004-05
Age (year)	58.1 (20–79)	58 (20–79)	56 (43–72)	59 (31–78)	58 (37–77)
Body weight (kg)	74.4 (40.4–136.4)	72.6 (42.7–117.0)	87.2 (40.4–136.4)	74.4 (50.0–104.0)	73.0 (45.0–120.0)
Body surface area (m^2)	1.84 (1.29–2.43)	1.81 (1.29–2.25)	1.96 (1.32–2.40)	1.82 (1.47–2.18)	1.85 (1.41–2.43)
ALP (xULN)	1.12 (0.26–8.34)	1.16 (0.26–8.34)	0.96 (0.45–2.38)	1.09 (0.45–2.18)	1.13 (0.39–3.45)
ALT (xULN)	0.61 (0.13–2.91)	0.56 (0.15–1.96)	0.45 (0.17–0.85)	0.65 (0.23–2.27)	0.66 (0.13–2.91)
AST (xULN)	0.80 (0.19–4.08)	0.62 (0.19–2.98)	0.69 (0.50–1.03)	0.89 (0.35–3.03)	0.95 (0.30–4.08)
Total bilirubin (xULN)	0.54 (0.16–1.52)	0.50 (0.16–1.52)	0.96 (0.45–2.38)	0.60 (0.18–1.30)	0.55 (0.18–1.40)
Serum creatinine (mg/dL)	0.92 (0.51–1.64)	0.93 (0.56–1.64)	1.01 (0.60–1.30)	0.95 (0.51–1.53)	0.94 (0.55–1.53)
Creatinine clearance (mL/min)[a]	86.4 (33.9–150.0)	85.5 (52.8–132.0)	89.3 (33.9–150.0)	82.5 (51.4–126.0)	89.2 (44.0–140.7)
Serum albumin (g/dL)	3.77 (2.40–6.40)	3.70 (2.40–4.90)	3.90 (3.00–4.50)	4.02 (3.29–4.46)	3.65 (2.50–6.40)
Total protein (g/dL)	7.09 (5.30–9.17)	7.13 (5.30–8.60)	7.10 (5.80–7.80)	7.13 (5.78–9.17)	7.02 (5.80–8.20)
Hemoglobin (g/dL)	12.5 (9.2–15.9)	12.6 (9.9–15.9)	11.6 (10.2–14.6)	12.7 (10.4–15.1)	12.4 (9.2–15.4)
Sex (*N*, %)					
Male	87 (64.4)	33 (71.7)	4 (36.4)	21 (67.7)	29 (61.7)
Female	48 (35.6)	13 (28.3)	7 (63.6)	10 (32.3)	18 (38.3)
Liver metastases (*N*, %)					
No	78 (58.2)	32 (71.1)	7 (63.6)	18 (58.0)	21 (44.7)
Yes	56 (41.8)	13 (28.9)	4 (36.4)	13 (42.0)	26 (55.3)
Performance status (*N*, %)					
0	55 (41.1)	25 (62.5)	5 (83.3)	19 (61.3)	6 (14.6)
1	79 (58.9)	20 (37.5)	6 (16.7)	12 (38.7)	41 (85.4)
Stable disease (*N*, %)					
No	119 (88.1)	39 (84.8)	8 (72.7)	29 (93.6)	43 (91.5)
Yes	16 (11.9)	7 (15.2)	3 (27.3)	2 (6.4)	4 (8.5)

Continuous variables are expressed as median (range), whereas categorical variables are expressed as counts, *N*, and percentage (%)

ULN Upper Limit of Normality, *ALP* alkaline phosphatase, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

[a] Missing covariates are expressed as percentage of subjects in the combined dataset with missing values

[b] Creatinine clearance was calculated using the Cockroft and Gault's formula and values higher than 150 mL/min were truncated to 150 ml/min

Model refinement

The distribution of the interindividual random effects and the correlation between them were examined graphically to evaluate the normality and the independence assumption, respectively. The random effects with the highest correlation were tested by including the corresponding non-diagonal elements in the matrix of random effects. If implementing a correlation significantly improved the fit ($\Delta MVOF > 10.83$), the off-diagonal element of the random effects matrix was kept in the model and the process was repeated until no further improvement of the fit could be achieved.

