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PHASE I STUDIES

Population pharmacokinetic analysis of AR-67, a lactone stable camptothecin analogue, in cancer patients with solid tumors

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Summary

Background AR-67 is a novel camptothecin analogue at early stages of drug development. The phase 1 clinical trial in cancer patients with solid tumors was completed and a population pharmacokinetic model (POP PK) was developed to facilitate further development of this investigational agent. *Methods* Pharmacokinetic data collected in the phase 1 clinical trial were utilized for the development of a population POP PK by implementing the non-linear mixed effects approach. Patient characteristics at study entry were evaluated as covariates in the model. Subjects ($N = 26$) were treated at nine dosage levels (1.2–12.4 mg/m^2/day) on a daily × 5 schedule. Hematological toxicity data were modeled against exposure metrics. *Results* A two-compartment POP PK model best described the disposition of AR-67 by fitting a total of 328 PK observations from 25 subjects. Following covariate model selection, age remained as a significant covariate on central volume. The final model provided a good fit for the concentration versus time data and PK parameters were estimated with good precision. Clearance, inter-compartmental clearance, central volume and peripheral volume were estimated to be 32.2 L/h, 28.6 L/h, 6.83 L and 25.0 L, respectively. Finally, exposure-pharmacodynamic analysis using E_{max} models showed that plasma drug concentration versus time profiles are better predictors of AR-67-related hematologic toxicity were better predictors of leukopenia and thrombocytopenia, as compared to total dose. *Conclusions* A POP PK model was developed to characterize AR-67 pharmacokinetics and identified age as a significant covariate. Exposure PK metrics C_{max} and AUC were shown to predict hematological toxicity. Further efforts to identify clinically relevant determinants of AR-67 disposition and effects in a larger patient population are warranted.

Keywords Camptothecins · AR-67 · Population pharmacokinetics · Performance status

Introduction

Topotecan and irinotecan, two FDA-approved camptothecins, are used clinically to treat cancer patients. They interact with topoisomerase-I, a nuclear enzyme responsible for relieving DNA torsional stress, and induce cell death by stabilizing the DNA-enzyme-drug complex [1, 2]. Camptothecin analogues are present in two forms, the charged carboxylate and the lipophilic lactone, in a pH- and protein-binding-dependent equilibrium [3, 4]. However, it has been shown that their cytotoxic action is mediated primarily via the lactone species [2].

Similar to most cytotoxic anticancer agents, camptothecins have a relatively low therapeutic index and their administration has been associated with life-threatening toxicities [5–7]. Identifying sources of variability between patients allows the establishment of an accurate dose-response relationship that could guide dosing resulting in enhanced efficacy and reduced toxicity. Population pharmacokinetic (POP PK) models of topotecan and irinotecan have incorporated patient characteristics as covariates in order to increase their predictive value [8–14]. Descriptors of renal function were important determinants of topotecan clearance [10–12, 15–17]. On the other hand, impaired liver function, UGT1A1*28 homozygosity

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and co-administration of UGT1A1*28 inducers or inhibitors were found to correlate with chemotherapy-related toxicity in patients treated with irinotecan [18–21].

AR-67 (7-t-butyldimethylsilyl-10-hydroxycamptothecin, DB-67) [22–25] is a novel camptothecin analogue, currently undergoing early phase clinical trials. AR-67 is a 3rd generation camptothecin with low carboxylate-to-lactone ratio, which characterizes it as blood stable [25–28]. It also has increased lipophilicity [29], attributed to its 7-silyl group, which is thought to improve cytotoxicity by increased diffusion through the membranes and thus allowing interaction with topoisomerase-I [25, 30, 31]. AR-67 was found to be bound to albumin and overall protein binding was determined to be 95.4% ±1.8 for the lactone and 89.7 ± 3.2 for the carboxylate [32]. Exposure to AR-67 correlated with hematological toxicity experienced by the study participants. Subsequent in vitro studies on the metabolic profile of AR-67 suggested drug elimination via extensive metabolism by uridinoglucuronosyltransferases (UGT) and CYP450 enzymes in the gut and liver, respectively [33]. Using recombinant microsomal enzyme systems, AR-67 was metabolized by CYP3A5, CYP3A4, CYP1A1 and CYP1A1, in the order of activity. UGT1A7 and UGT1A8 were identified as the primary extrahepatic (i.e., intestinal) phase-II metabolizing enzymes for AR-67 while the observed in vitro interaction with the primarily hepatic UGT1A1 was weak. These findings suggest that the extrahepatic expression of UGT1A7/1A8 may be responsible for the lack of diarrhea among patients that have received AR-67 [27, 28], whereas diarrhea has been identified as a dose limiting toxicity for irinotecan which is glucuronidated by UGT1A1 in the liver, but not in the gut [7, 34, 35]. As the development of AR-67 is advancing in the clinic, a POP PK model is likely to facilitate the process and potentially identify subpopulations with the highest risk of drug-related toxicities.

In this study, the pharmacokinetic data collected in a phase 1 clinical trial [27] were used to develop a POP PK model that describes the distribution and elimination profile of AR-67 lactone following intravenous (i.v.) infusion in cancer patients. Available covariates potentially contributing to PK parameter variability were tested for inclusion into the model. Additionally, the effect of drug exposure and PK parameters on reported AR-67 hematological toxicity was investigated.

Materials and methods

Study population

Data analysis included AR-67 plasma concentration versus time values from cancer patients with solid tumors that participated in a phase 1 clinical trial, which was designed to determine the maximum tolerated dose (MTD) and to identify the dose-limiting toxicities for this novel camptothecin analogue [27]. AR-67 was administered as a 1-h infusion daily for the first 5 days of a 21-day cycle. Patient eligibility criteria included age ≥ 18, ECOG PS ≤2, and adequate hematologic, renal and liver function and have been described elsewhere [27]. Patients allergic to other camptothecins or cremophor and patients receiving anticonvulsants or any other strong enzyme inducers were excluded from the study. The study was approved by the Institution Review Board of each participating institution and written informed consent was obtained from patients before entering the study [27].

