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ORIGINAL ARTICLE

Population pharmacokinetics of clofarabine and its metabolite 6-ketoclofarabine in adult and pediatric patients with cancer

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Abstract Clofarabine for injection is a second-generation nucleoside analog approved in the United States (Clolar®) and Europe (Evoltra®) for the treatment of pediatric relapsed or refractory acute lymphoblastic leukemia. This report describes the population pharmacokinetics of clofarabine and its metabolite 6-ketoclofarabine in adult and pediatric patients with hematologic malignancies or solid tumors. Clofarabine pharmacokinetics were best described by a 2-compartment model with linear elimination and first-order absorption after oral administration. Clofarabine was rapidly absorbed following oral administration with a mean absorption time of less than 2 h and bioavailability of 57.5%. The important covariates affecting clofarabine pharmacokinetics were age, weight, and estimated creatinine clearance (eCrCL). No difference in pharmacokinetics was observed between sexes, races, or disease type. The elimination half-life was dependent on all the covariates but was generally less than 7 h in all cases. A difference in clofarabine pharmacokinetics was observed between adults and children. For a pediatric patient 3 years old weighing 16 kg with an eCrCL of 138 mL/min/1.73 m^2, the population estimates for total systemic clearance and volume of distribution at steady-state were 18.3 L/h (1.14 L/h/kg) and 92.9 L (5.81 L/kg), respectively. α- and β-half-life were 0.9 and 4.4 h, respectively. For an elderly patient 82 years old weighing 96 kg with an eCrCL of 46 mL/min/1.73 m^2, the population estimates for CL and Vdss were 21.5 L/h (0.22 L/h/kg) and 257.4 L (268 L/kg), respectively. α- and β-half-life were 0.5 and 10.6 h, respectively. Because of the difference in pharmacokinetics, adults have higher exposure than children

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given a similar dose standardized to body surface area. The exact mechanism of this difference is not understood. As eCrCL decreased, exposure increased due to reduced total systemic clearance. In the case of moderate (eCrCL 30 to 60 mL/min/1.73 m^2) and severe (eCrCL <30 mL/min/1.73 m^2) renal impairment, dose reduction may be needed to maintain similar exposure in an equivalent patient of the same age, weight, and normal renal function after both oral and intravenous administration. 6-Ketoclofarabine was a minor metabolite with peak plasma concentrations occurring about 1 h after the start of the infusion and having a metabolite ratio averaging less than 5% and not more than 8% for any particular individual. 6-Ketoclofarabine was rapidly cleared from plasma with an average apparent half-life of 4.9 h (range 3.9 to 6.2 h). No accumulation of 6-ketoclofarabine was observed with predose samples all below the limit of quantification on Days 8 and 15. Further monitoring of 6-ketoclofarabine is not required in future studies.

Keywords Nucleoside · NONMEM · Renal impairment · Modeling and simulation · Hybrid estimation · Imputation

Abbreviations

α-Half-life	Distribution half-life
β-Half-life	Elimination half-life
ALB	Albumin
AUC(0–last)	Area under the curve from time 0 to the last measure concentration
AUC(0–6)	Area under the curve from time 0 to 6 h post-dose
BSV	Between-subject variability
CL	Total systemic clearance
C_{max}	Maximal concentration
Cr	Serum creatinine concentration
DLT	Dose-limiting toxicity
eCrCL	Estimated creatinine clearance
F1	Oral bioavailability
FOCE	First-order conditional estimation
IOV	Inter-occasion variability
IVI	Intravenous infusion
KPS	Karnofsky performance status
LRT	Likelihood ratio test
MAT	Mean absorption time
MTD	Maximum tolerated dose
Q	Intercompartmental clearance
RP2D	Recommended phase II dose
RECIST	Response evaluation criteria in solid tumors
T_{max}	Time to maximal concentration
V2	Central volume of distribution
V3	Peripheral volume of distribution
Vdss	Volume of distribution at steady-state
WBC	White blood cell

Introduction

Clofarabine for injection is a second-generation nucleoside analog approved in the United States and Europe for the treatment of pediatric relapsed or refractory acute lymphoblastic leukemia [1]. The precise mechanism of action of clofarabine on dividing and non-dividing cells is unknown. The purine nucleosides must be converted intracellularly to their respective 5′-triphosphate forms to exert their cytotoxic effects. Clofarabine has enhanced affinity for the activating phosphorylating enzyme, deoxycytidine kinase (dCK), exceeding that of cladribine and the natural substrate, adenosine [2]. In parallel, phosphorylated clofarabine acts to inhibit DNA polymerase α and ribonucleotide reductase [3]. Clofarabine disrupts the integrity of mitochondria, leading to the release of the pro-apoptotic mitochondrial proteins cytochrome C and apoptosis-inducing factor, leading to programmed cell death [4]. Clofarabine triphosphate is also incorporated into RNA at approximately 1% of the rate of incorporation into DNA [5].

Clofarabine is prodrug that is devoid of activity on its own. Clofarabine is transported intracellularly by equilibrative and concentrative nucleoside transporters [6]. Once inside the cell, clofarabine is sequentially metabolized to the 5′-monophosphate metabolite by deoxycytidine kinase and to the active 5′-triphosphate metabolite by mono- and di-phosphokinases [5]. Clofarabine is not metabolized in human microsomes or isolated hepatocytes to any significant extent (internal data). In preclinical studies, Fischer 344 male rats were administered clofarabine 25 and 50 mg/kg for 5 days [7]. On the 5th day, rats received a radioactive dose. 6-Ketoclofarabine (2-chloro-9-[3-fluoro-4-hydroxy-5-hydroxymethyl)tetrahydrofuran-2-yl]-1,9-dihydro-6H-purin-6-one), believed to be formed via adenosine deaminase, was the metabolite of greatest concentration found in urine and feces, but in each matrix accounted for only 7% of the daily recovery of radioactivity. 6-Ketoclofarabine was also found in myocardium and liver, but accounted for less than 2% of the total radioactivity in those tissues. No pharmacokinetic data on 6-ketoclofarabine has been reported in humans.

A previous population analysis for clofarabine has been reported in 32 pediatric patients with cancer [8]. A 2-compartment model with weight (scaled to a 40 kg reference patient) modeled as a power function on all pharmacokinetic parameters (0.75 on clearance-related terms and 1.0 on volume-related terms) was fit to plasma clofarabine concentrations. White blood cell (WBC) count, modeled as a power function (scaled to a WBC count of $10 \times 10^3/\mu L$), was a significant predictor of central volume with power term 0.128 ± 0.0314. A reference patient had a systemic clearance of 32.8 L/h

(27% between-subject variability, BSV), central volume of 115 L (56% BSV); intercompartmental clearance of 20.5 L/h (27% BSV), and a peripheral volume of 94.5 L (39% BSV). In adults, a population analysis has not been reported. Non-compartmental results in adults have only been reported in abstracts [9]. Clofarabine pharmacokinetics were dose proportional up to 103 mg/m^2 and were time-invariant. The average systemic clearance, volume of distribution at steady-state (Vdss), and half-life was 18.4 L/h (range 5.9 to 33.2 L/h), 73 L (range 5.6 to 181 L), and 4.0 (range 2.1 to 1 1.8 h), respectively. There was some evidence that Vdss was dose-dependent with Vdss decreasing with increasing dose.

Clofarabine is approved for use only in children and does not as of yet have an approved adult indication. As clofarabine is cleared mostly by the kidney ($\sim$49 to 60% of the drug is excreted unchanged in the urine [10]), there is a need to understand how to make dose recommendations in children with renal impairment. There are two generally acceptable approaches to determining dose adjustments in the presence of renal impairment. One way is to conduct a study in the population of interest with an *a priori* specified degree of renal impairment. Such a study might be a 4-arm study enrolling severely impaired, moderately impaired, and mildly impaired children with a group of age- and sex-matched normal controls. However, in this pediatric population with cancer, such a study would be almost impossible to actually enroll into and would be a very lengthy study. An alternative method would be to use a population approach in patients with varying degrees of renal impairment and model the relationship between renal function and clofarabine total systemic clearance. However, given the paucity of pharmacokinetic data in our pediatric database, such an approach might not be viable as there were only a few subjects with renal impairment. Pharmacokinetic data were available, however, in adults with varying degrees of renal impairment. If the adult and pediatric data could be bridged by modeling, then it would be possible to make dose adjustment recommendations based on the combined adult and pediatric database.

The purpose of this analysis was to characterize the pharmacokinetics of clofarabine in adult and pediatric patients with hematologic malignancies or solid tumors and to identify covariates that are predictive of its disposition with the goal of bridging the pharmacokinetics between adults and children so that dose adjustments in renal impairment could be made in a separate analysis. A secondary analysis was to characterize the pharmacokinetics of 6-ketoclofarabine in a select cohort of adult patients having solid tumors after intravenous administration of parent clofarabine.