Model qualification

A non-parametric bootstrap analysis was performed as an internal model evaluation technique, using the package Wings for NONMEM VI (N. Holford, Version 616, Auckland, New Zealand). A new replication of the original dataset (a bootstrap sample) was obtained by *N* random draws of individual data (with replacement) from the dataset including Phase I clinical studies. The final population pharmacokinetic model was re-fitted to each new dataset, and this process was repeated 500 times with different random draws. The stability of the final model was evaluated by visual inspection of the distribution of the model parameter estimates from the new datasets and compared with that obtained from the fit of the original dataset. Bootstrap runs with unsuccessful minimization were evaluated and excluded from further analysis, if deemed appropriate. The final model parameter estimates were compared to the mean and 95% confidence intervals of the non-parametric bootstrap replicates of the final model. If the parameter estimates fall into the 95% confidence interval obtained from the bootstrap analysis, the model was considered unbiased.

A visual predictive check was made using the technique described by Yano et al. [7] The parameter estimates obtained by fitting the final population pharmacokinetic

model to the data were used to simulate the population pharmacokinetic profile in plasma after the intravenous administration of PM00104 3 mg/m^2 as 1-h infusion and 4 mg/m^2 as 24-h infusion. A non-parametric 90% prediction interval around the median plasma concentration was constructed to quantify the variability in the model predictions and to visually compare with the observed dose-normalized plasma concentrations.

In addition, a standardized visual predictive check (SVPC) was also conducted and stratified by the duration of the infusion. This test was performed by plotting the percentile of each observation in the dataset in relation to its 100 simulated observations derived from the final model as a function of the predicted plasma concentrations. If the model and population parameter estimates are adequate, these percentiles should be uniformly distributed between 0 and 100. The percentage of points below 20th, 50th, and 80th percentile was calculated [8].

Results

An open three-compartment mamillary model with linear distribution and elimination from the central compartment provided a reasonable description of the time course of intravenous PM00104 plasma concentration (MVOF = −2,338.081). However, overprediction of the plasma concentrations observed during the first hours after the end of the infusion was noticed. The goodness of fit was improved by the inclusion of an additional route of elimination from a peripheral compartment (ΔMVOF = −17.763) or the switch from a mamillary to a catenary model structure (ΔMVOF = −37.624) or both (ΔMVOF = −59.916). Nevertheless, a four-compartment mamillary model provided a substantially better fit to the dataset than the initial three-compartment model (ΔMVOF = −366.283) or its modifications mentioned before. Additional further improvement of the fit was achieved by the catenary structure of the four-compartment model and the inclusion of an additional route of elimination from a peripheral compartment (ΔMVOF = −82.024). However, this model did not minimize successfully and showed signs of over-parameterization, probably due to the presence of several outlier concentrations. The exclusion of both the eight plasma concentrations with absolute weighted residuals higher than five and the elimination pathway from peripheral compartment provided an additional improvement in the goodness of fit (MVOF = −3,552.395). The residual variability was reduced by one-third after the exclusion of outliers. After excluding the outliers, the catenary structure of the four-compartment model still improved the goodness of fit as compared with the mamillary structure (ΔMVOF = −15.021). The residual variability was also

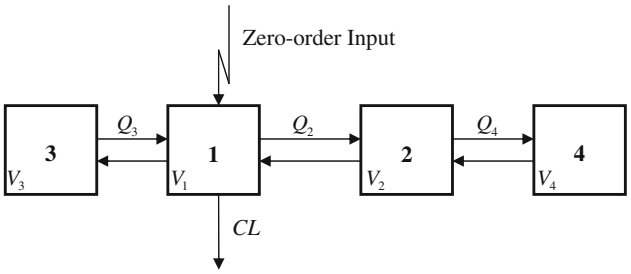

Fig. 1 Schematic of the pharmacokinetic model for PM00104

13% lower for the catenary structure of the four-compartment relative to the three-compartment model.