Body weight (kg) and height (cm) measurements from study participants were used to estimate body-size measures such as Body Surface Area (BSA, m^2) using the equation [36] below:

$$BSA = 0.007184 \times WT^{0.425} \times HT^{0.725} \quad (1)$$

All patients participating in the phase 1 clinical trial were closely monitored for hematological toxicity throughout Cycle 1. Measurements of the absolute neutrophil count (ANC, 10^3/ mm^3), white blood cell counts (WBC, 10^3/ mm^3), hemoglobin levels (Hgb, g/dL) and platelet counts (PLT, 10^3/mm^3) were taken on Days 1 (baseline), 8, 15 and 21 of Cycle 1. Nadir for every toxicity parameter was defined as the lowest measurement among the ones collected during Cycle 1 per study participant.

Drug administration

AR-67 was supplied by the National Cancer Institute (Rapid Access to Intervention Development program, RAID) and prepared for administration as described by Arnold et al. [27]. The investigational agent was administered at 9 dose levels ranging from 1.2 to 12.4 mg/m^2/day.

Pharmacokinetic sampling and AR-67 quantitation in plasma

Blood samples were collected from each patient at predose, 5, 45, 65 min and 1, 5, 2, 4, 6, 8 and 24 h after the start of the infusion on Days 1 and 4 of Cycle 1 and AR-67 lactone was quantified using a validated HPLC method, as described previously [37]. Lactone concentrations lower than 2.5 ng/mL were not included in the pharmacokinetic analysis as they were below the lower limit of quantification.

Population pharmacokinetic analysis

Population pharmacokinetic analysis was performed using the nonlinear mixed effects modeling approach implemented in NONMEM (version 7.3, Icon Development

Solutions, Hanover, MD). First order conditional estimation (FOCE) with interaction method was used for the pharmacokinetic population analysis. Statistical analysis, data graphical representation and data manipulation were performed using R Studio (version 1.1.442).

Base model

One- and two-compartment models were tested as structural models using NONMEM subroutines, ADVAN1 TRANS2 and ADVAN 3 TRANS 4, respectively. Interindividual variability (IIV, η, eta) on the population estimate (P_{pop}, θ, Theta) of the pharmacokinetic parameter P for subject (i) (P_i) was modeled as an exponential term as follows:

$$P_i = P_{pop} \times \exp(\eta_i) \quad (2)$$

Error was introduced due to interindividual and inter-occasion variability (IOV), while the remaining unexplained variability due to e.g., assay and sample handling/processing was designated as residual error (ε, epsilon). Different residual error models including additive, proportional, and combined error models were tested. The distributions of the η and ε parameters were assumed to be normal with zero as mean and variances equal to ω (omega) and σ (sigma), respectively.

Goodness of fit plots, likelihood ratio test (Objective Function Value, OFV), precision of parameter estimates, eta shrinkage and successful minimization were used to determine model superiority. A decrease by ≥3.84 units (1 degree of freedom in χ^2 distribution) of the OFV was considered statistically significant ($p < 0.05$).

Inter-occasion variability

Inter-occasion variability (IOV) between Day 1 (occasion 1) and Day 4 (occasion 2) of Cycle 1 was tested on the drug clearance (CL) from the central compartment and volume (V) in the base model prior to the inclusion of any covariates. IOV was modeled as described by Karlsson et al. [38]. Briefly, IOV was included in the model as an exponential term (η_{IOV}):

$$P_i = P_{pop} \times exp(\eta_i) \quad \times exp\eta(\eta_{IOV,1}) \quad (3)$$

$$P_i = P_{pop} \times exp(\eta_i) \quad \times exp\eta(\eta_{IOV,2}) \quad (4)$$

where $\eta_{IOV,1}$ and $\eta_{IOV,2}$ are the inter-occasion variability for subject (i) on occasion 1 (Day 1) and on occasion 2 (Day 4), respectively. IOV is lumped with the residual error of the model in NONMEM [38]. Therefore, IOV was included in the model when a decrease in σ was observed and all the aforementioned criteria for model acceptance were met.

Covariate model

Covariates considered during the analysis were both continuous and categorical and included age (yr), gender, weight (kg), height (cm), body surface area (BSA, m^2), body mass index (BMI, kg/m^2), creatinine clearance (CRCL, mL/min), blood urea nitrogen (BUN, mg/dL), albumin (ALB, g/dL), total bilirubin (BIL, mg/dL), alkaline phosphatase (ALP, U/L), alanine transaminase (ALT, U/L), aspartate transaminase (AST, U/L), and lactate dehydrogenase (LDH, U/L). CRCL (mL/min) was calculated using the Cockcroft-Gault equation [39]:

$$CRCL = \frac{(140 - Age) \times WT \times A}{72 \times SrCr} \quad (5)$$

where Age is in years, A is 1 for males and 0.85 for females, and SrCr is serum creatinine (mg/dL). To minimize the bias in CRCL calculation, WT (in kg) takes on the value of total body weight (TBW), ideal body weight (IBW) and adjusted body weight ($ABW_{0.4}$) in underweight patients, patients of normal weight, and overweight or obese patients, respectively [40]. IBW was calculated using Eq. 6 in males and Eq. 7 in females, whereas $ABW_{0.4}$ was calculated following Eq. 8 [40]:

$$IBW_{male} = 50 + 2.3\ kg\ (every\ inch\ over\ 5\ feet) \quad (6)$$

$$IBW_{female} = 45.5 + 2.3\ kg\ (every\ inch\ over\ 5\ feet) \quad (7)$$

$$ABW_{0.4} = IBW + 0.4 \times (TBW - IBW) \quad (8)$$

After the base model was selected, patient attributes were evaluated graphically for relationships with IIV terms. Missing covariate values were imputed with the median value of all treated subjects. Covariates were inserted in the model following Eqs. 9–10 for continuous and categorical variables, respectively:

$$\theta_i = \theta_{Typical} \left(\frac{COV_j}{median(COV_j)} \right)^{\theta_{covij}} \quad (9)$$

$$\theta_i = \theta_{Typical} * e^{(COV_j * \theta_{covij})} \quad (10)$$

where $\theta_{Typical}$ is the typical value of parameter θ_i; COV_j is the value of j^{th} continuous variable or an indicator variable with the value of 0 or 1 for a categorical variable; and θ_{covij} is the effect of the j^{th} covariate on the i^{th} parameter.

