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RESEARCH PAPER

Application of Hematological Toxicity Modeling in Clinical Development of Abexinostat (S-78454, PCI-24781), A New Histone Deacetylase Inhibitor

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ABSTRACT

Purpose A population pharmacokinetic/pharmacodynamic (PK/PD) model was developed to describe the thrombocytopenia (dose-limiting toxicity) of abexinostat, a new histone deacetylase inhibitor. An optimal administration schedule of the drug was determined using a simulation-based approach.

Methods Early PK and PK/PD data were analysed using a sequential population modeling approach (NONMEM 7), allowing for the description of a PK profile and platelet-count decrease after abexinostat administration with various administration schedules. Simulations of platelet count with several administration schedules over 3-week treatment cycles (ASC) and over a day (ASD) were computed to define the optimal schedule that limits the depth of thrombocytopenia.

Results An intermediate PK/PD model accurately described the data. The administration of abexinostat during the first 4 days of each week in a 3-week cycle resulted in fewer adverse events (with no influence of ASD on platelet count profiles), and corresponded to the optimal treatment schedule. This administration schedule was clinically evaluated in a phase I clinical trial and allowed for the definition of a new maximum tolerated dose (MTD), leading to a nearly 30% higher dose-intensity than that of another previously tested schedule. Lastly, a final model was built using all of the available data.

Conclusions The final model, characterizing the dose-effect and the dose-toxicity relationships, provides a useful modeling tool for clinical drug development.

KEY WORDS abexinostat · NONMEM · population PK/PD · simulations · thrombocytopenia

ABBREVIATIONS

ASC	Administration schedule over a cycle (3-week treatment)
ASD	Administration schedule over a day (*e.g.* once a day dosing, *bid* dosing, *et cetera*)
BASE	Baseline platelet count (x10^9/L)
CIRC	Compartment of circulating cells
EBE	Empirical Bayesian Estimates
HDACi	Histone deacetylase inhibitor
k_{el}	Constant rate of elimination
k_{prol}	Constant rate of proliferation
k_{tr}	Constant rate between transit compartments
MTT	Maturation time from PROL to CIRC (h)
NPDE	Normalized prediction distribution errors
PK/PD	Pharmacokinetic/Pharmacodynamic
PROL	Compartment of proliferative cells
SLOPE	Coefficient of drug decrease $(\mu g/mL)^{-1}$
TRAN	Transit compartment
VPC	Visual predictive check
γ	Power factor for the feedback mechanism

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INTRODUCTION

Abexinostat (S 78454, PCI-24781) is a new orally administered Histone Deacetylase inhibitor (HDCAi), currently in Phase I and II clinical trials for the treatment of solid tumors and lymphoma, respectively. Drugs of the HDACi class (1–4) increase gene expression through DNA acetylation, leading to a more open chromatin and the activation of genes involved in tumor suppression, cell cycle, cell division and apoptosis. The exact mechanism of action of the HDACi-induced anti-tumor activity is not yet fully characterized, but HDACi lead to an up-regulation of pro-apoptotic genes and a down-regulation of anti-apoptotic genes on tumor cells (5–7). The first phase I clinical studies (PCYC-401 [intra-venous administration] and PCYC-402 [oral administration]) in the USA showed that thrombocytopenia was the most frequent Dose-Limiting Toxicity (DLT) associated with abexinostat, consistent with other HDACi (8–11). It was suggested that HDACi decrease the transactivation function of GATA-1, leading to a delay in the maturation of megakaryocytes, resulting in thrombocytopenia (12). The administration schedules in PCYC-402 were: 5 days a week, 3/4 weeks and 5 or 7 days a week, every other week. Furthermore, a new phase I clinical trial (CL1-78454-002) was planned to be conducted in Europe in patients with solid tumors. For this new study, the clinical team decided to define a new cycle duration (3 weeks). Therefore, in parallel of the present work, a new administration schedule (14 consecutive days in a 3-week cycle) was implemented for the dose-escalation phase I study in Europe.

The development of an anticancer drug frequently begins with dose-escalation studies in order to determine the Maximal Tolerated Dose (MTD), which equates to the dose above which the occurrence of adverse events (AEs) is too high (13). This MTD is most often based on a "3+3" dose-escalation study design. That is, 3 patients are included per dose level. If one DLT is observed in 1 out of the 3 patients of the dose-level cohort, 3 more patients are included at the same dose level. If 2 DLTs appear, the dose-escalation study is stopped and the corresponding dose level is used to define the MTD. In such dose-escalation designs, the MTD is determined with a limited number of patients per dose (maximum 6 patients) and is strongly dependent on the administration schedule of the drug. These schedules are defined empirically using *a priori* knowledge (i.e. *in vitro* and/or *in vivo* preclinical studies and, from time to time, the outcome of previous clinical studies, when available). As the clinical evaluation of a sub-optimal administration schedule leads to the unethical (i.e. less effective) treatment of patients and a loss of time in drug development, the early determination of an optimal administration schedule is of major importance. Thus, the characterization of the toxicity profile of the drug is of compelling interest (14) as the determination of the MTD is the first endpoint in most phase I studies.

Hematological toxicity (*e.g.* thrombocytopenia, neutropenia) is frequently associated with chemotherapy and results in dose limitation in clinical trials. This toxicity could lead to bleeding or infections.

Semi-physiological pharmacokinetic/pharmacodynamic (PK/PD) models have been developed to analyze the hematological effect of cytotoxic drugs. In particular, Friberg *et al.* (15,16) purposed a model to describe the drug-related toxicity on neutrophils. This structural model was then applied to thrombocytopenia, as it had been previously shown capable of describing different drug induced hematology toxicities (15,17–21). With this model, for example, it was possible to determine the optimal area-under-the curve of plasma concentration (AUC) *versus* time of carboplatin, depending on concomitant administered chemotherapy (18).

Due to the fact that the dose-limiting toxicity of abexinostat is thrombocytopenia, in this present study we described the development of a simulation tool using PK and PK/PD data from patients with advanced solid tumors from the first two phase I clinical studies performed in the USA (PCYC-401 and PCYC-402). This tool, based on a population PK/PD modeling and simulation approach, aimed at characterizing platelet-count time-course after the administration of abexinostat (22,23). This PK/PD model was built in order to eventually influence, through numerous simulations, the administration schedule of abexinostat in patients with solid tumors in an ongoing phase I clinical study in Europe (CL1-78454-002). Second, upon completion of the European study, the final PK/PD model parameters were estimated using all the available data.

MATERIALS AND METHODS

Clinical Studies and Patients

Clinical studies were performed in accordance with the ethical principles stated in the Declaration of Helsinki 1964, as revised in Seoul 2008. All participants provided written informed consent. The studies were started after obtaining written approval from the corresponding Ethics Committee, complying with local regulatory requirements, and obtaining the signature of the clinical study protocol of each contractual party involved. All study designs are summarized in Table I.