A schematic representation of the final structural model is presented in Fig. 1. In this structural model, between-subject variability was estimated for CL, V_1, V_2, Q_3, and V_3. Since several subjects received PM00104 in more than one occasion, and pharmacokinetic data available evidenced random within-subject variability, the inclusion of within-subject variability in the model parameters that vary across subjects was subsequently tested. While the inclusion of within-subject variability in CL provides a better fit to the data (ΔMVOF = −156.163) and reduced the residual variability by 15.5%, adding within-subject variability terms for other model parameters did not further improve the model fit significantly. The graphical and statistical exploration of the correlations among the between-subject variability terms revealed a potential association between CL, V_2, and Q_3, and these correlations were further evaluated in NONMEM by estimating the non-diagonal elements of the variance–covariance matrix of the between-subject random effects. Including the correlation between CL, V_2, and Q_3, on top of the within-subject variability in clearance, improved significantly the model fit (ΔMVOF = −86.857) and stabilized the model convergence.

Collectively, these results suggest an open four-compartment catenary pharmacokinetic model with linear elimination from central compartment, linear distribution from central compartment directly into a shallow compartment, and also linear distribution from central compartment into a deep compartment through a catenary compartment is suitable to describe the PM00104 plasma pharmacokinetic profile in cancer subjects who receive intravenous administration of the dosing regimens evaluated in Phase I clinical studies. The parameter estimates and their associated precisions, measured as relative standard error (RSE), are presented in Table 3. All structural model parameters, so-called fixed effects, were estimated with high precision (RSE < 14%). Statistical model parameters, or random effects, were estimated with moderate precision (RSE < 42%), except the within-subject variability in CL probably due to the limited amount of subjects with data in more than one occasion (N = 33).

Table 3 Non-parametric bootstrap analysis and parameter estimates (relative standard errors) of the PM00104 population pharmacokinetic model

Model parameters	Original dataset	Non-parametric bootstrap ($N = 300$ replicates out of 500)	
	Estimate (RSE %)	Mean (RSE %)	95% confidence interval
CL (L/h)	43.7 (3.43)	43.6 (3.11)	40.9–46.5
V_1 (L)	32.7 (12.4)	33.3 (11.4)	26.5–41.4
Q_2 (L/h)	123 (5.76)	121 (6.41)	107–137
V_2 (L)	162 (8.33)	166 (9.77)	138–203
Q_3 (L/h)	11.3 (13.2)	11.4 (14.6)	8.0–14.4
V_3 (L)	388 (11.8)	389 (7.37)	332–443
Q_4 (L/h)	62.3 (9.00)	60.3 (9.43)	48.9–70.0
V_4 (L)	239 (9.00)	233 (9.24)	195–273
Interindividual variability (CV %)			
ω_{Cl}	34.1 (24.6)	33.7 (37.1)	28.9–39.9
ω_{V1}	82.5 (37.7)	78.7 (17.1)	50.8–100
ω_{V2}	65.1 (41.8)	64.0 (21.3)	48.9–76.1
ω_{Q3}	87.7 (31.2)	84.2 (30.9)	60.3–100
ω_{V3}	52.0 (25.2)	50.8 (24.2)	39.5–63.1
Interoccasion variability (CV %)			
π_{Cl}	14.1 (96.0)	14.0 (46.0)	8.88–21.1
Interindividual variability correlations (R^2)			
$\omega_{Cl,}\ \omega_{V2}$	0.555 (78.6)	0.681 (29.4)	0.503–0.911
$\omega_{Cl,}\ \omega_{Q3}$	0.572 (33.6)	0.599 (24.1)	0.453–0.749
$\omega_{V2,}\ \omega_{Q3}$	0.522 (84.0)	0.397 (61.1)	0.186–0.666
Residual variability (CV %)			
σ	18.3 (10.5)	18.3 (9.84)	16.8–19.9