Initial screening of covariates was conducted using univariate analysis, where a decrease by ≥3.84 units of the OFV was considered statistically significant ($p < 0.05$). A decrease in IIV was also required to retain a covariate into

the model. Selected covariates were tested for co-linearity by calculating the Pearson correlation coefficient in R Studio [41, 42]. After the initial screening, covariates were then subject to stepwise covariate model (SCM) selection in Perl speaks NONMEM (PsN) (version 4.7.0). *P*-values for forward selection and backward elimination in SCM were 0.05 and 0.01, respectively.

Model evaluation

Plots of individual (IPRED) or population predictions (PRED) versus observed drug concentrations were analyzed for closeness to the line of unity, and plots of individual weighted residuals (IWRES) or conditional weighted residuals (CWRES) versus IPRED/PRED or time after dose were analyzed for randomness. Precision of model parameter was expressed as coefficient of variation (CV) %, which was produced by dividing the standard error over the parameter value provided by NONMEM.

Model performance was assessed by performing prediction-corrected visual predictive checks where 500 replicates per time point were generated through resampling using PsN. The simulation-based 95% confidence intervals for the median and the 5th and 95th percentiles were overlaid with prediction-corrected observed concentrations. Model reliability was evaluated with bootstrap resampling approach ($n = 1500$ simulated datasets) in NONMEM where the first 1000 runs that minimized successfully were utilized to calculate 95% confidence intervals for the obtained parameter estimates.

Evaluation of relationships between PK and toxicity endpoints

Categorical toxicity endpoints investigated included grade 3 or 4 leukopenia and thrombocytopenia according to CTCAE version 3.0. Mann-Whitney test was used to compare the distribution of individual predicted PK parameters (clearance and volume) in patients with or without hematological toxicity, at a significance level of 0.05. Additionally, logistic regression was performed to evaluate the relationship between these categorical toxicity endpoints and metrics of drug exposure in R. Metrics of drug exposure investigated included total dose, area under the concentration versus time curve from 0 to infinity ($AUC_{0-\infty}$) and maximum concentration (C_{max}). To calculate the C_{max} and $AUC_{0-\infty}$, model predicted drug concentration versus time profiles for subjects included in the analysis were simulated using the final population PK model in NONMEM at 0.1, 0.2, 0.3, 0.4, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6 h after the first dose. C_{max} and $AUC_{0-\infty}$ were determined for each subject by using predicted concentrations in noncompartmental analysis with the PKNCA R package.

Continuous toxicity endpoints were defined as the percent decrease of WBC or platelets from baseline values to nadir values. Metrics of drug exposure were fit to the hyperbolic E_{max} model and the sigmoid E_{max} model in GraphPad Prism (version 7.04, GraphPad Software, La Jolla, CA) following Eqs. 11 and 12, respectively:

$$E = E_0 + \frac{E_{max} \times d}{ED_{50} + d} \tag{11}$$

$$E = E_0 + \frac{E_{max} \times d^H}{ED_{50} + d^H} \tag{12}$$

where d represents dose, $AUC_{0-\infty}$, or C_{max}, $E(d)$ is the toxicity effect as a function of d, E_{max} is the maximum effect, ED_{50} is the value of d associated with half the E_{max}, and H represents the Hill coefficient. As the hyperbolic E_{max} model is a special

Table 1 Patient characteristics at study entry

Characteristic	Median (range) or Counts
Age, year	62 (30–79)
Gender	
Male	15
Female	10
Race	
Caucasian	24
Black	1
Weight, kg	82.1 (48.9–171)
Height, cm	173 (151–192)
BSA, m^2	2.00 (1.48–2.44)
BMI, kg/m^2	27.8 (16.8–73.5)
CRCL, mL/min	88.6 (51.6–156.2)*
BUN, mg/dL	14 (4–35)*
ALB, g/dL	3.1 (1.2–4.0)[#]
BIL, mg/dL	0.6 (0.4–2.3)[$]
ALP, U/L	94.5 (45.0–299)[$]
ALT, U/L	20 (7–69)[$]
AST, U/L	28.5 (12–51)[$]
LDH, U/L	181 (101–768)[#]
Dose levels, mg/m^2/day	1.2 ($n = 1$) 1.67 ($n = 3$) 2.34 ($n = 3$) 3.23 ($n = 3$) 4.5 ($n = 1$) 6.3 ($n = 1$) 7.5 ($n = 7$) 8.9 ($n = 4$) 12.4 ($n = 2$)

BSA body surface area, *BMI* body mass index, *CRCL* creatinine clearance, *BUN* blood urea nitrogen, *ALB* albumin, *BIL* total bilirubin, *ALP* alkaline phosphatase, *ALT* alanine transaminase, *AST* aspartate transaminase, *LDH* lactate dehydrogenase

*Data were available from 23 patients

[#] Data were available from 21 patients

[$] Data were available from 22 patients

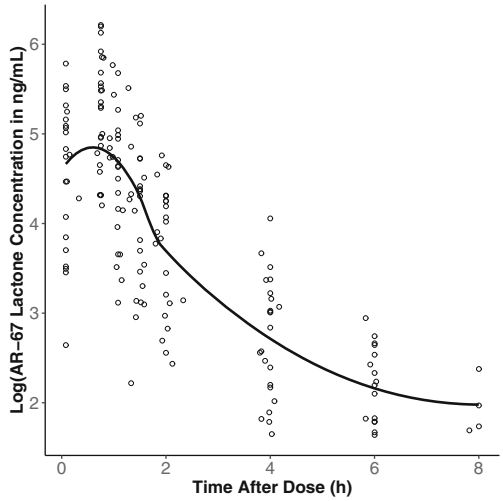


Fig. 1 Log AR-67 lactone concentration versus time after dose curve on Day 1. The solid line represents the LOESS smoothing curve

case of the sigmoid E_{max} model, extra sum-of-squares F test was used to choose the superior model. In the case where only one of the two models achieved convergence, the converged model was presented.

Results

Patient demographics

Of the 26 patients that participated in the phase 1 clinical trial, one patient did not receive all five days of therapy in Cycle 1. Therefore, 25 patients were considered for the development of the base and covariate models for AR-67 lactone. The demographics and clinical characteristics of the 25 patients are summarized in Table 1. The median (range) age and BSA were 62 years (30–79) and 2.00 m^2 (1.48–2.44), respectively. Fifteen of 25 patients (60%) were male.