Methods

Overall study designs

Details regarding the study design for Studies ID99-383, CLO-212, and CLO-222 were previously reported in our earlier manuscript in children [8]. Study CLO-151 was a Phase I, open-label, single-arm, dose-escalation studies of clofarabine administered to patients with advanced solid tumors who have either failed standard therapy or for whom no standard therapy exists. The primary objective of CLO-151 was to determine the MTD and DLT of clofarabine when administered as a once-weekly dose $\times$3 every 28 days to adult patients with solid tumors. Clofarabine was administered by intravenous infusion (IVI) over 0.5 to 2 h once a week for 3 weeks (Days 1, 8 and 15) followed by 1 week of rest. The starting dose of clofarabine was 4 mg/m^2/week, with subsequent dose escalation. At least 3 patients were treated at each dose level until the MTD/Recommended Phase II dose (RP2D) was reached. The MTD/RP2D was defined as the dose at which $\leq$1 of 6 patients experience a DLT with the next higher dose having at least 2 of 3 or 2 of 6 patients experiencing a DLT. Multiple cycles may have been administered until the patient was withdrawn from therapy or until a maximum of 12 cycles were administered.

Adult patients 18 years or older with a Karnofsky performance status (KPS) of $\geq$70 having a pathologic diagnosis of advanced solid tumors, having measurable disease per the Response Evaluation Criteria in Solid Tumors (RECIST) after the MTD was established, having a life expectancy of at least 12 weeks, having a negative serum pregnancy test (if female) within 7 days of enrollment, who agree to use an effective method of birth control to avoid pregnancy, who signed informed consent, have adequate organ function, and are able to comply with all the methods and procedures in the protocol were eligible for enrollment.

Patients who received previous treatment with clofarabine, who had active, uncontrolled systemic infection considered opportunistic, life threatening, or clinically significant at the time of treatment, who had prior malignancy with less than a 2-year disease-free interval, except for adequately treated basal cell or squamous cell skin cancer, or in situ cancer of the cervix, who were pregnant or lactating, who had psychiatric disorder(s) that would interfere with consent, study participation, or follow-up, who received any chemotherapy, major surgery, or irradiation, whether conventional or investigational, <4 weeks before enrollment in this study (6 weeks for mitomycin-C or nitrosourea) and/or had not recovered from acute toxicity of all previous therapy prior to enrollment, who had any other severe concurrent disease, which, in the

judgment of the investigator, would make the patient inappropriate for entry into this study (e.g., uncontrolled severe insulin-dependent diabetes, uncontrolled hypertension, transient ischemic attacks, uncontrolled symptomatic coronary artery disease), who received prior radiation therapy to ≥25% of the bone marrow (e.g., no whole pelvic irradiation was allowed) and had not recovered from the acute side effects of radiotherapy, who received prior radiation therapy to the mediastinal region, who had broncho-alveolar pattern evident on chest X-ray, or who had symptomatic or untreated central nervous system metastases were excluded from the study.

Blood samples were to be collected for pharmacokinetic evaluation prior to the first dose on Day 1, and at 0.5, 1, 2 (end of infusion), 3, 4, 6, 8 to 10 h post-dose, on Day 2 at 24 and 30 h, and on Day 3 at 48 h post-dose. Additional blood samples were to be collected prior to dosing on Days 8 and 15 of Cycle 1 and up to 24 h post-dose at the same nominal time points as listed for Day 1 sample collection. If samples on Day 8 and/or 15 of Cycle 1 were omitted due to any hematologic toxicity, samples were to be collected during Cycle 2 on Days 1–3 only.

Study CLO-152 was a Phase I, open-label, single-arm, dose-escalation study of oral clofarabine administered to patients with advanced solid tumors who have either failed standard therapy or for whom no standard therapy exists. The primary objective of CLO-152 was to determine the MTD and DLT of clofarabine when administered daily ×5 every 28 days in adult patients with locally advanced or metastatic tumors. Adults ≥18 years old having a pathologically confirmed diagnosis of a solid tumor malignancy that was refractory to conventional therapy or for which no therapy exists and measurable disease per RECIST criteria were eligible for enrollment. Patients were to have adequate organ function including renal and hepatic function. Clofarabine was administered in tablets of strength 0.5, 1, or 5 mg. Each dose of clofarabine was to be taken in the morning on an empty stomach, prior to intake of any food, with 4 oz of water. Food intake was not allowed for 1 h after dosing, while caffeinated beverages were not allowed before and 1 h after dosing. The following doses were studied: 1, 1.5, 2.25, 3.5, and 5 mg/m^2 based on body surface area (BSA) using the patient's actual height and weight with downward adjustment to the nearest 0.5 mg.

Adult patients 18 years or older with a KPS of ≥70 having a pathologic diagnosis of advanced solid tumors that were refractory to conventional therapy or for which no therapy existed, having measurable disease per the RECIST criteria, not eligible for therapy of higher curative potential, having a life expectancy of at least 12 weeks, being a male or a non-pregnant, non-lactating female, agreeing to use an effective barrier method of birth control (i.e., latex condom, diaphragm, cervical cap) to avoid pregnancy, having a negative serum or urine pregnancy test within 10 days of study treatment (if patient was a female of childbearing potential), having signed a written Informed Consent Form, and having adequate organ function as indicated by the specified laboratory values, obtained within 2 weeks prior to registration were eligible for enrollment.

Patients who had received previous treatment with clofarabine, had an active, uncontrolled systemic infection considered opportunistic, life threatening or clinically significant at the time of treatment, who were pregnant or lactating, who had a psychiatric disorder(s) that would interfere with consent, study participation, or follow up, who had received any chemotherapy, major surgery, or irradiation, whether conventional or investigational 28 days before treatment in this study (42 days for mitomycin-C or nitrosourea), who had not recovered from acute toxicity of all previous therapy prior to enrollment, who had any other severe concurrent disease, which, in the judgment of the investigator, made the patient inappropriate for entry into this study (e.g., uncontrolled severe insulin-dependent diabetes, uncontrolled hypertension, transient ischemic attacks, uncontrolled symptomatic coronary artery disease), who had abnormal cardiac function who were in consideration for study participation were to be discussed with the Medical Monitor prior to enrollment, who had received prior radiation therapy to ≥25% of the bone marrow (e.g., no whole pelvic irradiation was allowed) and not recovered from the acute side effects of radiotherapy, and who had symptomatic or untreated central nervous system metastases were not eligible for participation in this study.

Blood samples for pharmacokinetic analysis were to be collected at 0 (immediately prior to dosing), 0.5, 1, 1.5, 2, 3, 4, 5, and 6 h after dosing on Day 1, at predose on Day 2, and at predose, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 24, and 48 h after dosing on Day 5. Urine samples were to be collected on Day 1 at 0 to 6 h post-dose and 6 to 24 h post-dose and on Day 5 at 0 to 6 h post-dose, 6 to 24 h post-dose, and 24 to 48 h post-dose.

All studies were conducted in accordance with the principles of Good Clinical Practice (GCP) and the Basic Principles of the Declaration of Helsinki.

Bioanalytical methods

Plasma concentrations of clofarabine were assayed using a validated method using high performance liquid chromatography (HPLC) with mass spectrometric (MS/MS) detection at MicroConstants, Inc. (San Diego, CA). Briefly, plasma was spiked with cladribine as the internal standard, extracted into acetonitrile, evaporated, and reconstituted

into water/acetonitrile/formic acid (90:10:0.1, v/v/v). The samples were analyzed by reversed phase HPLC and detected using a tandem quadrapole mass spectrometer (HPLC–MS/MS). The linear range of the method was from 1 to 500 ng/mL. Samples were shown to be stable for at least 938 days at −20°C, and all analyses were done within the stability window. The inter-day accuracy of the controls was within 20% of the target value for all controls and had a precision of <20% across all studies.

Plasma concentrations of 6-ketoclofarabine were assayed using a validated method using LC–MS/MS detection. The linear range of the method was from 0.1 to 20 ng/mL. Samples were shown to be stable for at least 377 days at −20°C, and all analyses were done within the stability window. The accuracy and precision of the quality control samples (0.3, 3.0, and 16.0 ng/mL) was ±6.0% and <12.9%, respectively. At the lower limit of quantification (0.1 ng/mL), the accuracy and precision were 4.0 and 11.6%, respectively.