The PCYC-401 study was carried out in the USA and was a mono-centric, 2-phase (accelerated and standard phase), open-label, sequential, dose-escalation phase I study investigating the tolerability, safety, and pharmacokinetics of abexinostat in patients with refractory solid or hematologic malignancies. In the accelerated phase, abexinostat was administered to patients (1 per dose level) as a 2-hour intravenous (IV) infusion on Day 1 - Day 3 (D1-D3) of a 21-day cycle. The administered dose ranged from 0.25 mg/kg to 3.0 mg/kg

Table I Clinical Study Designs and Different Datasets

	Clinical study	PCYC-401			PCYC-402	CL1-78454-002
		Accelerated phase	Standard phase			
Clinical study	Treatment cycle duration	3-week-cycle	Single oral dose	3-week cycle	4-week cycle	3-week cycle
	Administration days (number of patients)	• D1-D3 (5)	7 days before the first IV dosing (9*)	• D1-D3, D8-D10, D15-D17 (9*)	• D1-D5, D8-D12, D15-D19 (21) • D1-D7, D15-D21 (6) • D1-D5, D15-D19 (8)	• D1-D14 (15) ***• D1-D4, D8-D11, D15-D18 (20)***
	Route of administration	2-hour IV infusion	Orally (solution or capsule)	2-hour IV infusion	Orally (*bid* or *tid*)	Orally (*bid*)
	Dose treatment	0.25 to 3.0 mg/kg	2.0 mg/kg (solution) 200 mg (capsule)	2.0 to 2.4 mg/kg	30 to 75 mg/m^2	30 to 90 mg/m^2 ***60 to 105 mg/m^2***
Datasets	Intermediate PK dataset	X	X	X	X	
	Final PK dataset	X	X	X	X	X
	Intermediate PK/PD dataset				X (cycle1 only)	
	Intermediate evaluation PK/PD dataset				X	
	Final PK/PD dataset				X	X

Italic and bold inscriptions correspond to the information related to the CL1-78454-002 protocol amendment

9*: same 9 patients

D days, *IV* intra-venous infusion, *bid* twice a day, *tid* three times a day

over 2-hour infusion. In the standard phase, abexinostat was administered to patients (3 per dose level+3 further patients in the occurrence of toxicity) as a 2-hour IV infusion on D1-D3, D8-D10 and D15-D17 of a 21-day cycle. The same patients were also given abexinostat as a single oral dose (solution or capsule) 7 days before the first IV administration. In the standard phase, the IV dose ranged from 2.0 mg/kg to 2.4 mg/kg over a 2-hour infusion and the oral dose ranged from 2.0 mg/kg to 200 mg.

The PCYC-402 study was carried out in the USA and was a multi-centric, open-label, dose-escalation phase I study in advanced solid tumors, non-Hodgkin's lymphoma, Hodgkin's disease, chronic lymphoid leukemia, or multiple myeloma. Abexinostat was administered to patients (3 per dose level+3 further patients in the occurrence of toxicity) orally (capsule) *bid* (twice a day) or *tid* (three times a day) 4-hours apart, according to three different administration schedules in 4-week cycles (administration on D1-D5, D8-D12, D15-D19 [ASCP1]; administration on D1-D7, D15-D21 [ASCP2]; administration on D1-D5, D15-D19 [ASCP3]). The oral dose ranged from 30 mg/m^2 to 75 mg/m^2.

The CL1-78454-002 study was carried out in France and was a mono-centric (Institut de Cancérologie Gustave-Roussy), open-label, dose-escalation phase I study in advanced solid tumors which had relapsed or were refractory to conventional, standard forms of therapy. Abexinostat was administered to patients (3 per dose level+3 further patients in the occurrence of toxicity) orally, twice daily 4-hours apart in 3-week cycles (administration on D1-D14). The oral dose ranged from 30 mg/m^2 *bid* to 90 mg/m^2 *bid*.

Pharmacokinetic Data

Specific blood samples were obtained for either PK (plasma abexinostat concentration) or PD (platelet count).

Blood samples for PK were planned during the PCYC-401 clinical study accelerated phase at D1 pre-infusion and at 0.5, 1, 2, 2.5, 3, 4, 6, and 8 h after the start of infusion, and at D2 and D3 pre-infusion and 2 h after the start of infusion.

Blood samples for PK were planned during the PCYC-401 clinical study standard phase after oral dose pre-dose and at 0.5, 1, 2, 4, 6, 8 and 24 h after dose and after IV dose at D1 pre-infusion and at 0.5, 1.5, 2.5, 3, 4, 6, and 8 h after the start of infusion, and at D2, D3, D8, D9, D10 pre-infusion and 1.5 h after the start of infusion.

Blood samples for PK were planned during the PCYC-402 clinical study at D1 pre-dose and 0.5, 1, 2, 3, 4, 6 h after first dose, D2 pre-dose, D3, D4, or D5 pre-dose, D8 pre-dose and 2 h after first dose (ASCP1) and D15 pre-dose and 2 h after first dose (ASCP2 and ASCP3).

Blood samples for PK were planned during the CL1-78454-002 clinical study at D1 pre-dose and 0.5, 1, 2, 3, and 4 h after the first dose and 0.5, 1, 2, and 4 h after the second dose, D2 pre-dose, D4 pre-dose, 0.5, 1, 2, 3, 4 h after the first dose, D14 pre-dose.

The plasma samples were analysed using a validated method of liquid chromatography with tandem mass spectrometric detection (LC-MS/MS) in multiple analytical centers. The response of the detector to abexinostat was linear over the calibration range in human plasma, from 1.00 (lower limit of quantification: LLOQ) to 250 ng/mL (upper limit of quantification: ULOQ), with a R^2 of 0.9986. The analyte response (peak height) at LLOQ was greater than five times the blank response. Over the six replicates of each quality controls (low, medium and high), precision (percentage of standard deviation) did not exceed 7.6% and accuracy was not less than 91%.

The PK data of the two previous clinical trials (PCYC-401 and PCYC-402) were included in an intermediate PK dataset (Table I). At the completion of the CL1-78454-002 clinical study, PK data from the study was added to the intermediate PK dataset to form the final PK dataset (Table I).

Pharmacodynamic Data

Blood samples for platelet counts in the PCYC-402 clinical study were planned 14 days before treatment start, within 3 days before treatment commenced, and once per week during cycle 1, every other week in cycle 2, and on the first week of subsequent cycles.

Blood samples for platelet counts in the CL1-78454-002 clinical study were planned before treatment commenced, during cycle 1 on D1, D8, D12, D15 and D18 and subsequent cycles on D1, D8 and D15.

The platelet count data from cycle 1 of the PCYC-402 study were included in the intermediate model-building PK/PD dataset (Table I). Data from the later cycles of the PCYC-402 clinical study formed the intermediate evaluation PK/PD dataset (Table I). Thereafter, a final PK/PD dataset was built, that contained all platelet-count data from both the PCYC-402 and CL1-78454-002 clinical studies (Table I).

Modeling Strategy

A sequential PK/PD modeling approach was performed, in compliance with the Individual PK Parameters (IPP) approach described by Zhang *et al.* (23). A population PK model of abexinostat plasma concentration after oral or IV administration was built using PK data obtained in the PCYC-401 and PCYC-402 clinical studies (intermediate PK dataset). No covariate analysis was planned for the present work, as the aim of this PK analysis was to obtain individual PK predictions in order to estimate PK/PD

parameters. Individual Empirical Bayesian Estimates (EBE) of PK parameters from a modal posterior Bayesian estimate with prior derived only from the PK data and obtained using the POSTHOC method in NONMEM, were then input into the intermediate model-building and intermediate evaluation PK/PD datasets. They were used as an input into a PKPD model of platelet dynamics in order to fit platelet counts and to perform external evaluation respectively.