A total of 328 plasma samples, collected on Days 1 and 4 of Cycle 1 following AR-67 administration, were included in the analysis. Dose levels 2.34 mg/m^2/day ($n = 3$), 3.23 mg/m^2/day (n = 3), 7.5 mg/m^2/day ($n = 7$) and 8.9 mg/m^2/day ($n = 4$) contributed 34, 38, 101 and 57 PK samples, respectively. The mean number of samples per patient included in the analysis was 13.

Population pharmacokinetic analysis of AR-67 lactone

Plot of the log transformed AR-67 lactone concentration versus time curve with LOESS smoothing shows a biphasic pattern, favoring selection of a two-compartment model over a one-compartment model (Fig. 1). The OFV values were 2797.956 and 2412.494 for one-compartment and two-compartment models, respectively, confirming the appropriateness of a two-compartment model. The lowest value for the OFV was observed with the selection of a combined error model consisting of both proportional and additive error compared to other error models. Inclusion of IIV terms for intercompartmental clearance (Q) and volume of the peripheral compartment (V2) resulted in poor parameter estimates with CV% > 50%, so these two terms were fixed to zero.

Incorporation of IOV on CL decreased the OFV significantly; however, it only decreased proportional error from 30.6% to 28.2% and produced large eta shrinkage (>40%). Incorporation of IOV on central volume (V1) did not change

Table 2 Pharmacokinetic parameter estimates and bootstrap results

Original Dataset			Bootstrap Results ($n = 1000$ datasets)	
Parameter	Estimate	CV%	Median	95% CI
Structural Model				
CL, (L/h)	32.2	6.55	31.9	27.8–36.2
V1, (L)	6.83	22.8	6.65	3.34–10.0
Q, (L/h)	28.6	15.2	29.0	20.9–41.4
V2, (L)	25.0	12.5	25.8	20.3–32.9
Inter-subject Variability				
ω^2_{CL}	0.0849	38.9	0.0726	0.0265–0.146
ω^2_{V1}	0.957	39.0	0.856	0.272–1.85
Covariate Model				
AGE on V1	3.36	16.0	3.48	1.34–11.1
Residual error				
σ_{prop}	0.306	10.8	0.302	0.238–0.365
σ_{add}	6.73	29.1	6.59	1.40–9.64

CV coefficient of variance, *CI* confidence interval, *CL* clearance, *V1* central volume, *Q* intercompartmental clearance, *V2* peripheral volume, ω^2 interindividual variability, σ_{prop} proportional error, σ_{add} additive error

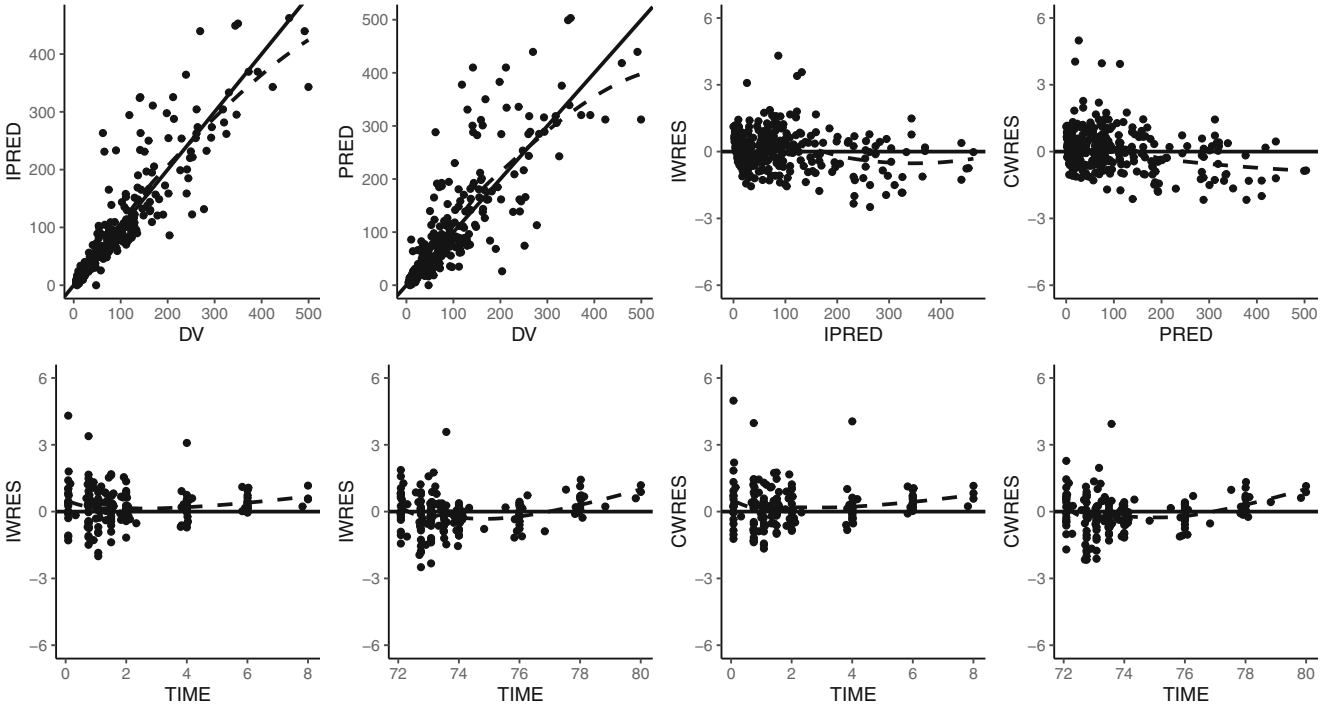


Fig. 2 Goodness-of-fit plots for the final population pharmacokinetic model of AR-67 lactone in cancer patients. The solid line indicates the line of unity for IPRED vs DV and PRED vs DV and zero for the others. The dashed line represents the loess curve. DV: dependent variable, IPRED: individual predicted concentration, PRED: population predicted concentration, IWRES: individual weighted residuals, CWRES: conditional weighted residuals

the OFV or the residual error. Therefore, IOV was not included in the model.

In univariate analysis, BSA and body weight were significant covariates on CL; age, height, gender and BUN were significant covariates on V1. These covariates were incorporated in covariate model selection except for weight, as weight was highly correlated with BSA and was associated with a smaller decrease in OFV and interindividual variability of CL than BSA. Following SCM, age on V1 was the only covariate retained in the final model (OFV = 2401.885) as follows:

$$V1 = V1_{TV} \times \left(\frac{AGE}{62}\right)^{\theta_{AGE\_V1}} \quad (13)$$

where $V1_{TV}$ represents typical population value of V1 in reference subjects aged 62 years, and θ_{AGE_V1} is used to describe the effect of age on V1.