Methods for clofarabine pharmacokinetics

The general modeling approach to be taken followed the guidelines set forth by Bonate [10] and Bruno et al. [11]. Model selection was based on physiological and pharmacological rationale and the principle of parsimony—simpler models were chosen over more complex models when statistically justified [12]. First, exploratory data analysis was undertaken to examine the basic structure of the concentration–time data and to identify any outliers. Second, a structural model was developed. Because of the pediatric and adult ages of the patients, weight was built into the model *a priori* in accordance with allometric theory. Third, covariate models were developed using the base model as a reference in a stepwise forward–backward approach and using the likelihood ratio test (LRT) with a p value of 0.01 for significance to be included in the model and 0.001 to remain in the model. Fifth, appropriate methods were used to evaluate the performance of the final covariate model. Lastly, simulations were used to explore the effect of the covariates on measures of exposure.

Base model development

Clofarabine plasma concentration–time data were analyzed by non-linear mixed-effects modeling (NONMEM program, Version VI) to develop a base structural population pharmacokinetic model [13]. The times used in the model were exact times based on infusion length, infusion rate, and time from the start of infusion; no nominal times were used in the analysis. The base model was identified by comparing different structural pharmacokinetic models, e.g., a one-compartment model vs. a two-compartment model. Random effects associated with pharmacokinetic parameters assumed a log-normal (Ln) distribution consisting of between-subject variability. The coefficient of variation for a log-normal was calculated as

$$\mathrm{CV}(\%) = \sqrt{\exp(\sigma^2) - 1} \times 100\% \quad (1)$$

where σ^2 is the variance component for the random effect. At this stage, inter-occasion variability was not considered. In keeping with current pharmacokinetic theory, weight was built into the structural model parameters *a priori*. Hence, pharmacokinetic parameters were defined as

$$\mathrm{P_{ij}} = \theta_1 \left(\frac{\mathrm{Weight}}{70\ \mathrm{kg}}\right)^{\theta_2} \exp(\eta_{ij}) \quad (2)$$

where $\mathrm{P_{ij}}$ is the ith parameter of interest for subject "j", θ_1 is the estimate of the population intercept for a 70 kg adult, θ_2 is a power term fixed to 0.75 for clearance-related terms and 1.0 for volume-related terms, η_{ij} is the deviation from the population mean for subject "j" under the assumption that $\eta_{ij} \sim N(0, \sigma_i^2)$ [14, 15, 16].

The basis for addition or removal of model terms was based on whether the models were nested or non-nested, all other things being equal (e.g., precise standard error of the parameter estimates, unbiasedness of residual plots, and precise estimation of the variability associated with the random effects). If multiple random effects were included in the model, a diagonal covariance matrix for the random effects was initially used. Block and unstructured covariances were examined as appropriate. Residual error was modeled using the logarithmic transform-both-sides approach

$$\mathrm{Ln}(\mathrm{Y_{ij}}) = \mathrm{Ln}(\mathrm{F_{ij}}) + \varepsilon_{ij} \quad (3)$$

where $\mathrm{Y_{ij}}$ is the observed concentration, F is the model predicted concentration, and ε_{ij} are the residuals under the assumption $\varepsilon_{ij} \sim N(0, \sigma^2)$.

Note that the NONMEM objective function value (OFV) is equivalent to −2 times the log-likelihood function. Model selection was dependent on whether models were nested or non-nested. For nested models, the following selection rationale and criteria were used. It has been shown that if two models are nested and the full model having p model parameters is compared to a reduced model with q model parameters, such that $q < p$ and the set of parameters in q is a subset of the parameters in p, then difference in the NONMEM OFVs between the two models (reduced–full) has a chi-squared distribution with p-q degrees of freedom under the null hypothesis that the additional model parameters in the full model are zero [17]. Thus, for this analysis if the difference between the OFVs for two nested models (reduced–full) was greater than the

critical value based on a chi-square test with P value 0.01, assuming both OFVs were obtained using the same estimation method, the full model was considered the superior model. Testing for variance components used a more rigorous P value, 0.001, because of the boundary issue and the LRT [18].

Covariate screening

Covariate screening was done directly within NONMEM using a forward–backward deletion algorithm. A model with (full model) and without (reduced model) the covariate was run and compared using the LRT with a 0.01 level significance to enter into the model. Continuous covariates were entered into the base model using a power model standardized to a reference value. In cases where the power model could not converge a linear model of the form scaled to the same reference values was tested. Categorical covariates were entered into the model using dummy variables with a fractional change model. All models were fit using first-order conditional estimation (FOCE) whenever possible.

The covariates to be screened were selected based on a scientific rationale following ICH guidelines [19]. The following covariates were to be tested: age, serum creatinine concentration (Cr), serum albumin concentration (ALB), race, estimated creatinine clearance (eCrCL), disease type (AML, ALL, or solid tumor), and presence/absence of diabetes. Weight was not screened as a covariate but was entered directly into the model *a priori*. The following rationale was chosen for the covariates:

- Age: age has been known to affect the pharmacokinetics of drugs outside of weight based influences;
- Cr: clofarabine is predominantly cleared through the kidney and Cr is widely used as a predictor of renal function. Cr was tested as a covariate only on CL.
- ALB: albumin is a measure of hepatic function and a measure of protein binding, both of which may influence clofarabine pharmacokinetics;
- Race: race has been known to affect the pharmacokinetics of drugs and race was tested herein as a dichotomous covariate taking values of "Caucasian" vs. "Not Caucasian";
- eCrCL: clofarabine is predominantly cleared through the kidney and eCrCL is widely used as a predictor of renal function. eCrCL was tested as a covariate only on CL.
- Disease type: It is possible that the pharmacokinetics of clofarabine may be different in patients with solid versus patients with hematologic tumors;
- Presence/absence of diabetes: preclinical studies have shown that clofarabine is cleared by the kidney [20], predominantly by the human organic cation transporter Type II (hOCT-2). Studies have shown reduced hOCT-2 gene expression in diabetics [21] and it may be that diabetics have decreased clofarabine clearance.

eCrCL was based on whether the patient was an adult. If the patient was enrolled in Study CLO-151 or CLO-152, which had an inclusion criteria of the patient being 18 years of age or older, then eCRCL (in mL/min/1.73 m^2) was estimated using the Modification of Diet in Renal Disease formula [22]. If the patient was enrolled in Studies CLO-212, CLO-222, or ID99-383, which had an inclusion criteria of $\leq$ 21 years of age, then eCrCL (in mL/min/1.73 m^2) was estimated using the Schwartz formula [23] which was shown by Rudd et al. [24] as being the most accurate and unbiased method for estimating creatinine clearance in children. In both adult and pediatric patients, eCrCL was capped at 200 mL/min/1.73 m^2. In some patients, height was not recorded on the Case Report Forms and for these patients, Cr and eCrCL was set to missing.

Some of the clinical chemistry values were not collected. Serum albumin concentrations were not collected in any patients in Study ID99-383. Cr was measured in some, but not all, patients in Study ID99-383. Also, in Studies CLO-212 and CLO-222 some patients had missing heights and eCrCL was not able to be calculated. Because of these missing values, the covariate models for albumin, Cr, and eCrCL used a conditional form which depended on whether the covariate was observed or missing. These models took the form

$$P_{ij} = \begin{cases} \theta_1 \left(\frac{\text{Weight}}{70\,\text{kg}}\right)^{\theta_2} \left(\frac{\text{Covariate}_i}{\text{Reference}}\right)^{\theta_3} \exp(\eta_{ij}) & \text{if covariate is observed} \\ \theta_4 \left(\frac{\text{Weight}}{70\,\text{kg}}\right)^{\theta_2} \exp(\eta_{ij}) & \text{if covariate is not observed} \end{cases} \quad (4)$$

In this manner, a model with the covariate is developed if the covariate is observed and the base model is used to estimate the pharmacokinetic parameter if the covariate is missing.

Covariates were tested one by one using a significance level of 0.01 to enter the model. The most significant covariate was entered first. Then, all remaining covariates were tested one by one for further improvement in the model. This process was repeated until no further covariates could be entered into the model. The significance of the covariates was then confirmed by removal one at a time from the full model. The significance of the reduced model without the covariate was tested against the full model criteria at a more stringent P value ($P < 0.001$) to avoid false positives. Likelihood profiling of the fixed effects

under the final model was used to generate 95% confidence intervals and confirm the significance of the covariates.

Population model performance and stability

Because of the small number of subjects in the final data set, data splitting was not done. Instead, model validation focused on internal and external methods. Stability of the parameter estimates was examined using the non-parametric bootstrap [25]. Briefly, subjects were resampled from the final data set with replacement. Because the dataset consisted of concentrations after oral and intravenous administration, the bootstrap datasets were balanced in the sense that the patients administered clofarabine intravenously were resampled with replacement independent of the patients administered clofarabine after oral administration. In each bootstrap dataset, the number of patients administered clofarabine orally was maintained relative to the original dataset. A total of 1,000 bootstrap datasets were generated and the final model was fit to the bootstrapped data using FOCE. The distribution of the bootstrapped parameter estimates was then examined graphically for precision and bias from the model building dataset.