In addition, diagnostic plots showed tight normal scatter around the line of identity and indicated an absence of bias (Fig. 2), while histograms of the EBE of pharmacokinetic parameters exhibited centered distribution around the population typical value (data not shown) and low shrinkage (<0.22). In addition, relatively low correlations ($R^2 < 0.15$) between interindividual random effects without non-diagonal elements of the variance covariance matrix were observed. In this model, continuous and categorical patient covariates were explored. Graphical and statistical screening analyses of covariates did not evidence any significant trend ($R^2 < 8\%$) and no covariates were further tested in NONMEM. Therefore, the model described above is considered the final model.

Non-parametric bootstrap analysis was used as an internal model evaluation technique to qualify the model developed. From the 500 bootstrap replicates, 40% failed to minimize successfully due to rounding errors and were excluded from the analysis. The average difference between the estimate of fixed and random effects obtained from the replicates that minimized successfully and those obtained from the replicates that failed to minimize successfully due to rounding errors was less than 6%, after excluding two replicates with V_3 estimates higher than 10,000 l and one replicate with IIV estimate of Q_3 higher than 1,600%. Consequently, the exclusion of the replicates that failed to minimize successfully due to rounding errors is not expected to generate any significant bias in the results of the non-parametric bootstrap. The analysis of the results from the 300 bootstrap replicates that minimized successfully is provided in Table 3. The population estimates for the final model were very similar to the mean of the 300 bootstrap replicates and were contained within the 95% confidence intervals obtained from the bootstrap analysis. Besides the accuracy, the precision of the NONMEM parameter estimates was also good, because the relative standard error from the bootstrap analysis for the fixed and random effects was lower than 15 and 47%, respectively, except for the correlation between V_2 and Q_3 (61.1%).

The results of the visual predictive check performed on the normalized PM00104 plasma concentration for 1- and 24-h intravenous infusion are presented in Fig. 3. In these plots, the percentiles 5th, 50th, and 95th percentiles of the model-based prediction for plasma concentrations are presented with the observed PM00104 plasma concentrations under the same conditions. The figures evidence that the model developed is appropriate to describe the time course of PM00104 plasma concentrations in cancer