Estimates for the population parameters in the final POP PK model are summarized in Table 2. They were obtained with satisfactory precision (CV% < 50%), and eta shrinkage estimates for CL and V1 were 4.1% and 11%, respectively. AR-67 lactone population CL, V1, Q and V2 parameter estimates were 32.2 L/h, 6.83 L, 28.6 L/h and 25.0 L, respectively. Interindividual variability (CV%) was 29.1% and 97.8% for the population CL and V1, respectively.

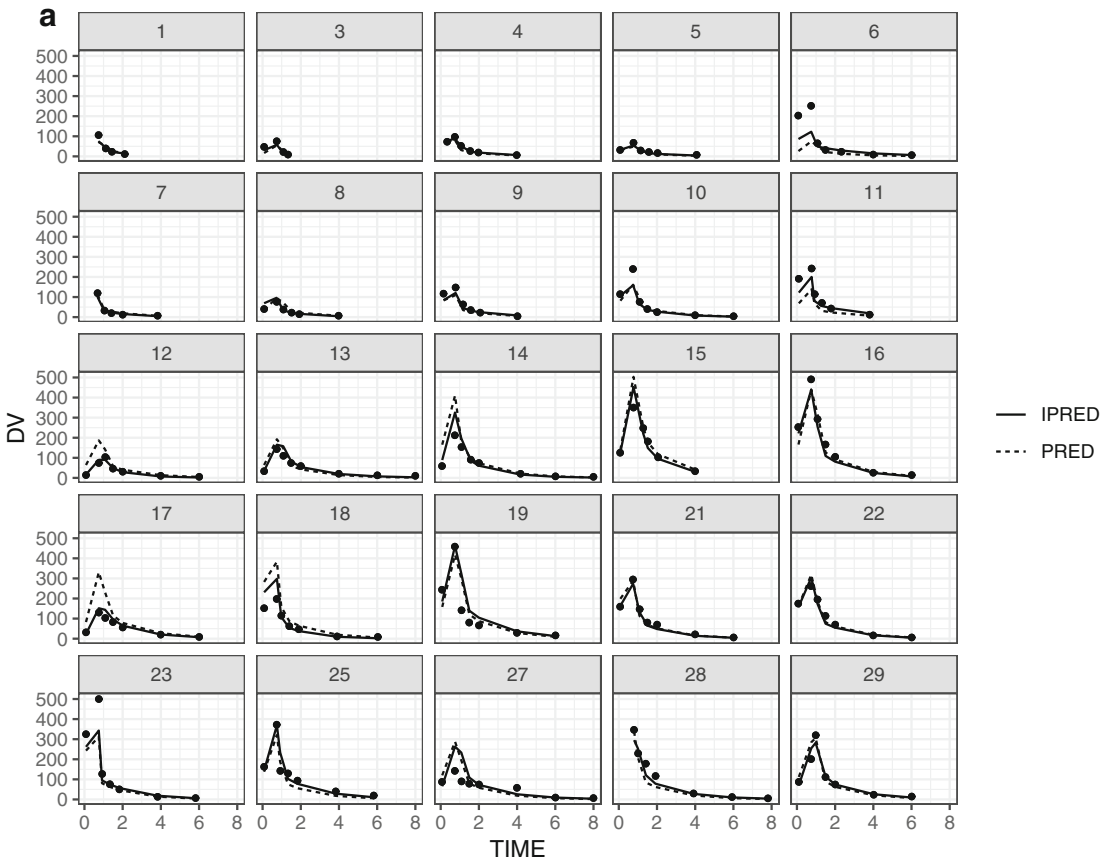
Diagnostic plots showed a close-to-symmetrical distribution around the line of unity when IPRED or PRED was plotted against observed concentration values (Fig. 2), but it could be seen that at high concentrations the model slightly underestimates the concentrations. No apparent model misspecification was detected when CWRES or IWRES was plotted against PRED/IPRED, as the loess smoothing lines lie closely to the horizontal line of 0. In plots of individual fits (Fig. 3), individual observed concentrations were in general well described by model predicted concentrations.

Evaluation of model stability was completed by performing bootstrap analysis. As shown in Table 2, median bootstrap estimates were similar to the estimates obtained from the POP PK model. Additionally, prediction-corrected visual predictive check presented in Fig. 4 demonstrated an overall lack of model misspecification, with the vast majority of prediction-corrected observations contained within model-based confidence intervals.

Evaluation of relationships between PK and toxicity endpoints

Values of nadir WBC and platelets counts were available from all 25 patients included in the POP PK analysis, so these