Influence analysis was undertaken by creating n new data sets where n is the number of subjects in the final data set. Each new data set has one subject removed so that each data set has $n - 1$ subjects. These new data sets are referred to as jackknifed data sets. Each jackknifed data set was used to fit the final model using FOCE. The percent change in parameter estimates between the final model and the jackknife data set was calculated and graphically examined for any subject who had a consistent effect on parameter values (as defined as a percent change from the final model parameter estimate of more than ±20%). If any such individuals were identified then they may have been removed from the final model data set.

Model stability was also assessed by calculating the condition number (the ratio of the largest to smallest eigenvalue of the covariance matrix) of the final model, which assesses the loss of degrees of precision in inverting the Hessian matrix during the optimization process. Log_{10} of the condition number represents the number of digits lost during matrix inversion. A condition number of more than 1,000 is generally indicative of ill-conditioning [26].

Lastly, model stability was examined using an external dataset. Study CLO-221 was a Phase II study in adults with relapsed or refractory AML. Patients were to receive a 40 mg/m^2 dose infused over 1 h once daily for 5 days every 28 days. A total of 102 observations were available from 12 patients (7 males, 5 females; 11 Caucasians, 1 black) ranging in age from 32.9 to 78.3 years (mean 56.1 years), ranging in weight from 53.9 to 144.3 kg (mean 76.3 kg), and who received a total daily doses ranging from 59.0 to 105.6 mg (mean 73.8 mg). Model evaluation was done by fixing the parameter estimates to their final values and setting the maximum number of model evaluations to zero in NONMEM. Each patient's pharmacokinetic parameters were estimated using *maximum a posteriori* estimation. Model evaluation was by examination of goodness-of-fit plot and summary statistics for the error and relative error between observed and individual predicted concentrations.

Methods for 6-ketoclofarabine pharmacokinetics

Only samples from patients enrolled at the 103, 129, and 148 mg/m^2 dose groups in Study CLO-151 were analyzed for 6-ketoclofarabine. Because of the limited pharmacokinetic data available for this metabolite, it was decided not to use the labor-intensive population pharmacokinetic approach for data analysis. 6-Ketoclofarabine pharmacokinetic parameters were estimated using non-compartmental methods using exact times of blood collection [27]. All parameters were calculated using SAS for Windows, Version 8.02 (SAS Institute Inc., Cary, NC). The following plasma pharmacokinetic parameters were calculated for 6-ketoclofarabine and clofarabine: area under the curve from time 0 to the last time point [AUC(0–last)], area under the curve from time 0 to 6 h post-dose [AUC(0–6)], maximal concentration ($C_{\max}$), time to maximal concentration ($T_{\max}$), terminal half-life, and metabolite ratio. $C_{\max}$ and $T_{\max}$ were determined from direct observation of the data. The terminal elimination rate constant and its corresponding half-life was based on 3 or more data points using linear regression of the log-transformed concentrations against time during the elimination phase of the concentration–time profile. The metabolite ratio was calculated 3 different ways using the ratio of 6-ketoclofarabine AUC (0–last), AUC(0–6), and $C_{\max}$ to the same clofarabine parameter. Area under the curve was estimated using the linear trapezoidal rule.

Clofarabine results

Demographics

A total of 1898 quantifiable concentrations from 144 patients (91 adults, 53 children) were used in the analysis. Patients ranged in age from 2.8 to 81.5 years and ranged in weight from 14.1 to 135.3 kg. A total of 81 males and 63 females were in the dataset with the majority being white (70.1%) and approximately the same percent of blacks (11.8%) and Hispanics (13.2%). Patients in Study

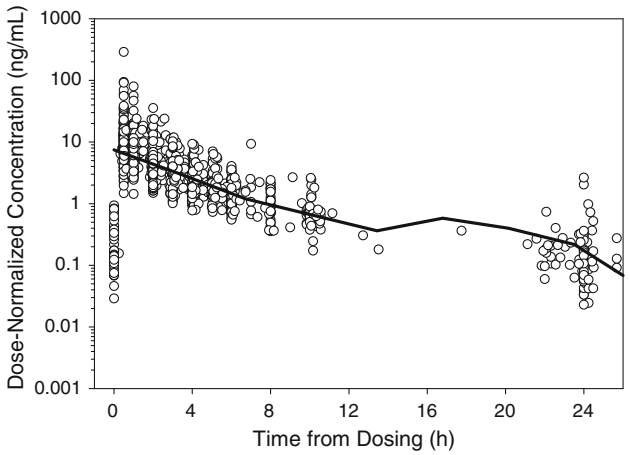

Fig. 1 Scatter plot and LOESS smooth to dose-normalized clofarabine plasma concentration–time profiles pooled across studies and day of administration after intravenous infusion

ID99-383 did not have any serum albumin concentrations measured and almost half did not have Cr measured. Patients with missing Cr had their creatinine values imputed using the mean baseline value from patients enrolled in Studies CLO-212, CLO-222, and ID99-383. Creatinine values ranged from 0.3 to 2.0 mg/dL with eCrCL values ranged from 33 to 200 mL/min/1.73 m^2. Serum albumin ranged from 2.2 to 5.2 mg/dL. The total dose of clofarabine administered ranged from 1.5 to 301.9 mg.

Population pharmacokinetics of plasma clofarabine concentrations

Descriptive analyses

Clofarabine concentrations ranged from the LLOQ to 6517.90 ng/mL. Most samples were collected within 10 h of the start of infusion with fewer samples collected out to 2 days post-dose. Figure 1 presents a scatter plot of dose-normalized clofarabine concentrations and time relative to the start of infusion pooled across studies and day of administration. After the end of infusion, concentrations appeared to decline multiphasically. Figure 2 presents a scatter plot of mean concentrations after oral administration in Study CLO-152. Concentrations appeared to peak about 1 to 2 h after administration and declined in a biphasic manner Table 1.

Best model

The best model was a 2-compartment model with first-order absorption and linear elimination kinetics. The power terms associated with weight were fixed to their theoretical values on all structural model parameters. Age was a significant covariate affecting both CL and V2. eCrCL was also a significant covariate affecting CL. BSV was observed with CL, V2, Q, and oral bioavailability (F1), whereas IOV was observed with CL, V2, and V3. A high correlation between Q and V3 was observed so a common-eta approach was used. The final model was given by Eq. 5.

$$
\begin{aligned}
\mathrm{MAT} &= \theta_1 \\
\mathrm{CL} &= \begin{cases} \theta_2 \left(\frac{\mathrm{Weight}}{70\,\mathrm{kg}}\right)^{0.75} \left(\frac{\mathrm{Age}}{30\,\mathrm{years}}\right)^{\theta_7} \exp(\eta_{\mathrm{CL}} + \kappa_{\mathrm{CL}}) & \text{if eCrCL is missing} \\ \theta_9 \left(\frac{\mathrm{Weight}}{70\,\mathrm{kg}}\right)^{0.75} \left(\frac{\mathrm{Age}}{30\,\mathrm{years}}\right)^{\theta_7} \left(\frac{\mathrm{eCrCL}}{90\,\mathrm{mL/min/1.73\,m^2}}\right)^{\theta_{10}} \exp(\eta_{\mathrm{CL}} + \kappa_{\mathrm{CL}}) & \text{if eCrCL is observed} \end{cases} \\
\mathrm{V2} &= \theta_3 \left(\frac{\mathrm{Weight}}{70\,\mathrm{kg}}\right) \left(\frac{\mathrm{Age}}{30\,\mathrm{years}}\right)^{\theta_8} \exp(\eta_{\mathrm{V2}} + \kappa_{\mathrm{V2}}) \\
\mathrm{Q} &= \theta_4 \left(\frac{\mathrm{Weight}}{70\,\mathrm{kg}}\right)^{0.75} \exp(\eta_{\mathrm{Q}}) \\
\mathrm{V3} &= \theta_5 \left(\frac{\mathrm{Weight}}{70\,\mathrm{kg}}\right) \exp(\eta_{\mathrm{Q}} \times \theta_{11} + \kappa_{\mathrm{V3}}) \\
\mathrm{F1} &= \theta_6 \exp(\eta_{\mathrm{F1}})
\end{aligned}
\tag{5}
$$

The residual variance model used was a transformed both sides approach stratified by study

$$
\begin{aligned}
\mathrm{Ln(Y)} &= \mathrm{Ln(F)} + \varepsilon_1 \quad \text{if study} \neq \text{ ID99-383} \\
\mathrm{Ln(Y)} &= \mathrm{Ln(F)} + \varepsilon_2 \quad \text{if study} = \text{ID99-383.}
\end{aligned}
\tag{6}
$$

Due to convergence problems with the final model, the hybrid estimation algorithm was used to estimate the model parameters with the IOV term associated with CL estimated using first-order approximation and all other terms estimated using FOCE.