Data obtained from the first treatment cycle (cycle 1 only: about 21 first days of treatment) of the PCYC-402 clinical study (intermediate model-building PK/PD dataset) were used to build the intermediate PK/PD model, as data from later cycles (intermediate evaluation PK/PD dataset) were added to an external simulation-based evaluation (Table I). Using this intermediate PK/PD model, simulations of numerous administration schedules were carried out in order to ascertain the safest schedule for abexinostat. Finally, the PK data from patients included in both the PCYC-402 and CL1-78454-002 clinical studies were pooled together (final PK dataset) in order to estimate the individual EBE of PK parameters for all patients. These parameters were input, together with all platelet-count data from all cycles of all studies, into the final PK/PD model dataset. The final PK/PD model was built using this final pooled dataset.

For graphical evaluation, all pre-dose samples (complete blood counts realized before inclusion) were set at $t=0$, as platelet count was assumed to remain constant in the absence of treatment.

Model Building

Model parameters were estimated with the first order conditional estimation method with interaction (FOCEI) as implemented in the NONMEM 7 software. Discrimination between hierarchical models was based on the objective function value (OFV) of NONMEM using the Likelihood-Ratio-Test (LRT). A difference greater than 3.84 for one additional parameter, corresponding to a significance level of 5%, was used for discrimination between two nested models. Non-nested models were discriminated by computing the Bayesian Information Criterion ($BIC = OFV - k \times Ln(n)$, with n, the number of observations, and k, the number of estimated parameters). The model with the lowest BIC value was selected.

The structural PK model was selected between 1, 2, or 3 distribution compartments, zero-order or first-order oral absorption, with or without a lag-time.

The structural PK/PD model (Fig. 1), for both the intermediate and final PK/PD models, was based on the original semi-mechanistic PK/PD myelosuppression model of Friberg *et al.* (15,16). In this PK/PD model, the drug effect (E), depending on drug concentrations, affects the progenitor cell proliferation rate constant. Different drug effects (linear, imax or sigmoid) were tested. MTT represents the mean maturation time, expressed in hours, for a cell to mature between the proliferation compartment (PROL) to the circulating compartment (CIRC) and is defined as $MTT = 4/k_{tr}$, where k_{tr} is the transit constant rate.

Inter-individual variability on each parameter was investigated, assuming it to be exponential (log-normal distribution of parameters): the individual value θ_i of a parameter is $\theta_i = \theta \times \exp(\eta_i)$ where θ is the typical (population) value and η_i is the inter-individual variability, corresponding to the discrepancy between θ_i and θ. η_i is normally distributed with a mean 0 and a variance ω^2.

The residual error model that accounts for the difference between model prediction F and observed data Y was selected between additive ($Y = F + a \times \varepsilon$), proportional ($Y = F \times (1 + b \times \varepsilon)$) and combined $Y = F + (a^2 + bF^2)^{1/2} \times \varepsilon$ where a and b are respectively the additive and proportional components of the error and ε is assumed to be normally distributed with a mean 0 and a variance 1. (a_{PK}, b_{PK}) are used for PK model and (a_{PD}, b_{PD}) are used for PD models.

Model Evaluation

PK Model

The intermediate PK model was evaluated using standard goodness-of-fit plots, evaluation of parameter precisions of estimation in order to assess the ability of the model to describe the intermediate PK data. When performing Bayesian estimation of the individual parameters using the final PK dataset, the intermediate PK model's ability to describe the new data was assessed through goodness-of-fit plots and external Visual Predictive Checks (VPCs) (24).

Intermediate and Final PK/PD Model Evaluation

Large inter-individual and intra-individual (due to missed doses or dose adaptation) heterogeneity in administered doses and administration schedules rendered VPCs inappropriate, therefore an internal model evaluation was carried out using normalized prediction distribution errors (NPDE) generated by NONMEM, which are assumed to be normally distributed. Five hundred simulations based on the dose regimen and sampling time-points of each patient were performed using the PD parameter estimates. NPDE were computed and plotted *vs.* time in order to identify possible bias in the structural model (25,26). By construction, the NPDE followed a standard normal distribution (25,26). Therefore, a Kolmogorov-Smirnov test was performed in order to test this assumption.

An initial external evaluation of the intermediate PK/PD model was carried out using each individual's EBE parameters, obtained using the POSTHOC option in NONMEM,

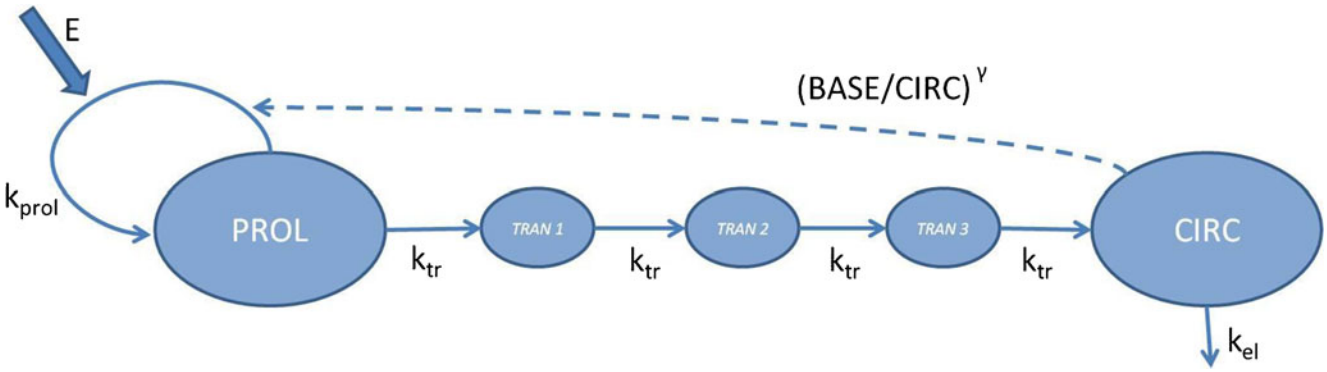

Fig. 1 Representation of the PK/PD model structure as published by Friberg [15;16]. PROL stands for proliferation compartments, TRAN stands for transit (maturation) compartments, and CIRC stands for circulating (blood) compartment. Initially, amounts in each compartment are all set to BASE. Rate constants for proliferation of progenitor cells k_{prol} and platelet elimination k_{el} are set to k_{tr} for identifiability. Drug effect E as well as feedback term $(BASE/CIRC)^{\gamma}$ are multiplicative on progenitor cells proliferation rate constant.

to simulate each patient's predicted platelet-count time profiles during the entire treatment period, and then comparing observations to simulation during the later cycles (intermediate evaluation PK/PD dataset). External individual VPCs were then carried out with data from the first fourteen patients included in the CL1-78454-002 clinical study. Five hundred simulations based on the dose regimen and sampling time-points of these patients were performed using the PK and PD parameter estimates. Then, the 10th, 50th, and 90th percentiles of simulated data were calculated for each sampling time-point and were plotted together with the observed data in order to evaluate the model. The model was considered correct if less than 20% of the observed data were located outside the 80% prediction interval. The baseline parameter was fixed to the last individual observed value before treatment. This external evaluation was carried out in order to assess the intermediate PK/PD model's ability to describe new data.