Fig. 3 Plots of individual fits. Numbers on the top of each plot represent patient ID. DV: dependent variable, IPRED: individual predicted concentration, PRED: population predicted concentration ▶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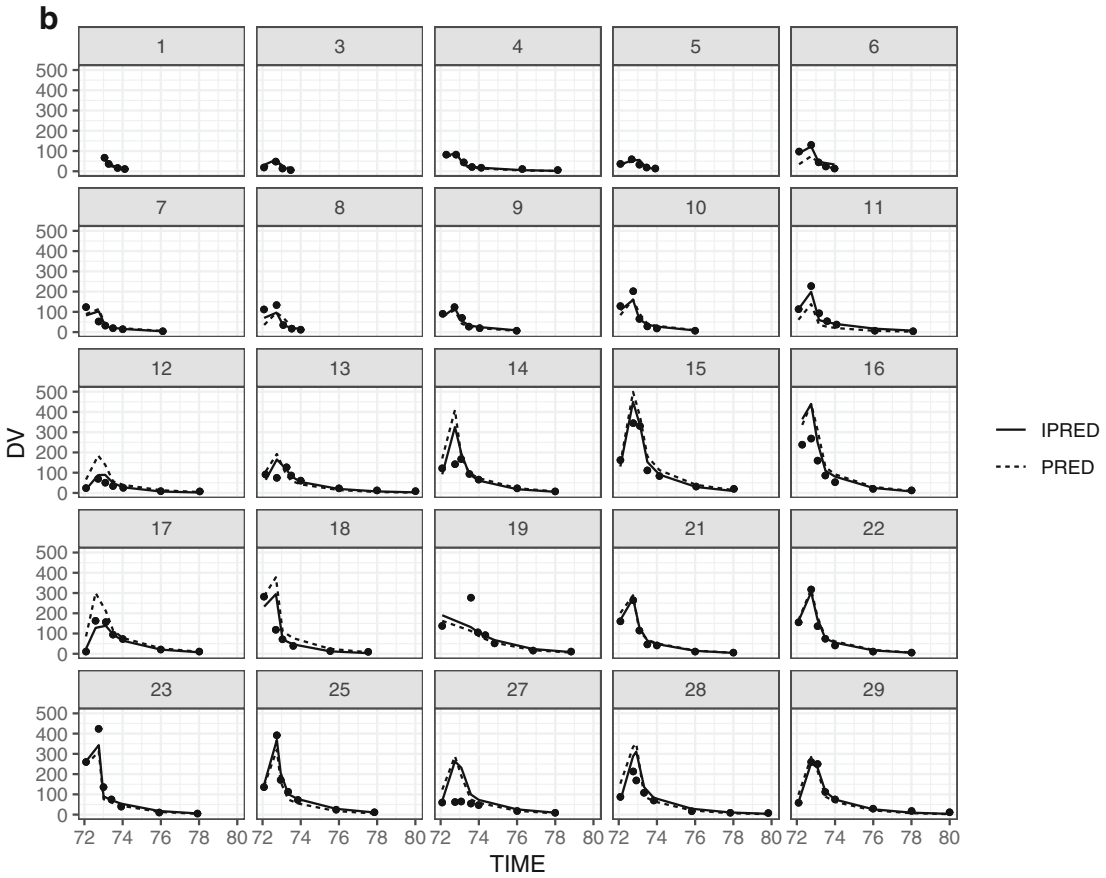
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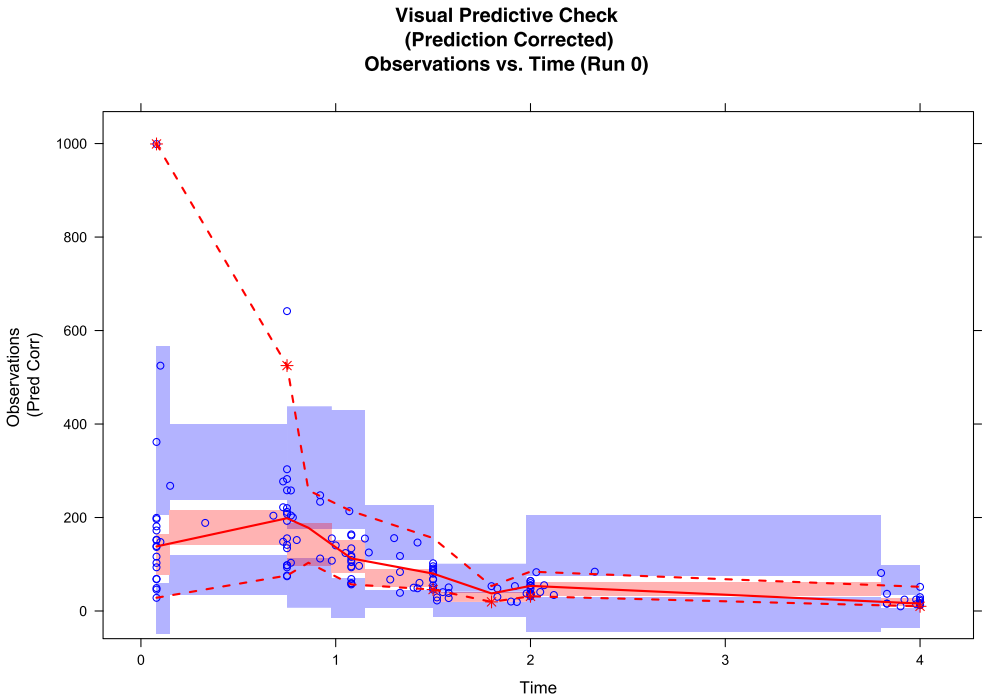

Fig. 4 Prediction-corrected visual predictive checks for AR-67 lactone using 500 replicates per time point. Data depicted for the first 4 h following dosing as this time window captures the majority of the available observations. The blue half-circles are the prediction-corrected observations. The solid line represents the median and the dashed lines the 5% and 95% percentiles of prediction-corrected observations. The light areas and dark areas represent the simulation-based 95% confidence interval for the median and the 5% and 95% percentiles, respectively

patients were included in the analysis of categorical toxicity endpoints. Using the Mann-Whitney test, no statistically significant difference was detected in the distribution of CL, V1 and V_{ss} (volume at steady state, sum of V1 and V2) in patients with or without grade 3 or 4 leukopenia and thrombocytopenia. Results from fitting categorical toxicity endpoints with logistic regression are shown in Fig. 5. Similar to the findings from noncompartmental analysis [27], logistic regression showed that occurrence of grade 3 or 4 leukopenia was more likely than thrombocytopenia at the same level of $AUC_{0\text{-}\infty}$, C_{max}, and total dose. *P*-values for logistic regression modeling leukopenia were 0.06, 0.02 and 0.02 when $AUC_{0\text{-}\infty}$, C_{max}, and total dose were used as independent variables, compared to *p*-values of 0.45, 0.07 and 0.11 when thrombocytopenia was modeled as the endpoint. Multivariate logistic regression models were attempted, but model convergence was not achieved due to the limited sample size.

Baseline WBC and platelet counts were available in 22 out of the 25 patients, so continuous toxicity endpoints were studied in these patients. $AUC_{0\text{-}\infty}$, C_{max}, and total dose were fitted to E_{max} models using the percent decrease of WBC or platelets from baseline values to nadir values as the response variable. When $AUC_{0\text{-}\infty}$ was used as the independent variable, the hyperbolic E_{max} model was favored in describing percent decrease of WBC, while the sigmoid E_{max} model was favored in describing percent decrease of platelets using the extra sum-of squares F test (Fig. 6a). The AUC ED_{50} values were 397.6 and 276.8 ng*hr./mL for WBC and platelets, respectively. When C_{max} was used as the independent variable, only the sigmoid E_{max} model achieved convergence for both WBC and platelets, and the C_{max} ED_{50} values were 326.8 and 281.1 ng/mL (Fig. 6b). When total dose was used as the independent variable, though the hyperbolic E_{max} model achieved convergence for both WBC and platelets, neither model produced physiologically reasonable ED_{50} values, which was indicative of model misspecification (Fig. 6c).