Table 2 presents a summary of the model parameter estimates and derived pharmacokinetic parameters for the final model. Figure 3 presents the goodness-of-fit plots for the final model. The model showed good predictability although it slightly underpredicted at very high concentrations. No systemic trend was observed in the residuals

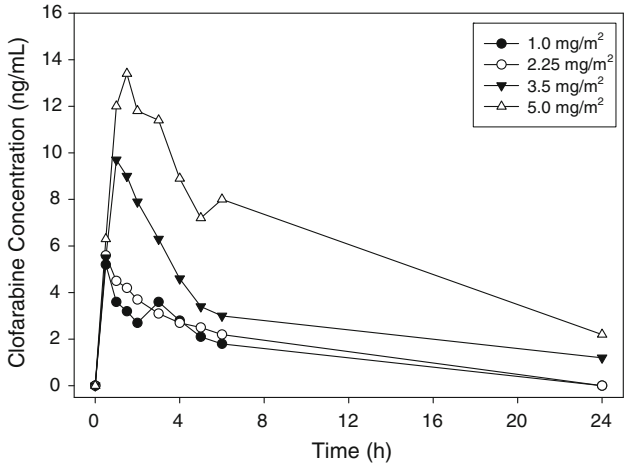

Fig. 2 Scatter plot of mean concentrations after oral dosing in study CLO-152

over time or against predicted concentrations. The residuals themselves and the weighted residuals were not exactly normally distributed, but were symmetric and centered at zero, which is more important than strict normality. A total of 5 observations had weighted residuals exceeding ±5, the largest of which was 6.2. These observations were retained in the model development dataset and not removed as outliers. All the random effects were approximately symmetric and normally distributed. The shrinkage of the random effects was minimal. The shrinkage for CL, V2 and Q as calculated was 13%, 20%, and 10%, respectively.[1] The totality of the model diagnostics indicated the final model reasonably predicted clofarabine concentrations under both IV and oral administration and across a wide range of doses.

The only important covariates in the model were age, eCrCL, and weight. CL, V2, Q, and V3 increased with increasing weight. Table 3 presents a summary of clofarabine pharmacokinetic parameters for a typical 30 year old patient (the reference patient) and at the extremes of the age range studied (3 years and 82 years old). CL increased with increasing eCrCL but decreased with increasing age. V2 decreased with increasing age. As a result of the changes in CL and volume of distribution, both the distribution and elimination half-life increased with increasing age. The net effect of decreasing CL and V2 with increasing age resulted in higher clofarabine concentrations in adults compared to children when administered equivalent doses.

After oral administration, clofarabine was rapidly absorbed and had good bioavailability. The mean absorption time (MAT, calculated as the inverse of the absorption rate constant) was 1.56 h with an oral bioavailability (F1) of 57.5%. No lag-time was apparent. Because of the limited data no estimate of BSV or IOV for F1 could be determined.

Model stability

The condition number of the final model was 54 (the largest and smallest eigenvalues were 4.17 and 0.0769, respectively) indicating that the parameter estimates were stable and the model did not appear to be overparameterized.

Examination of the principal components analysis revealed that 1 patient from Study CLO-152 appeared to be different from the remainder of the patients in the dataset. Examination of the index plots did not indicate that this patient had undue influence on the parameter results. When this patient was removed from the dataset and the final model refit to the reduced dataset, the parameter estimates did not change by any significant degree except for the IOV terms associated with V2 and V3, which did change by an appreciable amount. Since the fixed effects did not change to any appreciable extent this patient was not deemed an outlier and was kept in the dataset. In toto, no patients appeared to exert undue influence on the model parameter estimates.

A total of 1,000 balanced bootstrapped datasets were generated, of which 92.7% minimized without errors. All parameters were significant and unbiased. The bias of the fixed effects was <5% in all cases. The bias of the random effects were also <5% except in the case of the correlation term between V2 and Q, which was ~25% and the IOV of V2 which was ~17%. The bootstrap results indicate that the final model was unbiased in terms of the fixed effects and had some bias in the variance estimates.

Study CLO-221 was used as an external validation dataset. The range of observed concentrations ($n = 102$) was 2.90 to 1923 ng/mL with a mean of 240 ng/mL. The final pharmacokinetic model for clofarabine was fixed to the final parameter estimates and applied to the Study CLO-221 dataset. Each individual's pharmacokinetic parameters were estimated using maximum *a posteriori* estimation by setting MAXEVAL equal to 0 and using the POSTHOC option within NONMEM. Figure 4 presents a scatter plot of individual predicted concentrations versus observed concentrations. Although the model fails to predict the highest concentration observed, the remainder of the concentrations seem to be adequately predicted as there was random distribution around the line of unity with no systematic biases. Although there was 1 weighted residual greater than ±5, there did not appear to be any systematic trends in the residuals. The weighted residuals had a range of −6.4 to 1.8 with mean of −0.30 and standard deviation of 0.98. The weighted residuals were centered near zero and mostly symmetric with some degree of left skewness. The mean

[1] Reference PSN File: \final.nmn.dir.1\raw_results.csv columns CA2 to CS2.

Table 1 Demographic summary

Parameter	ID99-383 ($n = 12$)	CLO-212 ($n = 22$)	CLO-222 ($n = 19$)	CLO-151 ($n = 68$)	CLO-152 ($n = 23$)
Disease	Hematologic malignancies	Refractory or relapsed acute lymphoblastic anemia	Refractory or relapsed acute myelogenous anemia	Advanced solid tumors	Refractory solid tumors
Age (years)	13.0 (4.0–17.4)	12.3 (2.9–18.1)	13.3 (2.8–21.3)	64.5 (24.1–79.9)	64.8 (45.3–81.5)
Weight (years)	42.5 (15.3–72.0)	43.2 (15.6–129.8)	51.3 (14.1–82.3)	75.2 (44.1–135.3)	71.4 (43.9–113.6)
Sex	6 Males	12 Males	12 Males	40 Males	11 Male
	6 Females	10 Females	7 Females	28 Females	12 Females
Race	6 Caucasian	9 Caucasian	9 Caucasian	60 Caucasian	17 Caucasian
	5 Hispanic	8 Hispanic	4 Hispanic	7 Blacks	4 Blacks
	1 Other	4 Blacks	3 Others	1 Hispanic	1 Hispanic
		1 Other	2 Blacks		
Total dose per cycle (mg)	65.0 (12.3–112.0)	71.7 (34.0–134.0)	80.6 (31.7–108.0)	66.5 (6.3–301.9)	5.5 (1.5–12.0)
Doses tested	11.25, 30, 40, 52, 70 mg/m^2 daily ×5 as a 1 or 2 h infusion every 2 to 4 weeks	52 mg/m^2 daily ×5 as an infusion over 2 h every 2 to 6 weeks	52 mg/m^2 daily ×5 as an infusion over 2 h every 2 to 6 weeks	4, 6, 10, 14, 18, 22, 27.5, 34, 42.5, 53, 66, 82.5, 103, 129, 148 mg/m^2 once weekly every 3 weeks	1.5, 2.25, 3.5, and 5.0 mg/m^2 orally daily ×5 every 28 days
eCrCL (mL/min/1.73 m^2)	122.8 (61.0–148.7)	135.3 (91.0–200.0)	140.9 (78.8–200.0)	88.1 (36.6–172.6)	68.7 (33.1–90.7)

Data are reported as median (range). eCrCL was capped at 200 mL/min/1.73 m^2

error was −10.3 ng/mL with a standard deviation of 154.4 ng/mL. The mean error was not different than zero. The mean relative error was +47.1% with a standard deviation of 281.2%. The mean relative error was not different than zero. While the mean relative error may seem large this was largely due to the skewness of the distribution of the data. The median error and relative error were even lower, 2.6 ng/mL and 2.7%, respectively. Examination of the random effects associated with CL, V1, and Q were all normally distributed and centered at zero ($P > 0.01$). In total, the final pharmacokinetic model for clofarabine did an adequate job of predicting the pharmacokinetics in the external dataset without any real systematic biases.

The results of the model evaluation and stability diagnostics suggested that the model was adequate for the prediction of clofarabine concentrations.

Effect of covariates on clofarabine pharmacokinetics

The important covariates shown to affect clofarabine pharmacokinetics were age, weight, and eCrCL. Figures 5, 6, and 7 present box and whisker plots and scatter plots of each individual's CL against eCrCL, age, and weight, respectively. As eCrCL increases, CL increases. As age increases, CL decreases and as weight increases CL increases. Because of the multifactorial nature of the effect of the covariates it is difficult to distinguish how any particular covariate affects clofarabine pharmacokinetics without controlling for the effect of the other covariates.

eCrCL was an important predictor of clofarabine CL. Of particular interest was how clofarabine exposure changes as a function of eCrCL. Deterministic simulation was used to determine how clofarabine AUC (0–∞) changed in a pediatric and adult/elderly population as a function of eCrCL.