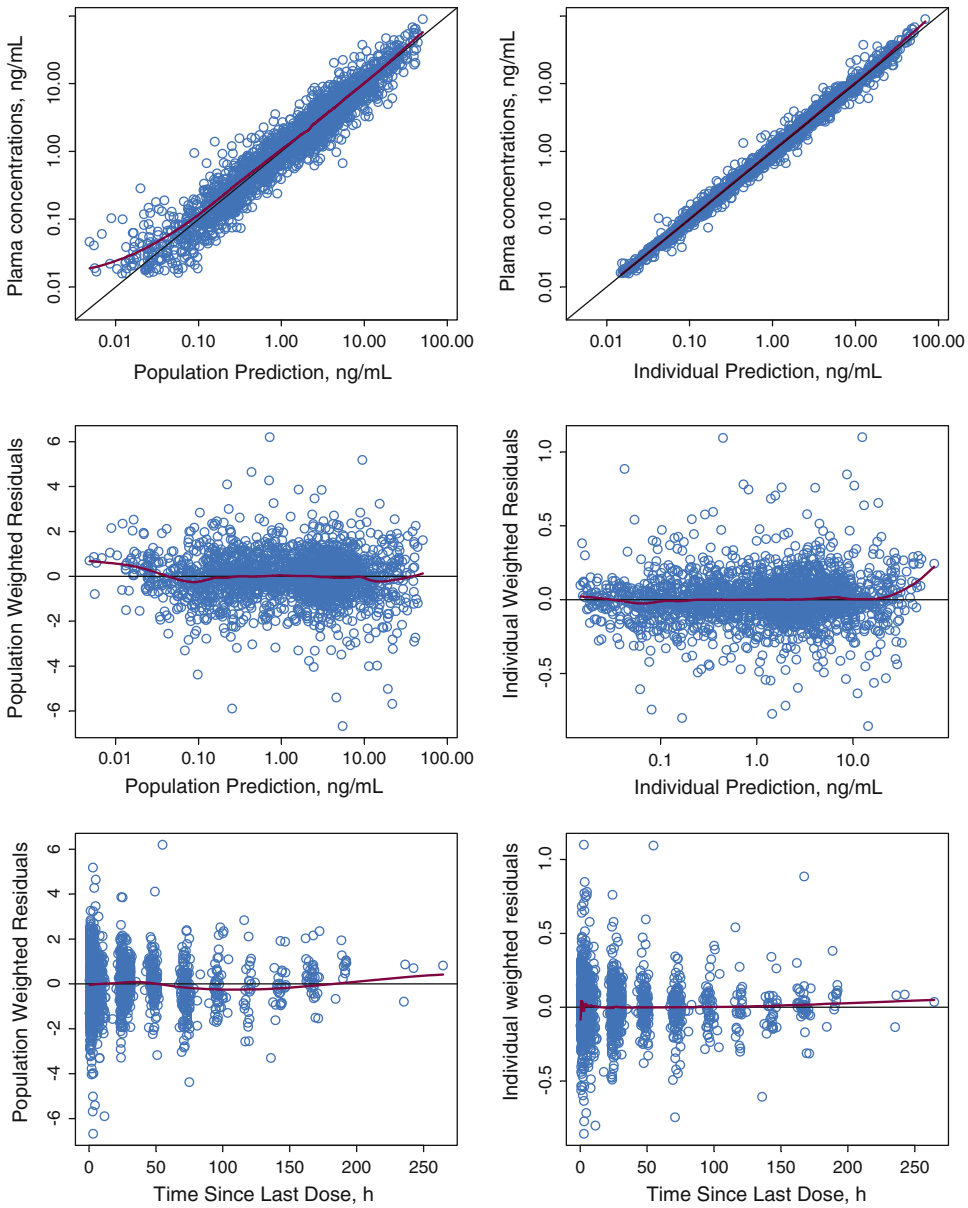

Fig. 2 Diagnostic plots of the population pharmacokinetic model of PM00104

patients. Furthermore, the results of the SVPC presented in Fig. 4 also confirm the adequacy of the model to describe the data. Overall, the validation results are satisfactory for this large and diverse dataset.

Discussion

The primary goal of this analysis was to characterize the concentration–time profile of PM00104 in plasma following different intravenous dosing schedules evaluated in Phase I clinical studies. As PM00104 plasma concentrations decline in a multi-exponential manner after intravenous administration, an open four-compartment catenary pharmacokinetic model with linear distribution and elimination from central compartment was used to describe the pharmacokinetics of PM00104 in plasma. The model assumes a direct distribution to the shallow compartment and a distribution to the deep peripheral compartments through a catenary compartment off the central compartment. This model has been also applied to other cancer treatments with drugs such as trabectedin [9].

A single clearance parameter described the linear elimination of PM00104 by all routes of elimination, including renal and non-renal pathways. In cancer subjects, the typical value of the estimated PM00104 plasma clearance was 43.7 L/h and there was a moderate between-subject (34%) and a relatively smaller within-subject variability (14%). The estimates of clearance and its variabilities are consistent with the estimates obtained in previous non-compartmental