Discussion

Here we studied the plasma pharmacokinetics of AR-67 lactone, a novel camptothecin analogue, in cancer patients participating in a phase 1 clinical trial. A POP PK model was developed and covariates were tested with the intent to explain the inter-subject variability associated with the obtained PK parameter estimates. Our analysis showed that disposition of AR-67 lactone following i.v. infusion was well characterized by a two-compartment model, similar to other camptothecin analogues [12–14]. Clearance was estimated to be 32.2 L/h. Comparable clearance values were obtained with other camptothecins, including irinotecan and the lipophilic karenitecin [13, 14, 20, 43]. Although this formulation contains Cremophor EL, the amount in present in the formulation is well below what would be required for micelle formation and therefore would not have influenced the disposition of AR-67.

As AR-67 is undergoing clinical development, it is critical to describe its disposition and elimination in humans as well as potential sources of inter-subject variability observed with these processes. Following covariate model selection, age effect on central volume was retained, with older age associated with larger central volume. Incorporation of age into the model reduced interindividual variability in V1 from 118% to

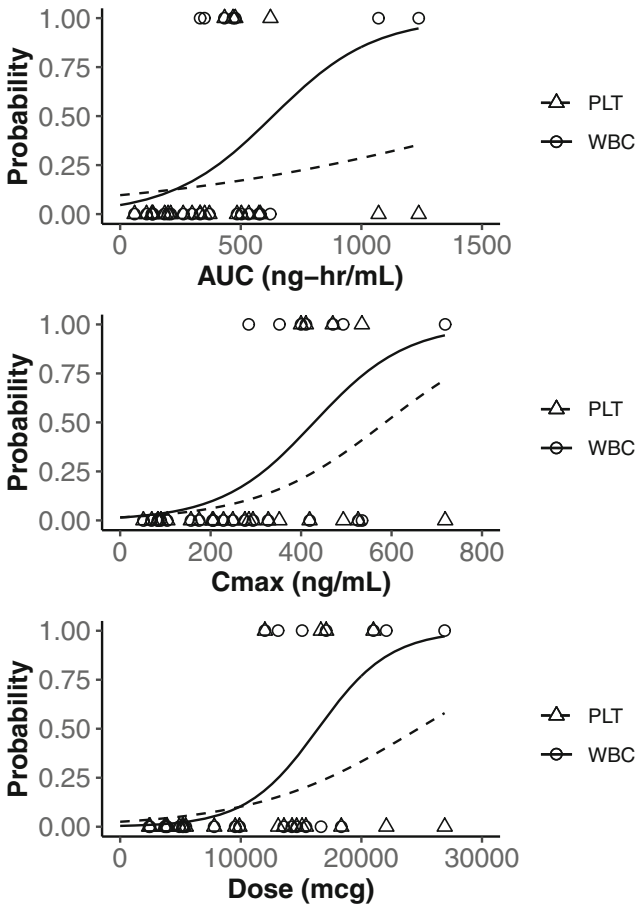

Fig. 5 Logistic regression analysis showing the probabilities of grade 3/4 leukopenia and thrombocytopenia in relation to AUC0-∞, C_{max}, and dose. Solid and dotted lines represent the regression lines for grade 3/4 leukopenia and thrombocytopenia, respectively. Circles and triangles represent the actual data points of the occurrence of grade 3/4 leukopenia and thrombocytopenia, respectively

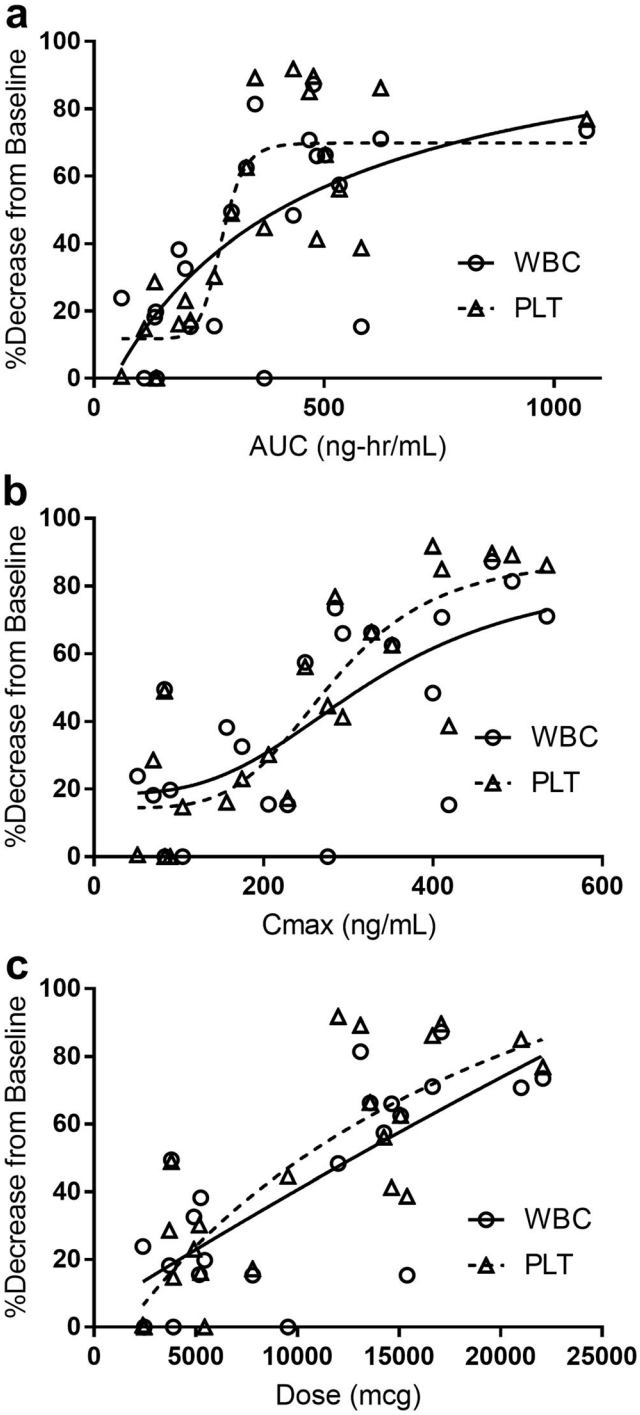

Fig. 6 Fitting of E-max models to percent decrease of white blood cells and platelets from baseline to nadir values in relation to AUC0-∞, C_{max}, and dose. Solid and dotted lines represent the regression lines for percent decrease in white blood cells and platelets, respectively. Circles and triangles represent the actual data points of percent decrease in white blood cells and platelets, respectively