In the first simulation, weight was fixed to 70 kg and the total dose administered was 52 mg (30 mg/m^2 in a 1.73 m^2 person). eCrCL was varied from 30 to 150 mL/min per 1.73 m^2 and age was varied from 20 to 80 years. The percent difference in AUC (0–∞) was calculated relative to a person of the same age and weight having a eCrCL of 90 mL/min per 1.73 m^2. Figure 8 presents a line plot of exposure by eCRCL at different ages. For any given age, as eCrCL decreases exposure increases. Across all ages, only when eCrCL decreases to a moderate (eCrCL 30 to 60 mL/min/1.73 m^2) and severe (eCrCL < 30 mL/min/1.73 m^2) degree of renal impairment does exposure increase by more

Table 2 Final model summary for plasma clofarabine pharmacokinetics based on (Eq. 5)

Parameter (units)	Estimate	Standard error	*T*-test	Change in OFV when parameter was set equal to 0
θ_1 (h)	1.56	0.214	7.3	–
θ_2 (mL/h)	32700	3490	9.4	–
θ_3 (mL)	71500	5310	13.5	–
θ_4 (mL/h)	40600	3510	12.6	–
θ_5 (mL)	147000	9130	11.6	–
θ_6	0.575	0.0660	8.7	–
θ_7	−0.181	0.0488	4.1	31.46 ($P < 0.001$)
θ_8	−0.560	0.0902	3.7	48.71 ($P < 0.001$)
θ_9 (mL/h)	29100	1060	27.5	–
θ_{10}	0.533	0.0739	7.2	51.28 ($P < 0.001$)
θ_{11}	1.00	0.0960	10.4	122.82 ($P < 0.001$)
Variance (CL)	0.0731 (28%)	0.0138	–	–
Variance (V2)	0.350 (65%)	0.118	–	–
Corr (CL, V2)	0.469	–	–	–
Variance (Q)	0.338 (63%)	0.0728	–	–
Corr (CL, Q)	0.577	–	–	–
Variance (V3)**	0.338 (63%)	0.0728	–	–
Corr (V2, Q)	0.497	–	–	–
IOV (CL)	0.0185 (14%)	0.00564	–	–
IOV (V2)	0.244 (53%)	0.145	–	–
IOV (V3)	0.0193 (14%)	0.00897	–	–
σ_1^2	0.0904 (31%)	0.0122	–	–
σ_2^2	0.163 (42%)	0.0373	–	–

T-test was calculated as the estimate/standard error

Variance components are reported as estimate (CV%) where CV% was calculated using (Eq. 1)

** The estimate for Variance (V3) was calculated using the equation $\omega_{V3}^2 = \theta_{11}^2 \times \omega_Q^2$

– not tested or computed

than 20% suggesting that dose adjustment is necessary in this population.

In the second simulation, the effect of age, weight, and eCrCL on clofarabine exposure was simulated in pediatric patients. In this simulation, age was varied from 2 to 20 years. Weight was determined based on the United States National Health and Nutrition Examination Survey age–weight tables [28].[2] The median weight was used for a given age and was converted to a BSA using the formula presented by Livingston and Lee [29]. eCrCL was then varied from 30 to 150 mL/min per 1.73 m^2. For simplicity, the approximate average of males and females was used for the weight estimate. The total dose administered was 52 mg/m^2. Figure 9 presents a line plot of exposure for pediatric patients by age at different eCrCL categories. For any given age, as eCrCL decreases exposure increases and as eCrCL increases exposure decreases. Across all ages, only when eCrCL decreases to a moderate and severe degree of renal impairment does exposure increase by more than 20% suggesting that dose adjustment is necessary in this population.

These simulations illustrate that clofarabine exposure does not increase significantly until moderate or severe renal impairment is observed within a population. An equivalent degree of impairment may be observed in both pediatric patients and adult patients and is expected to apply to both oral and intravenous administration. It is expected a decrease in renal impairment of moderate severity, regardless of age, may require a 25% dose reduction to maintain equivalent exposure to similar patient with normal renal function.

6-Ketoclofarabine results

6-Ketoclofarabine pharmacokinetic data were obtained from 9 patients (7 females and 2 males) who were enrolled

[2] Equivalent tables are not available for Europe, so there may be a certain degree of bias in these simulations in some populations, for example populations that are on average taller than the US population.

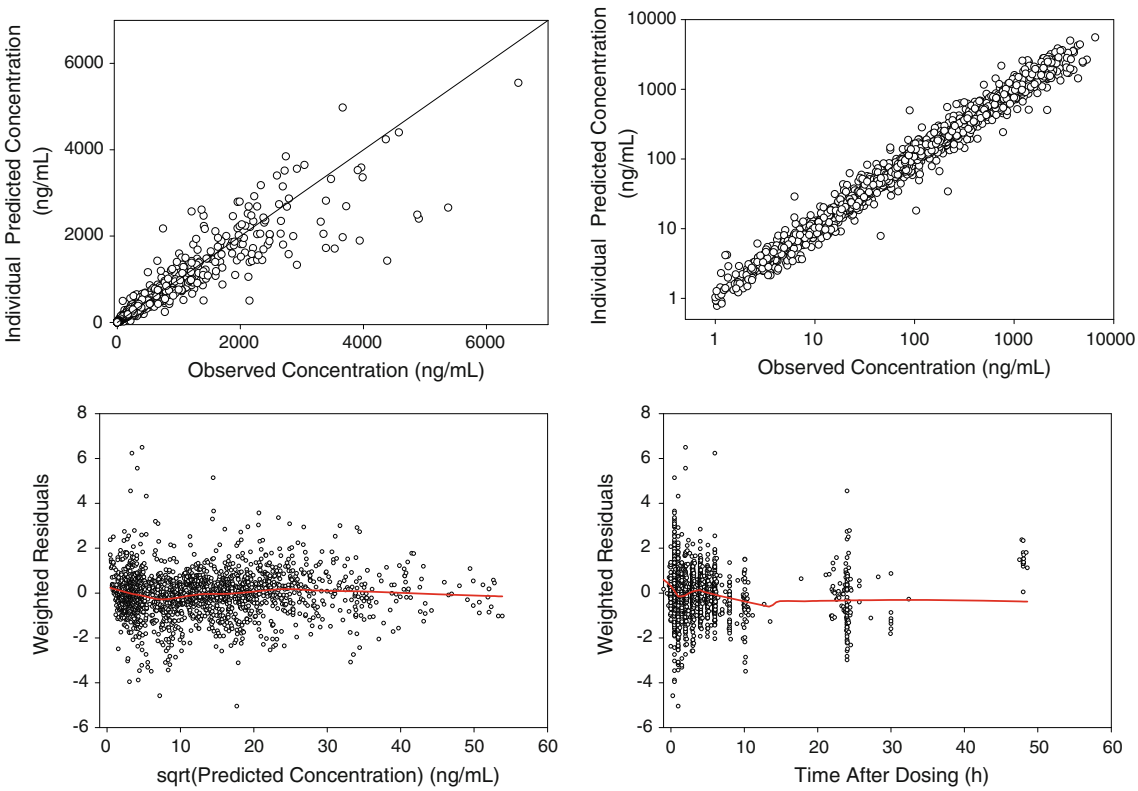

Fig. 3 Goodness-of-fit plots for final model of clofarabine plasma concentrations

Table 3 Typical pharmacokinetic values for different ages

Parameter	Age		
	3 years old	30 years old (reference patient)	82 years old
eCrCL (mL/min/1.73 m^2)	138	90	46
Weight (kg)	16	70	96
CL (L/h)	18.3	29	21.5
V2 (L)	59.3	71.5	55.8
Q (L/h)	13.4	40.6	51.5
V3 (L)	33.6	147	202
Vdss (L)	92.9	219	257
CL (L/h/kg)	1.44	0.41	0.22
Vdss (L/kg)	5.8	3.1	2.7
α-half-life (h)	0.9	0.6	0.5
β-half-life (h)	4.4	7.1	10.6

CL total systemic clearance, *V2* central volume, *Q* intercompartmental clearance, *V3* peripheral volume, *Vdss* volume of distribution at steady-state

in Study CLO-151 at the following dose cohorts: 103 ($n = 2$), 129 ($n = 6$), and 148 mg/m^2 ($n = 1$). The total clofarabine dose administered ranged from 237 to 284 mg. 6-Ketoclofarabine concentrations peaked near the end of infusion, averaging about 1.1 h after the start of the infusion (the per protocol length of the infusion was to be 1 h) (Fig. 10). The mean 6-ketoclofarabine AUC(0–last) pooled across visits was 346 ng h/mL (range 108 to 854 ng h/mL). The mean clofarabine AUC (0–6) was 6238 ng h/mL (range 2545 to 12693 ng h/mL). An equivalent 24 h AUC was not available for clofarabine since the 24 h samples were not analyzed for clofarabine. Nevertheless, the metabolite ratio for 6-ketoclofarabine was small, averaging less than 5% and not more than 8% for any particular individual. 6-Ketoclofarabine was rapidly cleared from plasma with an average apparent half-life of 4.9 h (range 3.9 to 6.2 h). No accumulation of 6-ketoclofarabine was observed with predose samples all below the limit of quantification on Days 8 and 15.