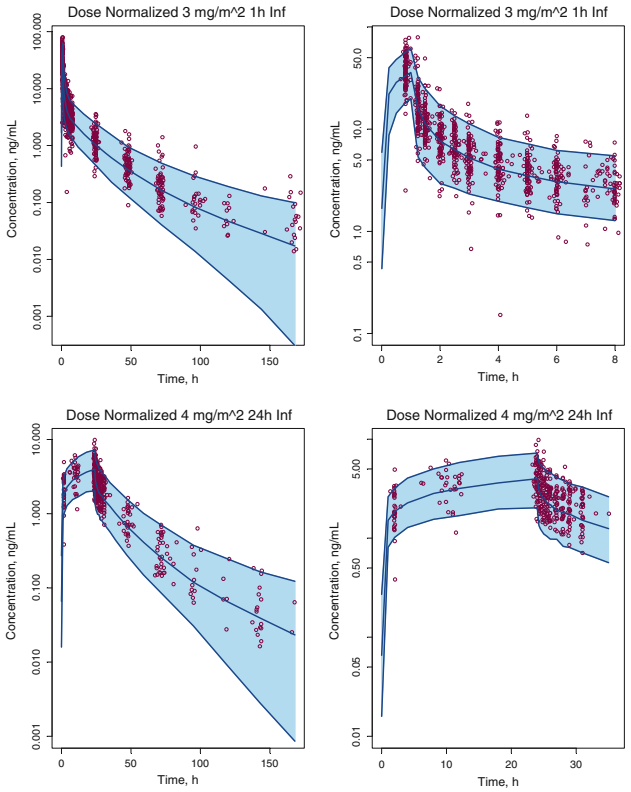

Fig. 3 Visual predictive check for the PM00104-normalized plasma concentrations stratified by 1-h (*upper panels*) or 24-h (*lower panels*) intravenous infusion

analysis and also relatively lower to what has been observed for other anticancer agents [5].

The typical volume of the central compartment (between-subject variability) in cancer subjects was estimated to be 32.7 L (82.5%). PM00104 was found to be widely distributed and the volume of distribution at steady state was estimated to be 821.7 L, which is consistent with the results of the non-compartmental analysis [5]. The central volume of distribution was larger than the extracellular fluid and the steady-state volume of distribution clearly exceeded the total body water, reflecting the large distribution to peripheral tissues consistent with the lipophilicity of the PM00104 and indicating an extensive tissue binding. This finding is consistent with the volume of distribution observed with other highly lipophilic drugs such as trabectedin [9] or plitidepsin [10]. In addition, no evidence of time-dependent kinetics was found and PM00104 systemic exposure is consistent following repeat intravenous administration.

The following covariates were evaluated as potential factors that may contribute to the between-subject variability of PM00104 pharmacokinetic parameters: age, sex, body weight, AST, ALT, ALP, total bilirubin and creatinine clearance, serum concentration of total protein and serum albumin, lactate dehydrogenase, hemoglobin, performance status, and the presence of liver metastases, dose-limiting toxicity or stable disease after 3 month of treatment. Within the range of values analyzed, the covariates evaluated did not influence the pharmacokinetics of PM00104 to a significant extent. The lack of statistical significant covariate effect on model parameters is unlikely due to a lack of power of detecting such a difference. The low relative standard error on the parameter estimates suggested that the sample size was good enough to detect clinically relevant (i.e. 20%) covariate effects on model parameters.

Since, generally, body weight is related to the amount of extracellular body water, the volume of the central compartment would be expected to be directly proportional to body weight. However, no association between body weight and central volume of distribution was identified, but currently all regimens with PM00104 contain a dose adjustment to patients body surface area. In theory, with the current dosing practice, plasma concentration at the end of the short intravenous infusion could potentially be associated with body weight. In any case, given the large variability in the volume of distribution, these potential effects would be expected to be negligible and no clinically relevant. Actually, the lack of sex- and body weight-related differences in the population pharmacokinetic analysis suggests that the scientific rationale for dosing PM00104 according to body surface area is beyond the pharmacokinetic considerations and, as consequence, PM00104 could potentially be in a long list of oncology drugs where dosing according to body weight might not be necessary, unless that efficacy and safety data evidenced otherwise.

Notably, no difference in PM00104 clearance between the cancer patients older than 65 years relative to younger subjects was observed. In addition, no correlation between PM00104 clearance and creatinine clearance was identified. Consequently, dose adjustments within the range of creatinine clearance evaluated might not be needed. Further analysis of pharmacokinetic data collected in Phase II program should confirm or refute this hypothesis. Furthermore, limited accumulation is expected with the weekly of every 3-week dosing schedules administered in Phase II because these dosing intervals are relatively longer than the terminal half-life.