PK/PD Simulation Plan

The PK model and the intermediate PK/PD model were used to simulate platelet-count time profiles after the administration of abexinostat with the 60 mg/m^2 dose *bid*, corresponding to the recommended dose in PCYC-402.

Four administration schedules over a 3-week treatment cycle (ASC) were evaluated: 14 days (d) on treatment/7 days off treatment (ASC1); 10d on/11d off (ASC2); 5d on/2d off during the first 2 weeks, then 7d off (ASC3); 4d on/3d off during 3 weeks (ASC4). In addition, four administration schedules over a day (ASD) detailing the administration of abexinostat on each treatment day were evaluated: *bid* 4 h apart (ASD1); *bid* 12 h apart (ASD2); *qd* (once a day) (ASD3); *tid* 4 h apart (ASD4). As no interaction was expected between ASD and ASC, and as ASD1 and ASC1 correspond to the original schedules used in the CL1-78454-002 clinical study, each ASD was evaluated using ASC1 and each ASC was evaluated using ASD1 (Table II).

For each condition, 500 replication simulations were performed and the median, 10th percentile, and 90th percentile of the predicted platelet-count time profiles were computed. The evaluation criterion was the median nadir (lowest value of platelet count) during the first cycle of treatment, leading to the selection of the optimal administration schedule with the less pronounced nadir. The selected ASD and ASC

Table II Description of the Administration Schedules Clinically Evaluated in PCYC-402 (*) or Simulated. Seven Different Administration Schedules of Abexinostat with the 60 mg/m^2 Dose *Bid* over a 3-week Treatment Cycle (ASC) and over a Day (ASD) were Simulated using the Intermediate PK/PD Model

Administration schedule over a treatment cycle (ASC)		Administration schedule over a day on treatment (ASD)	
ASCP1*	D1-D5, D8-D12, D15-D19, 4 week-cycles	ASD1*	*bid* 4 h apart
ASCP2*	D1-D7, D15-D21, 4 week-cycles	ASD2	*bid* 12 h apart
ASCP3*	D1-D5, D15-D19, 4 week-cycles	ASD3	*qd*
ASC1	D1-D14, 3 week-cycles	ASD4*	*tid* 4 h apart
ASC2	D1-D10, 3 week-cycles		
ASC3	D1-D5, D8-D12, 3 week-cycles		
ASC4	D1-D4, D8-D11, D15-D18, 3 week-cycles		

D day, *qd* once a day, *bid* twice a day, *tid* three times a day

Table III PK and PK/PD Model Parameters and their Respective Inter-individual Variability, Quantified as Coefficient of Variation (CV) Expressed as a Percentage (for example, $CV_{CL} = 100 \times \omega_{CL}/\theta_{CL}$). Combined Residual Error Model for PK and PK/PD Models are Composed of a (Additive Part) and b (Proportional Part)

Models	Parameters	Population mean estimates (RSE)	BSV (RSE)	Sh. Std.
PK model	ka (h^{-1})	0.763 (13.1%)	72% (30.1%)	13%
	F (%)	31.7 (22.1%)	34% (42%)	5.8%
	V1 (L)	36.2 (29.3%)	58% (175.1%)	22%
	V2 (L)	561 (41.9%)	–	–
	V3 (L)	54.4 (13.8%)	42% (37.7%)	12%
	Q2 (L/h)	11.5 (18.3%)	–	–
	Q3 (L/h)	39 (14.3%)	–	–
	CL (L/h)	57.4 (8.1%)	35% (51.4%)	3.2%
	a_{PK} (ng/mL)	3.8 (29.2%)		
	b_{PK} (%)	30.8 (6.5%)		
	ε-shrinkage (%)	13.7		
Intermediate PK/PD model	MTT (h)	97 (3%)	–	–
	γ (−)	0.288 (10.3%)	41.2% (64.1%)	18.5%
	BASE (×10^9/L)	267 (8.7%)	39.6% (41.4%)	2.1%
	SLOPE $((\mu g/mL)^{-1})$	3.98 (14.5%)	60.2% (26.7%)	16.7%
	a_{PD} (×10^9/L)	23.7 (14.6%)		
	b_{PD} (%)	13.8 (20.9%)		
	ε-shrinkage (%)	25		
Final PK/PD model	MTT (h)	94.1 (3%)	9.8% (52.1%)	22.8%
	γ (−)	0.278 (7.5%)	27.6% (43.9%)	21.8%
	BASE (×10^9/L)	260 (5.7%)	37.3% (27.8%)	4.2%
	SLOPE $((\mu g/mL)^{-1})$	4.28 (9.2%)	58.6% (31.5%)	12%
	Cov. SLOPE-MTT	0.0401 (56.6%)	–	–
	Cov. SLOPE-γ	0.0172 (334.3%)	–	–
	Cov. SLOPE-BASE	−0.0492 (91.9%)	–	–
	Cov. MTT-γ	0.006 (204%)	–	–
	Cov. MTT-BASE	0.0109 (114.7%)	–	–
	Cov. γ-BASE	0.0108 (314.8%)	–	–
	a_{PD} (×10^9/L)	14.3 (15.1%)		
	b_{PD} (%)	27.7 (2.8%)		
	ε-shrinkage (%)	10.9		

MTT (h) Mean maturation time, calculated as $MTT = 4/k_{tr}$ where k_{tr} is the transit constant rate

RSE (%) Relative Standard Error, calculated as RSE = (Standard Error/Final parameter estimate)*100

BSV (%) Between Subject Variability, calculated as BSV = ω*100

Sh. Std. (%) Shrinkage calculated on standard deviation as Sh. Std. = (1 − Standard deviation(η))/ω*100

Cov covariance estimates

allowed the highest daily dose of abexinostat to be defined as MTD, based on the model.

Throughout this present work, the simulations were based on 500 replicates, allowing for proper estimation of inter-subject variability.

RESULTS

PK Model

The intermediate PK dataset contained 724 concentration measurements from 49 patients, the age ranged from 23 to 81 years (mean: 58 years) and the weight ranged from 40 to 149 kg (mean: 82 kg). As less than 10% of non-predose samples were below the LLOQ, those were ignored in the PK analysis. The PK of abexinostat after oral and IV administration were best described by a mammillary 3-compartment model (Table III) parameterized with central volume V1, peripheral volumes V2 and V3, inter-compartment constants Q2 and Q3, with a first order elimination from central compartment (plasma clearance CL), a first order absorption (rate constant ka) and absolute bioavailability (F) for oral administration, and a direct input into central compartment for IV infusion. Inter-individual variability was estimated on clearance, central volume, absorption rate constant, bioavailability and V3. A combined residual error model best fit the data.

Goodness-of-fit plots (Fig. 2), individual fit, and parameters precision of estimation were satisfactory (lower than 42% for fixed effects parameters and lower than 52% for random effects except for ω_{V1}.