Discussion

Clofarabine pharmacokinetics were best described by a 2-compartment model with linear elimination and first-order absorption after oral administration. Clofarabine pharmacokinetics were time-invariant and dose proportional. Weight, age, and eCrCL were the most important covariates affecting clofarabine pharmacokinetics. It is difficult to evaluate how these 3 covariates interact without making at least one of the covariates fixed to some value, i.e., the results of any 2 covariates must be made conditional on the other covariate. Using 3-dimensional scatter plots, within

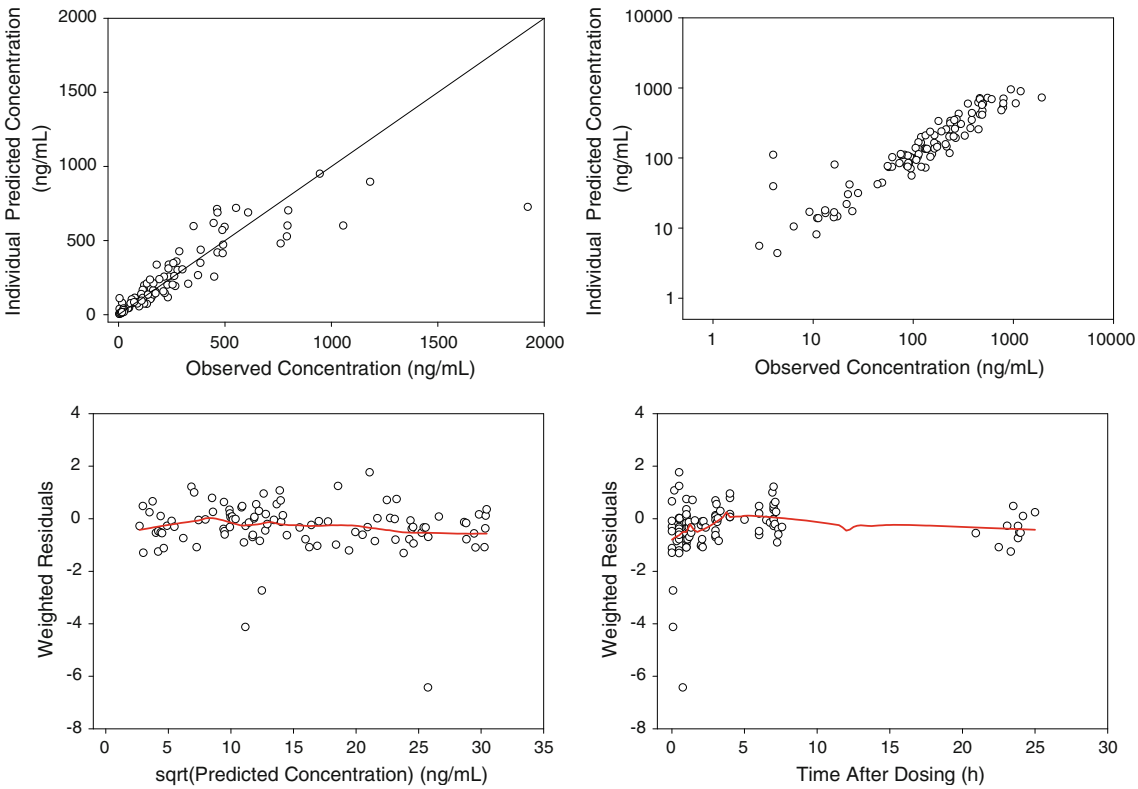

Fig. 4 Goodness-of-fit plots for the external validation dataset under the final model for clofarabine

both age groups it appears that CL increases with increasing weight and eCrCL decreases with age. Further, Vdss increased with weight and decreased with age. The net effect of these changes was that exposure increased with increasing age—adults had higher exposures than children for a given dose. It is difficult to reconcile why clofarabine exposure is different in adults and children. It may be that the transporters responsible for clofarabine distribution and elimination, such as the nucleoside and human organic cation transporters, are different between the age groups. This age difference in transporter function has not been reported in the literature and is speculative.

Importantly, no difference was seen in clofarabine pharmacokinetics between patients having a hematologic malignancy and those with solid tumors. Further, no difference was observed between males and females or between races. While it is impossible to prove a null hypothesis of no difference, these results appear reasonable and not the result of low statistical power as the sample size was fairly large both within and pooled across subgroups.

One of the limitations of the model is its reliance on weight as a covariate. Indeed, since dosing is standardized to BSA it makes more sense to use BSA as a covariate in the model. However, allometric theory is based on the size of the animal, on its weight, not on its BSA [30]. In this analysis, the relationship between weight and every pharmacokinetic parameter in the model was fixed to theoretical values using a power function with the exponents fixed to 0.75 for clearance terms and 1.0 for volume terms [31]. This was necessary to allow exploration of the impact of other potential covariates, like age and eCrCL, on the pharmacokinetic parameters in the model. Since age and weight are correlated, to allow both parameters to be treated as estimable would have led to high collinearity; hence the need to fix weight to its theoretical value. Unlike weight, there is no theoretical relationship between BSA and pharmacokinetic parameters. To allow BSA into the model would have required the parameter estimates associated with BSA to be estimated and in the presence of other correlated covariates may have led to high collinearity and parameter estimate instability [26].

These results did show that with decreasing renal function, clofarabine exposure increased due to decreased systemic clearance. Clofarabine is primarily excreted by the kidney unchanged with about 50 to 70% of the dose excreted unchanged in the urine [1]. When renal impairment is moderate or severe, dose reductions may be necessary to maintain equivalent exposure to a patient having the same weight, age, and normal renal function. Simulations have shown that the degree of impairment appears to be the about the same between adults and pediatric patients

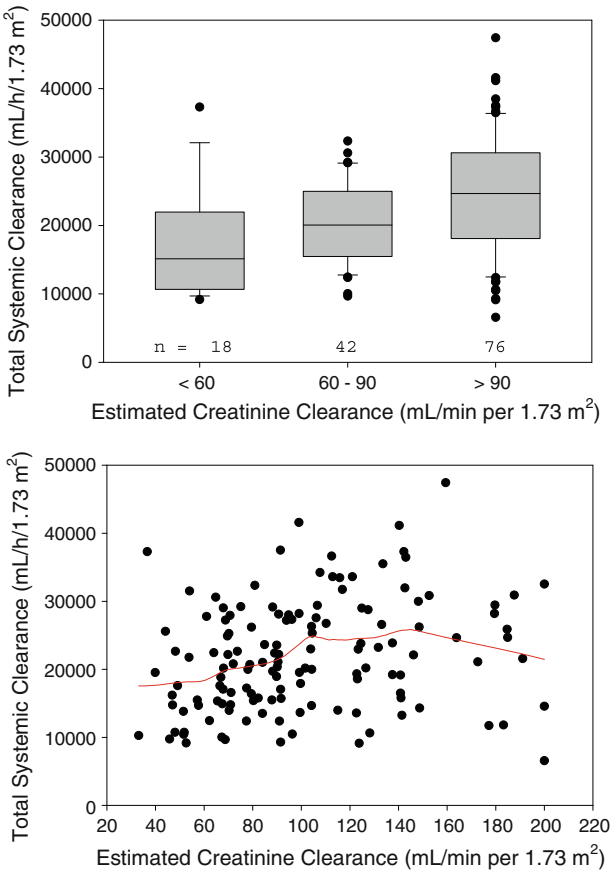

Fig. 5 Plots of the effect of eCrCL on clofarabine total systemic clearance. *Solid line* is the non-parametric smooth to the residual plot using a linear LOESS smoother with 0.4 sampling proportion

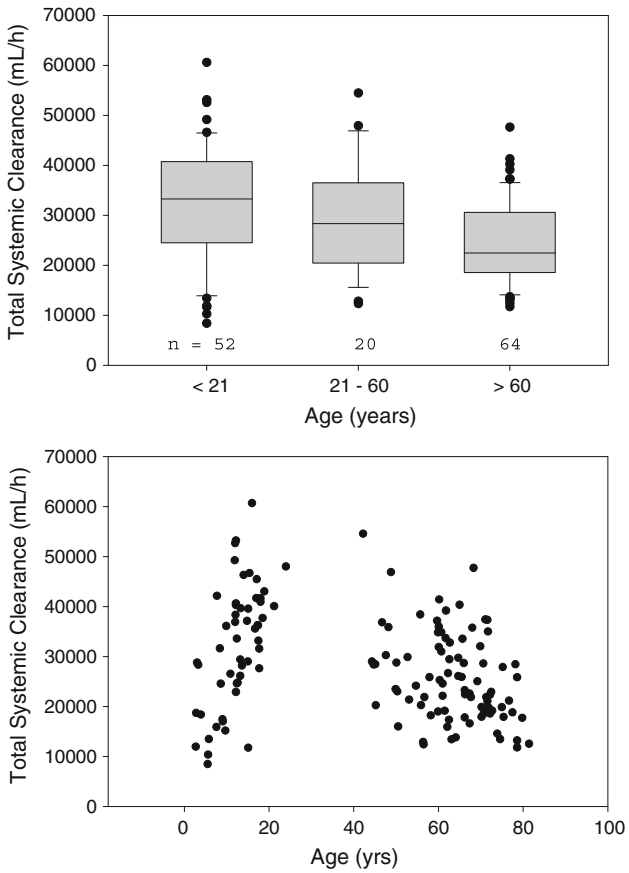

Fig. 6 Plots of the effect of age on clofarabine total systemic clearance

and that the same degree of dose adjustment would appear to be necessary in both populations.