97.8%, though the interindividual variability remained large. Age effect on volume of distribution has not been shown with two other marketed camptothecin analogs, irinotecan and topotecan [11, 12, 14]. However, it must be noted that while irinotecan and topotecan are both hydrophilic camptothecin analogs, AR-67 is about 25 times more lipophilic than camptothecin [22, 44]. Drug distribution of drugs has been shown to vary with age potentially following two patterns: 1) for hydrophilic drugs, volume of distribution decreases with age due to the decline in total body water content, and 2) for hydrophobic drugs, volume of distribution increases with age due to the increase in total body fat [45]. As AR-67 is a lipophilic compound, the observed relationship between age and volume is as expected.

In spite of the high AR-67 lipophilicity [22, 44], the model-predicted distribution of AR-67 lactone appeared to be limited; V_1 and V_2 estimates were 6.83 L and 25.0 L, respectively. These estimates are in agreement with the high protein binding observed with AR-67 as reported by Leggas et al., 2009; overall protein binding was found to be 95.4% ±1.8 for the lactone form [32]. Another factor potentially contributing to the limited tissue distribution may be the rapid clearance of the carboxylate form from

the systemic circulation compared to the lactone form, essentially leading to constant lactone-to-carboxylate conversion rather than effective lactone distribution in the peripheral tissues such as the adipose [26]. Finally, protein binding was evaluated for potential effect on the volume of distribution. However, when albumin was evaluated as a covariate on V1, no statistically significant improvement in the model performance was observed. As such, albumin was not included in the final model.

A correlation has been established between high bilirubin blood levels and increased exposure in patients with hepatic impairment that could lead to high grade toxicity, resulting in recommendations for dose adjustments for irinotecan, an extensively metabolized camptothecin [18, 19, 46, 47]. In vitro studies and clinical data have suggested that liver/gut metabolism is the major route of elimination for AR-67 [27, 33]. Thus, for the development of the covariate POP PK model, baseline liver enzymes and other indicators of liver function, such as albumin and bilirubin levels, were tested as covariates. However, inclusion of these covariates did not improve the model. As expected, CRCL, a well-established indicator of kidney function, did not reduce the IIV of clearance.

Body surface area has been identified as a determinant of topotecan and irinotecan total body clearance [12–14]. Mathijseen et al. [48] introduced the concept of flat or fixed irinotecan dosing after showing that normalizing irinotecan clearance to body-size measures did not reduce the observed IIV of this pharmacokinetic parameter. In our study, inclusion of BSA into the covariate model led to statistical significant decrease in OFV and a decrease in IIV of clearance from 29.2% to 25.5% in univariate analysis, suggesting that flat dosing of AR-67 may not be appropriate. However, this observation requires further validation under an appropriately designed clinical study.

Hematological toxicities, including leukopenia and thrombocytopenia, were dose limiting toxicities in the AR-67 phase 1 clinical trial. Therefore, the relationship between PK and hematological toxicity endpoints were investigated. Using logistic regression, C_{max} and total dose were shown to be significant predictor of grade 3/4 leukopenia, while $AUC_{0-\infty}$ was borderline significant. When grade 3/4 thrombocytopenia was modeled, only C_{max} was borderline significant. Multivariate logistic regression would allow evaluation of variables other than metrics of drug exposure in relationship to the likelihood of toxicity. However, due to the limited sample size, multivariate analysis was attempted but not successful. Fitting continuous toxicity endpoints to E_{max} models was successful when percent decrease of blood cells was expressed as functions of $AUC_{0-\infty}$ and C_{max}, but not the total dose. Together, these results showed that plasma drug concentration versus time profiles are better predictors of AR-67-related hematologic toxicity than drug dose. These findings are in agreement with early development studies on toxicity profiles of other camptothecins showing a relationship between exposure and hematological toxicity [49–51].

There are several limitations with our study. Unexplained interindividual variability in clearance could possibly be explained by differences in the expression or function levels of transporters and metabolic enzymes such as OATP1B1/1B3, P-gp, BCRP, MRP2, UGT1A7/1A8/1A1 and CYP3A4/5 [52–54] involved in the disposition and elimination of AR-67. Future studies should focus on exploring the impact of genetic polymorphisms of the aforementioned transporters and metabolic enzymes on AR-67 pharmacokinetics and potential drug-drug interactions between AR-67 and enzyme inducers/inhibitors. Tobacco and obesity have been shown to exacerbate the activity of metabolic enzymes such as CYP1A1, CYP1A2 and UGTs [55–57] that are assumed to play a role in the elimination of AR-67 in the liver and gut [33]. Although van der Bol et al. [58] showed that exposure to irinotecan and SN-38 was lower in smokers than patients that did not smoke, smoking status was missing for some of the subjects in this analysis and therefore was not tested as a covariate during the POP PK model building process. The impact of smoking and obesity on AR-67 disposition should be evaluated further in a larger patient population.

In conclusion, a POP PK model for AR-67 lactone was developed using the nonlinear mixed effects approach. A two-compartment model was fit to AR-67 lactone plasma concentrations from 25 subjects. After covariate selection, age effect on central volume was incorporated in the final model. The final model provided a satisfactory fit for the PK observations, and PK parameters were estimated with good precision. Relationships between PK and hematological toxicities were explored using logistic regression and E_{max} models, and plasma concentrations versus time profiles (C_{max} and AUC) were shown to be a better predictor of hematologic toxicity than total dose. As this is the first POP PK analysis on AR-67, more extensive studies need to take place towards the identification of clinically relevant individual predictors of AR-67 disposition and pharmacodynamic effects.

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Compliance with ethical standards

Conflict of interest S.M. Arnold, and M. Leggas have received research funding from Arno Therapeutics. The authors have no other conflicts of interest to declare.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent Informed consent was obtained from all individual participants included in the study.

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