Intermediate PK/PD Model

The intermediate model-building PK/PD dataset (i.e. PCYC-402, first treatment cycle) contained 181 platelet counts from 35 patients, and the intermediate evaluation PK/PD dataset (i.e. PCYC-402, later treatment cycles) contained 86 platelet counts from 21 patients. The structural PK/PD model (Fig. 1) was not modified from Friberg's original publication (16). A linear drug effect on proliferation rate, E = SLOPE × C(t) where SLOPE is the coefficient of drug decrease, expressed in $(\mu g/mL)^{-1}$, was selected. The estimated parameters of this intermediate PK/PD model are presented in Table III. Inter-individual variability was estimated on BASE, γ, and SLOPE. A combined residual error model best fit the data. Parameter precisions of estimation were satisfactory (lower than 15% for fixed effect parameters and lower than 65% for random effects). Goodness-of-fit plots (Fig. 3), NPDE (Fig. 4a), and individual fits (Fig. 5a and b, solid line) were computed and displayed in a graph. The power of the model to fully predict thrombocytopenia data, as the data were centered and randomly distributed around the *x*-axis, is shown in the Fig. 4a. The majority of the data were included in the 80% prediction interval. The *p*-value of the Kolmogorov-Smirnov test was approximately 0.66, indicating that the null hypothesis of a standard normal distribution for NPDE could not be rejected.

The external evaluation showed that the intermediate PK/PD model was able to predict the individual platelet time-course for the subsequent cycles (Fig. 5a and b, dashed line). All individual external VPCs were satisfactory (Fig. 5c and d), as less than 20% of observed data were outside the 80% prediction interval.

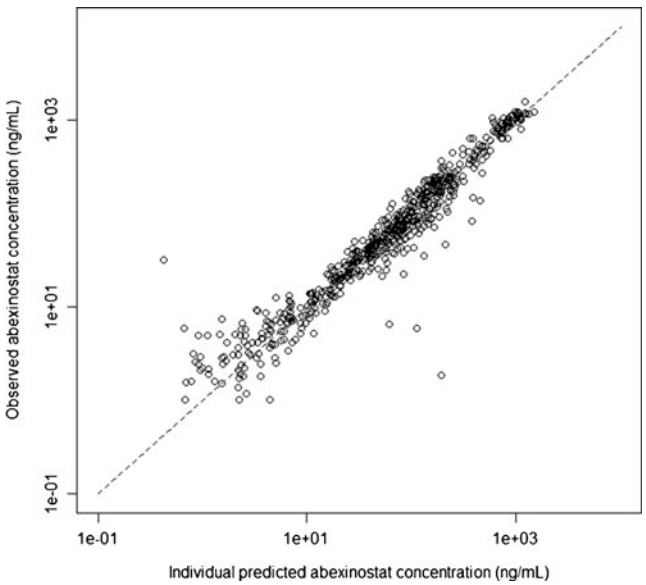

Fig. 2 Goodness-of-fit plot of the PK model: PCYC-401 and PCYC-402 observed *vs.* individual predicted concentrations of abexinostat. The *dashed line* represents the identity line.

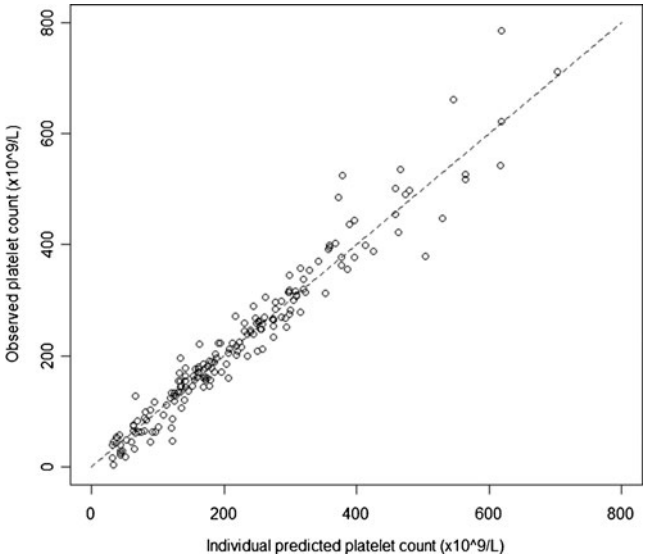

Fig. 3 Goodness-of-fit plot of the intermediate PK/PD model: PCYC-402 observed *vs.* individual predicted platelet count after the administration of abexinostat. The *dashed line* represents the identity line.

Simulations

Simulations performed with the PK and intermediate PK/PD models are presented in Fig. 6 and Table IV.

Evaluation of ASC (Fig. 6a) showed that the decrease in platelet count was more pronounced using ASC1 and ASC2, and that ASC3 and ASC4 showed the best safety profile. Evaluation of ASD (Fig. 6b) showed a very minor influence of daily administration on platelet count.

CL1-78454-002 Protocol Amendment

These results led to a protocol amendment of the CL1-78454-002 study allowing patients to be treated in accordance with the optimal administration schedule: administration of abexinostat during the first 4 days of each week in a 3-week cycle (ASC4). Twenty more patients were evaluated clinically and treated according to this optimal administration schedule. The capacity of the intermediate PK/PD model to predict and describe the platelet profiles of these patients was verified by computing external individual VPCs (Fig. 7). Five hundred simulations were carried out based on the dose regimen and sampling time-points of each patient using individual observed baselines, individual PK parameters and final PD parameter estimates. The 10th, 50th and 90th percentiles of simulated data were then calculated for each sampling time-point and plotted together with the observed data. As the observed data were located inside the 80% prediction interval the intermediate PK/PD model was deemed adequate for predicting the platelet time course of these patients (Fig. 7). Finally, Bayesian predictions of data from the CL1-

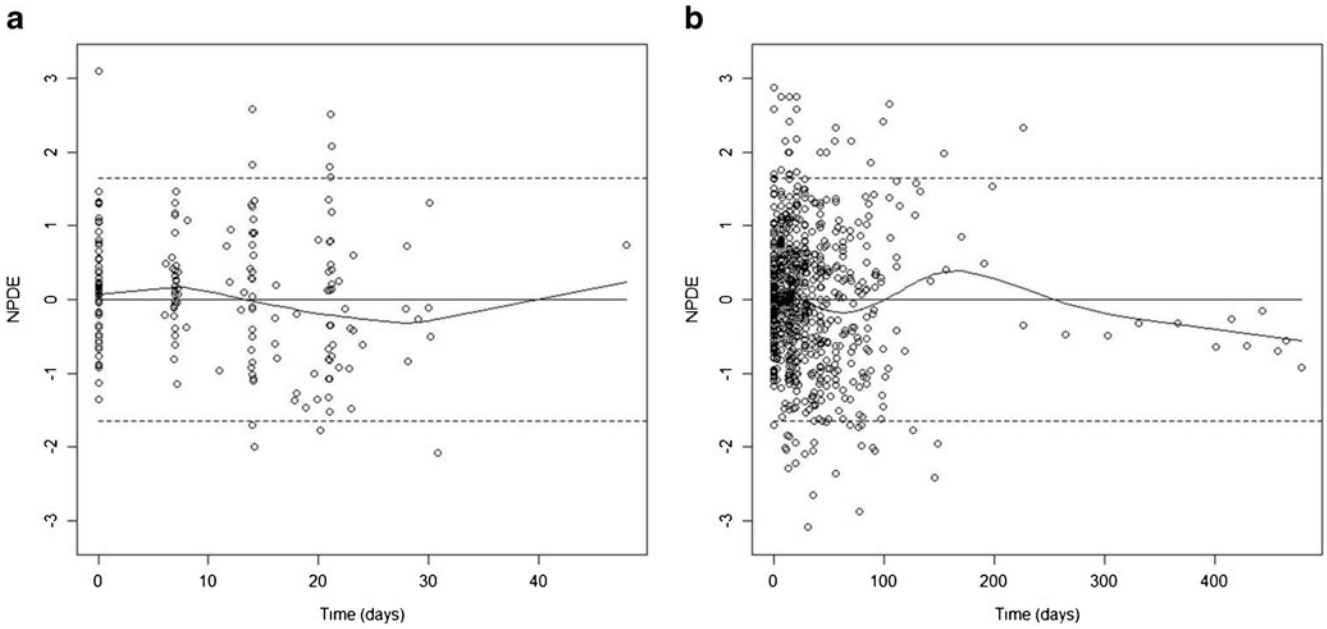

Fig. 4 Predictive evaluation of the intermediate PK/PD model (**a**) and the final PK/PD model (**b**): NPDE *versus* TIME (days). Five hundred simulations based on the dose regimen and sampling time-points of each patient were performed using the PD parameters estimates. The *dashed lines* represent the 80% Prediction Interval (PI). The *solid line* represents the trend curve.