After oral administration, clofarabine was rapidly absorbed and had good bioavailability. Absorption was complete within a few hours of dosing (the MAT was 1.56 h) with an oral bioavailability (F1) of 57.5%. No lag-time was apparent. No BSV or IOV could be estimated in the model, perhaps because of the few number of patients who received oral clofarabine and the few number of samples collected in the absorption phase. Given the MAT and moderate bioavailability, these results suggest that clofarabine is a Class III drug (high solubility, low permeability) under the Biopharmaceutics Classification System.

In animal studies, the metabolite of greatest concentration was 6-ketoclofarabine, which was believed to be formed via adenosine deaminase [7]. 6-Ketoclofarabine metabolite ratio was $< 8\%$ in all cases and averaged less than 5%. The apparent half-life of 6-ketoclofarabine was 4.9 h, which was near the reported 4.6 h half-life for clofarabine [1]. 6-Ketoclofarabine concentrations peaked near the end of the infusion. No accumulation of 6-ketoclofarabine was observed with repeated once-weekly dosing and based on the apparent half-life, even with repeated once-daily dosing, accumulation is not expected. Based on these results, 6-ketoclofarabine showed formation-rate limited clearance [32], had limited exposure compared to parent clofarabine, and does not warrant further monitoring in future studies.

In summary, the pharmacokinetics reported for clofarabine were consistent with previous publications. The covariates identified as being important predictors were consistent with clinical and preclinical observations. Weight was the most important predictor and its inclusion was consistent with allometric theory. eCrCL was shown to impact systemic clearance—renal function directly impacts clofarabine pharmacokinetics. And age affected systemic clearance and volume of distribution which was consistent with the observation that adults have higher exposure to clofarabine than pediatric patients. After oral administration, clofarabine has good exposure, absorption is rapid, and bioavailability is modest (a little more than half the dose is absorbed). Using simulations it was shown that patients with reduced renal function of moderate

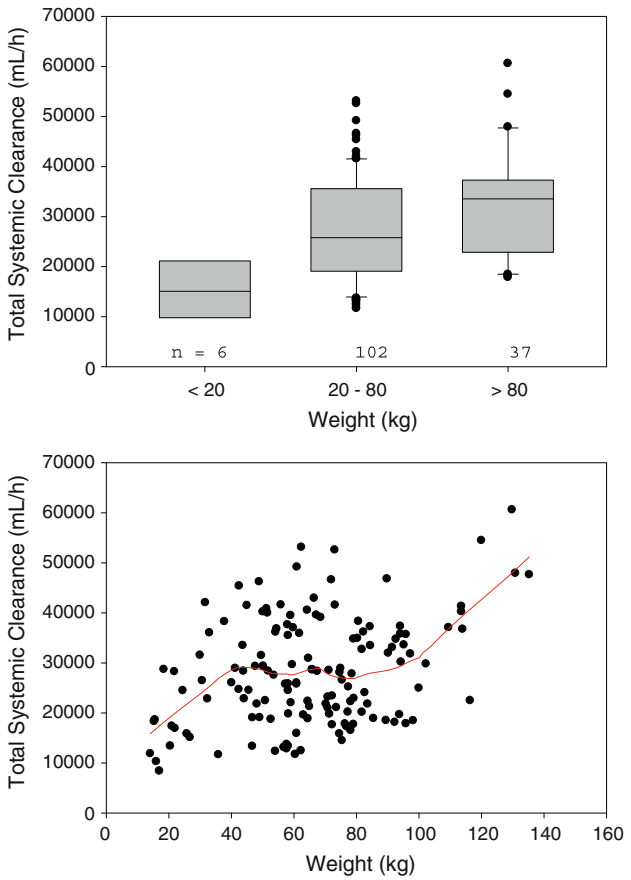

Fig. 7 Plots of the effect of weight on clofarabine total systemic clearance. *Solid line* is the non-parametric smooth to the residual plot using a linear LOESS smoother with 0.4 sampling proportion

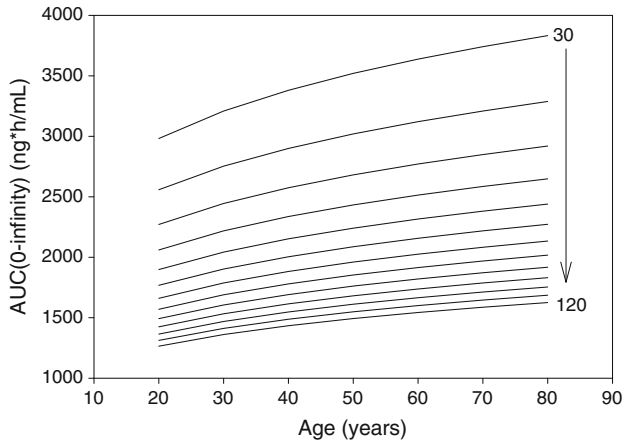

Fig. 8 Line plot of clofarabine exposure in an adult as a function of eCrCL (mL/min/1.73 m^2) and age for a 70 kg adult administered a total dose of 52 mg

impairment may require dose adjustment and that similar reductions are needed in adults and children. There was insufficient information to understand dose adjustment requirements in patients with severe renal impairment. 6-Ketoclofarabine, the metabolite of greatest exposure in

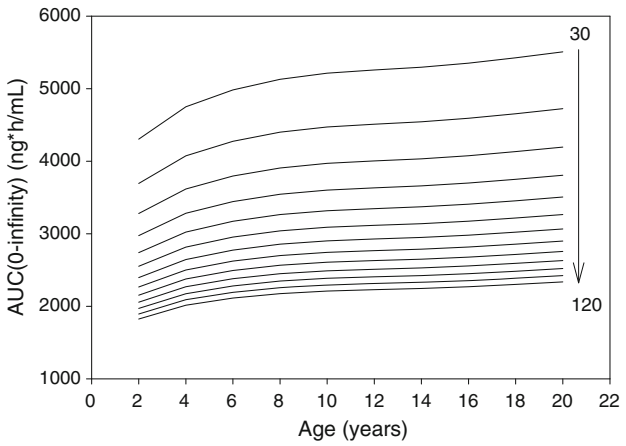

Fig. 9 Line plot of clofarabine exposure in a pediatric population as a function of eCrCL (mL/min/1.73 m^2) and age administered 52 mg/m^2

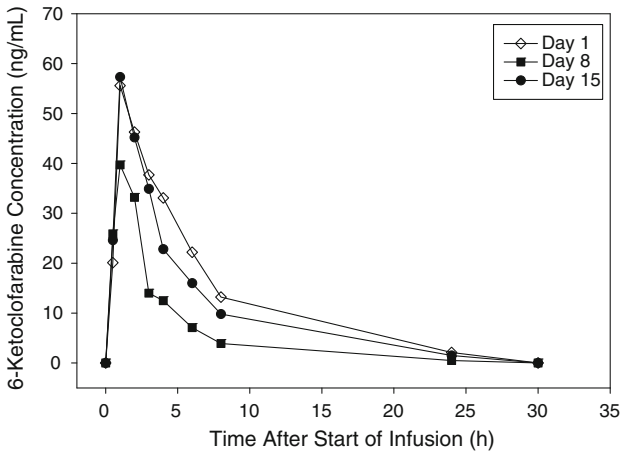

Fig. 10 Mean 6-ketoclofarabine concentrations over time after intravenous administration of clofarabine (52 mg/m^2) pooled across doses

preclinical studies, was formed in insufficient quantity in humans to warrant further monitoring in future clinical studies.

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