In the absence of a new dataset, non-parametric bootstrap, visual predictive check and standardize visual predictive check were used as complementary tools to internally evaluate the model developed. The bootstrap analyses yielded mean model parameters that were comparable to the estimates of the original dataset, although with better precision in the estimates of within-subject variability and the correlations between random effects. These findings indicate the stability of the developed model and are further supported by the predictive checks performed, which confirmed the model developed is suitable

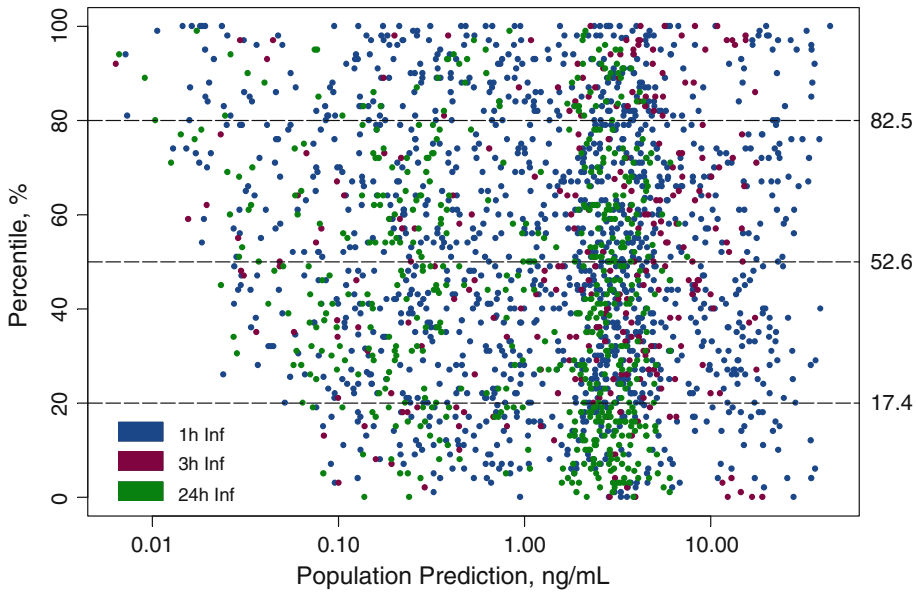

Fig. 4 Standardized visual predictive check stratified by infusion duration

to describe the time course of PM00104 plasma concentration following 1-, 3-, and 24-h intravenous infusion.

In summary, the integration of the Phase I pharmacokinetic data demonstrated PM00104 linear elimination from plasma, dose proportionality up to 5,000 μg/m^2, and time-independent pharmacokinetics. No clinically significant covariates were identified as predictors of PM00104 pharmacokinetics. The present model manages to well characterize the population and individual pharmacokinetics of PM00104, making it suitable for performing a model-based optimization of the pharmacokinetic sampling times, exploring the pharmacokinetic profiles under different dosing regimens, assessing the potential impact of concomitant administration of *Cytochrome P450* inducers and inhibitors in the PM00104 pharmacokinetics, and understanding the PM00104 pharmacokinetic and pharmacodynamic relationships for toxicity and efficacy endpoints.

Acknowledgments The authors thank Dr. Ricardo Nalda Molina for his comments and support at the beginning of this project. In addition, the authors would like to thank the patients, investigators, and their medical, nursing and laboratory staff who participated in the clinical trials included in the present study. In particular, we recognize the effort from the laboratory staff of ICON development solutions, which participated in the bioanalytical analysis of PM00104. Carlos Fernandez Teruel, Bernardo Miguel-Lillo, and Arturo Soto-Matos are employees of Pharma Mar SA, which supported this study. Consulting Projects for Research SL is consultant for Pharma Mar SA and received consultation fees for contributing to the current analysis.

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