78454-002 clinical study were performed in NONMEM using the POSTHOC option, with the intermediate PK/PD model parameters, in order to confirm, by an external posthoc evaluation, that such model could predict platelet profiles of these new patients (Fig. 8).

Final PK and PK/PD Models

The final PK and PK/PD datasets were thereafter built using the data from the 35 supplementary patients from the CL1-78454-002 study. Respectively, 15/20 patients were treated according to the CL1-78454-002 pre/post-amendment administration schedules. Overall the final PK dataset contained 1061 concentration measurements from 70 patients. Lastly, the final PK/PD model of abexinostat in patients with advanced solid tumors was built based on the 743 platelet samples (all cycles) from the 70 patients available in the final PK/PD dataset. A full variance-covariance matrix was estimated. The parameter precision of estimation was very satisfactory (less than 10% for fixed effect parameters and less than 53% for random effects). A slight ε-shrinkage value (10.9%) was noticed. Goodness-of-fit plots, individual fit and NPDE were computed and displayed in Fig. 4b. NPDE *versus* TIME plot (Fig. 4b) showed the strength of the model in fully predicting thrombocytopenia data as the data were centered and randomly distributed around the *x*-axis. The majority of the data were included in the 80% prediction interval. The *p*-value of the Kolmogorov-Smirnov test was approximately 0.17, implying non-rejection of the NPDE normality.

DISCUSSION

A PK model was built and described the data adequately according to goodness-of-fit plots (Fig. 2). All parameters were estimated accurately (Table III) except for the inter-subject variability of V1, but this was considered acceptable due to the fall in OFV and improvement in individual fit. At this stage of work, no covariate analysis was carried out as the aim of this PK model building step was to obtain reasonable individual PK parameters in order to estimate PK/PD parameters. As this PK model was challenged by external evaluation (results not shown), the PK parameters were not re-estimated when new PK data (from the CL1-78454-002) were available and EBEs of PK parameters were also obtained by a Bayesian method using the POSTHOC option in NONMEM. An intermediate PK/PD model of thrombocytopenia after the administration of abexinostat was developed using the first available PK and PK/PD clinical data. This model (Table III) adequately described the data according to goodness-of-fit plots (Fig. 3), and individual fits (Fig. 5a and 5b, solid line), and could fully predict thrombocytopenia data (Fig. 4a). External validation showed that the model was able to properly match platelet count time profile during further treatment cycles (Fig. 5a and b [dashed line] and Fig. 5c and d).

In parallel to the modeling and simulation work, the CL1-78454-002 study was initiated using a different administration schedule over a treatment cycle (ASC1) than that in the PCYC-402 clinical study.

An early decrease in platelet count was observed quickly after starting treatment and was maintained monotonously

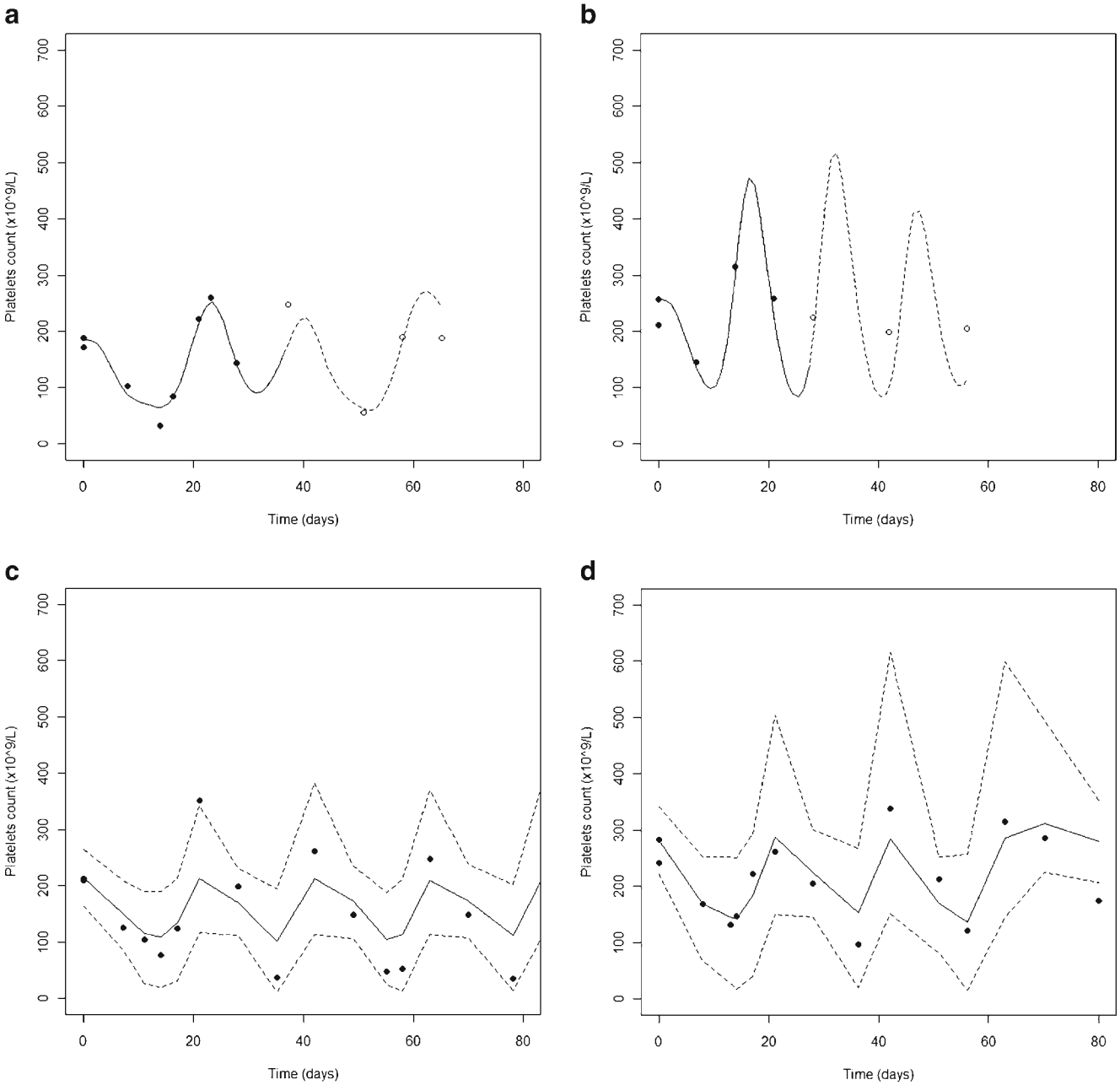

Fig. 5 Individual observed platelet count (*circles*), individual predicted platelet count (*solid line*) and external evaluation (*dashed line*) for 2 randomly selected patients from the PCYC-402 clinical study, using individual PK and PK/PD parameters combined with individual dosing information to predict each individual's platelet time-course during the whole treatment duration for different administration schedules and dose of PCYC-402 (**a and b**). External evaluation by individual VPCs of 2 randomly selected patients from the CL1-78454-002 study (pre-amendment). *Points* represent the observed data. *Solid and dotted lines* represent respectively the median and the 80% prediction interval of simulated data for the intermediate PK/PD model (**c and d**).

until a treatment break of several days occurred. Consistently, the nadir was lower when the administration schedule started with more consecutive days on treatment. Due to feedback mechanism, recovery was quite fast upon cessation of treatment. Although restarting the treatment again decreased platelet count, the stimulation of progenitor cells in PROL after the first consecutive days of treatment induced a less stringent decrease in platelet count. The model-building step of this work showed that Friberg's model adapted to abexinostat data was able to describe platelet decrease after the administration of abexinostat under various administration schedules. Consistent with previous uses of this model, this provides evidence of the robustness of this method in describing hematological toxicity. Nevertheless, refining the model based on pharmacological or physiological knowledge could improve its descriptive and predictive abilities (12,27,28).

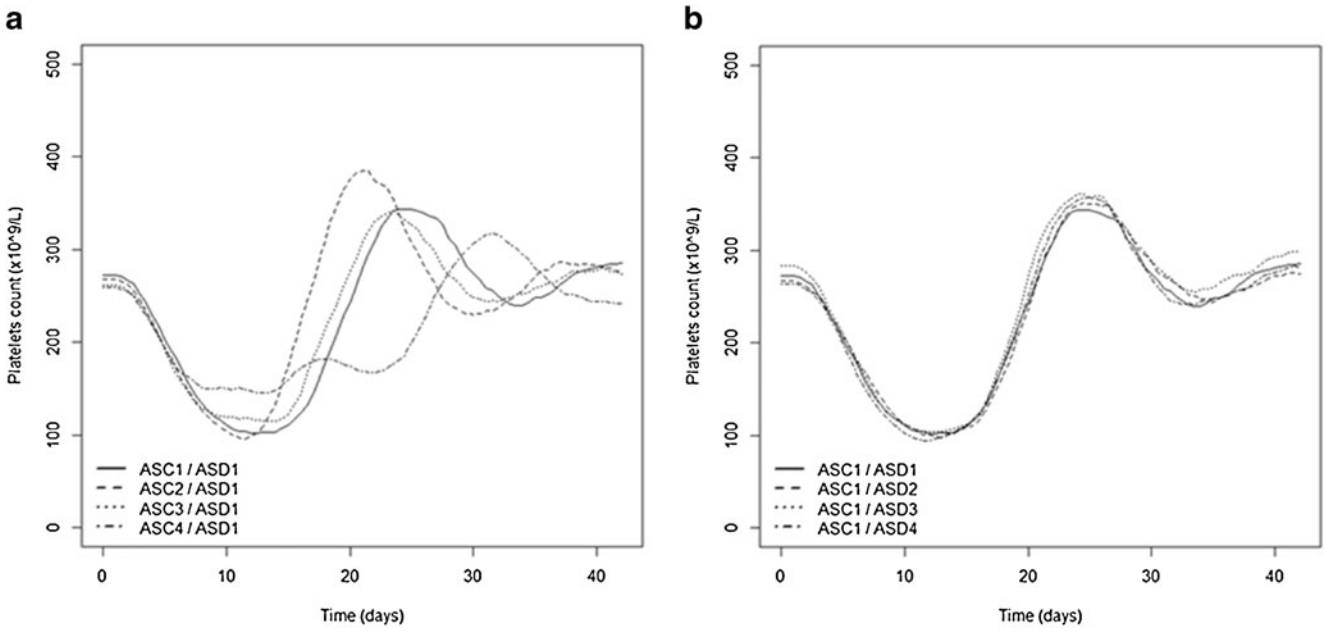

Fig. 6 Simulation of different administration schedules over a cycle (**a**, variation of ASCs) and over a day (**b**, variation of ASDs). Simulated platelet counts under different conditions are plotted during the first treatment cycle (median profiles only for readability reasons). ASC1: 14d (days) on treatment/7d off treatment; ASC2: 10d on/11d off; ASC3: 5d on/2d off during the first 2 weeks, then 7d off; ASC4: 4d on/3d off during 3 weeks; ASD1: *bid* 4 h apart; ASD2: *bid* 12 h apart; ASD3: *qd* (once a day); ASD4: *tid* 4 h apart.

Recent results on HDACi toxicity mechanisms suggest a non-central toxicity, in contrast to usual cytotoxic compounds, therefore, the implementation of such a known pharmacological drug effect to a physiological system may assist in the description of platelet counts when several drugs are co-administered.

ASC evaluation showed that the number of consecutive days of treatment at the start of the schedule had a significant impact on nadir values. ASD were shown to have a minor influence on thrombocytopenia mainly because the dynamic mechanisms of hematopoiesis (platelet production, maturation and elimination) could be characterized by MTT, around 100 h. Therefore, a daily modification of PK profile through a change in daily drug administration is less influential than a modification in the number of consecutive days of treatment, due to the short half-life (approximately 4 h) of abexinostat.

Table IV Median Simulated Nadir Values and 80% Prediction Interval over the First Treatment Cycle for each ASC (Combined with ASD1) and each ASD (Combined with ASC1)

ASC (+ ASD1)	Median nadir [80% PI]	ASD (+ASC1)	Median nadir [80% PI]
ASC1	88 [63–109]	ASD1	88 [63–109]
ASC2	103 [75–117]	ASD2	88 [79–102]
ASC3	125 [113–153]	ASD3	88 [72–106]
ASC4	132 [123–152]	ASD4	90 [79–103]

ASC administration schedule over a 3-week treatment, *ASD* administration schedule over a day, *PI* prediction interval

This work emphasizes the importance of administration schedule choice over a treatment cycle for this drug. *In silico* simulations are also a fast and ethical way to perform such comparisons, and permit a standardized and objective evaluation of different administration schedules using early collected data (29). One of the simulated administration schedules (combining ASD1 and ASC1) was being clinically evaluated in the CL1-78454-002 phase I study when this work was performed. After evaluation of ASC and ASD, the best administration schedule selected was the treatment of patients during the first 4 days of each week in a 3-week cycle (ASC4 combined with any ASD). Even though the platelet count was constantly inhibited with this administration schedule compared to the others during the continuous 3-week treatment, a rapid recovery, of about 1 week, was observed after treatment stops to return to baseline platelet count (Fig. 6). Moreover, during the treatment period, smaller variations in platelet count were observed with this administration schedule, which is clinically easier to manage. As a result of this work the CL1-78454-002 study was amended, and abexinostat was administered to 20 further patients employing the optimal administration schedule.

This new administration schedule made it possible for dose-escalation to increase two dose stages and, consequently, to define a new, higher MTD of abexinostat. Therefore MTDs were 60 mg/m^2 *bid* and 90 mg/m^2 *bid,* pre and post-amendment, respectively. This modeling and simulation work led to a gain of about 30% in terms of dose-intensity.

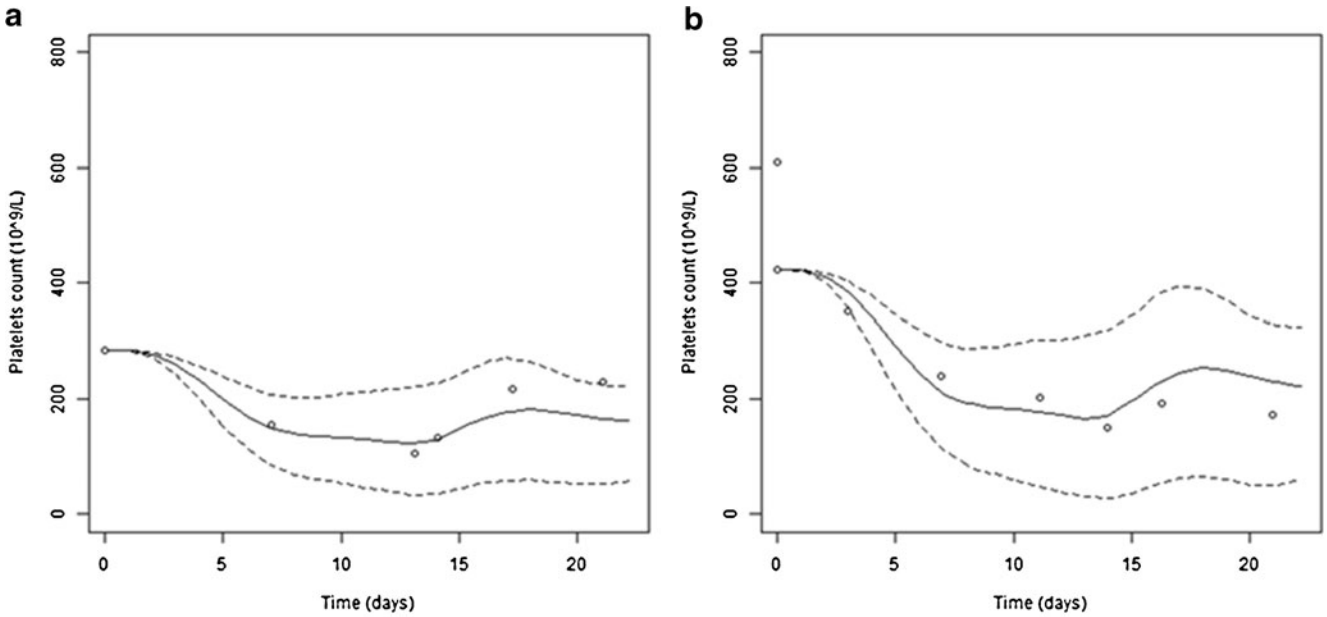

Fig. 7 External individual visual predictive checks of platelet count time profile of two randomly selected patients from CL1-78454-002 clinical study treated according to the optimal administration schedule of abexinostat determined using the intermediate PK/PD model. Individual observed baseline values and PK parameters are used as an input for the PK/PD model. *Dashed lines* (10th and 90th percentiles) represents the 80% predictions interval and *solid line* the median of simulated platelet counts at each time point. *Open circles* represent the observed platelet count.

At the end of this clinical study, all available pharmacodynamic data were used to estimate the final PK/PD model parameters, which could represent a very useful tool for designing future clinical studies (*e.g.* according to an optimal administration schedule) in patients with advanced solid tumors. Final PK/PD model parameters were consistent with those previously estimated with the intermediate PK/PD model (Table III), revealing the sufficient quantity of information contained in the intermediate PK/PD model, to accurately estimate parameters. Although RSE covariance values were not satisfying (greater than 300%), their inclusions in the final structural model were associated with an important drop in the objective function value and were therefore retained in the final PK/PD model. NPDE *versus* TIME plot (Fig. 4b) showed the power of the model to fully predict thrombocytopenia data over time in patients with solid tumors. The smallest parameter precisions of estimation and a considerable decrease in ε-shrinkage (−14.1%) in the final PK/PD model, compared with the intermediate PK/PD model, convey greater confidence in individual platelet-count predictions.

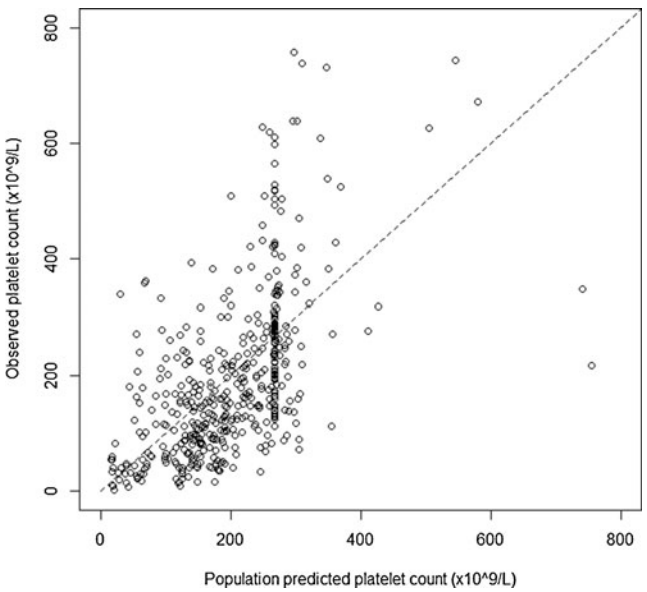

Fig. 8 Posthoc external evaluation of intermediate PK/PD model predictive ability based on Bayesian predictions of the CL1-78454-002 data using the POSTHOC option in NONMEM. CL1-78454-002 observed *vs.* population predicted platelet count. The *dashed line* represents the identity line.

These results highlight the impact that a modeling and simulation approach may have in clinical development (30). The integration of early PK and PD data trials facilitates the development of powerful tools that can be used to quantify dose-toxicity relationship and select the best dosing regimen of a given drug. In the context of oncology, where phase I studies usually aim at finding the MTD, the choice of an administration schedule is determinant as it has a major impact on the MTD. In the more general context of the search for an optimal treatment, the use of quantitative models may help in designing model-based dose escalation algorithms, such as continuous reassessment methods (31). Such algorithms require the definition of the dose-toxicity relationship, which can be determined using such a PK/PD model.

The determination of an optimal administration schedule of abexinostat in patients with advanced solid tumors, was facilitated by the availability of PK and PK/PD data from previous clinical studies, allowing for rapid Modeling and Simulation work. In order for model-based approaches to benefit drug development, early trial data is necessary, as well as a collaborative interaction between pharmacometricians and clinicians to facilitate for rapid data analysis and decision-making.

CONCLUSION

This work shows a clinical application of early PK and PKPD modeling of a new HDACi as an influential development tool for the selection of an optimized administration schedule. A wide range of simulation conditions were evaluated, in terms of platelet-count decrease, and an optimized administration schedule was determined. This treatment schedule was clinically evaluated after a CL1-78454-002 protocol amendment and a new MTD was defined.

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Quentin Chalret du Rieu and Sylvain Fouliard contributed equally to this work.

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