Biol Blood Marrow Transplant 25 (2019) 1603–1610

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Population Pharmacokinetics of Clofarabine as Part of Pretransplantation Conditioning in Pediatric Subjects before Hematopoietic Cell Transplantation

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Article history:
Received 3 January 2019
Accepted 12 April 2019

Keywords:
Clofarabine
Fludarabine
Pharmacokinetics
Pediatric
Allogeneic
Hematopoietic cell transplantation

A B S T R A C T

The primary objective of this work was to characterize the pharmacokinetics (PK) of systemic clofarabine (clo-fara) in pediatric allogeneic hematopoietic cell transplantation (HCT) recipients receiving either nucleoside monotherapy or a dual nucleoside analog preparative regimen. Fifty-one children (median age, 4.9 years; range, .25 to 14.9 years) undergoing allogeneic HCT for a variety of malignant and nonmalignant disorders underwent PK assessment. Plasma samples were collected over the 4 to 5 days of clo-fara treatment and quantified for clo-fara, using a validated liquid chromatography/tandem mass spectrometry assay. Nonlinear mixed-effects modeling was used to develop the population PK model, including identification of covariates that influenced drug disposition. In agreement with previously published models, a 2-compartment PK model with first-order elimination best described the PK of clo-fara. Final parameter estimates for clo-fara were consistent with previous reports and were as follows: clearance (CL), 23 L/h/15 kg; volume of the central compartment, 42 L/15 kg; volume of peripheral compartment, 47 L/15 kg; and intercompartmental CL, 9.8 L/h/15 kg. Unexplained variability was acceptable at 33%, and the additive residual error (reflective of the assay) was estimated to be 0.36 ng/mL. Patient-specific factors significantly impacting clo-fara CL included actual body weight and age. The covariate model was able to estimate clo-fara CL with good precision in children spanning a wide age range from infancy to early adulthood and demonstrates the need for variable dosing in children of different ages. For example, the dose required for a 6-month and 1-year old was approximately 43% and 17% lower, respectively, than the typical 40 mg/m^{2}dose to achieve the median $AUC_{0\text{-}24}$of 1.04 mg·h/L in the study population. Despite the known renal elimination of clo-fara, no significant clinical parameters for renal function were retained in the final model ($P > .05$). Coadministration of fludarabine with clo-fara did not alter the CL of clo-fara ($P > .05$). These results will help inform individualized dosing strategies for clo-fara to improve clinical outcomes and limit drug-related adverse events in children undergoing HCT.

INTRODUCTION

Although major advancements have been made in recent years through improvements in supportive care, high rates of engraftment failure and disease relapse remain prominent clinical problems in allogeneic hematopoietic cell transplantation (HCT). Historically, fludarabine has been included in most reduced-intensity conditioning regimens allogeneic HCT in combination with an alkylating agent, such as busulfan (BuFlu). More recently, combining clofarabine (clo-fara) with BuFlu has been evaluated in an attempt to improve efficacy in children undergoing allogeneic HCT to treat leukemia and various nonmalignant disorders [1].

Clo-fara is a newer-generation nucleoside analog with enhanced antitumor activity and an improved safety profile compared with fludarabine [2]. Evidence for dual nucleoside analog therapy as part of pretransplantation combination conditioning for high-risk malignancies is supported by both in vitro and in vivo research. Valdez et al [3] investigated the in vitro cytotoxic properties of clo-fara alone and in combination with fludarabine and busulfan in a human cell line model of busulfan-resistant acute myelogenous leukemia. In these

Financial disclosure: See Acknowledgments on page 1610.
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https://doi.org/10.1016/j.bbmt.2019.04.017
1083-8791/

models, the inhibitory concentrations of clo-fara and fludarabine were .06 μM and 3.0 μM, respectively, suggesting an approximate 50-fold difference in their cytotoxic activity. At low concentrations, the combination of clo-fara, fludarabine, and busulfan showed a higher degree of synergistic cytotoxicity compared with either nucleoside alone in combination with busulfan [4].

A similar response was observed in other acute myelogenous leukemia (AML) cell lines and in the isolated peripheral blood mononuclear cells of patients with leukemia. Andersson et al [4] reported the results of a 4-arm clinical trial in which escalating doses of clo-fara were combined with deescalating doses of fludarabine plus standard busulfan doses in primarily adult patients with high-risk myeloid malignancies. The clo-fara dosage was 10 to 40 mg/m^2/day for 4 to 5 consecutive days. All 51 patients engrafted, and there were no significant differences among the 4 arms in overall or progression-free survival. However, an early trend toward improved overall survival and event-free survival was observed for AML patients in the arms treated with higher clo-fara doses. In abstract form, the combination of Bu/Flu/clo-fara has demonstrated good response and low rates of relapse and toxicity in children treated for myeloid and lymphoblastic disease compared with historical TBI-containing regimens [1]. Unfortunately, no concentration-response data were reported with either clinical trial [1,4].

To date, only 1 published study has described the PK of clo-fara in the setting of pediatric allogeneic HCT [5]. Sixteen patients received a single nucleoside analog conditioning regimen consisting of clo-fara, alemtuzumab, melphalan, and total body irradiation (TBI) for the treatment of high-risk inherited metabolic disorders. Clo-fara (40 mg/m^2/day) was administered daily, and a 2- to 3-fold variability was observed in clo-fara PK parameters, including clearance (CL), area under the curve (AUC), and volume of distribution. PK variability was not sufficiently explained by markers of renal function and body size, and due to the small sample size, relationships between PK parameters and clinical outcomes were not evaluated.

Currently, no PK data are available for a combination nucleoside analog regimen consisting of both clo-fara and fludarabine. Given that clo-fara and fludarabine share a similar metabolic pathway, drug disposition may be affected via several mechanisms, including altered drug CL. Moreover, PK-guided dosing of nucleoside analogs has the potential to improve survival and reduce toxicity in children at high risk for graft rejection and disease relapse [6]. The primary objective of the present work was to characterize the PK of systemic clo-fara in pediatric allogeneic HCT recipients receiving either nucleoside monotherapy or a dual nucleoside analog preparative regimen.

METHODS

Study Population

This was a multicenter PK study of clo-fara in children who underwent allogeneic HCT for a variety of malignant and nonmalignant pediatric disorders. Patients were eligible to participate in PK analysis if they were between 0 and 18 years of age, met institutional- and protocol-specific eligibility criteria for HCT, and were scheduled to undergo allogeneic HCT that included receipt of single nucleoside therapy with clo-fara or dual nucleoside analog therapy (fludarabine plus clo-fara) as part of their conditioning regimen. Patients who received clo-fara alone or in combination with fludarabine over 4 to 5 days were eligible to participate. Clo-fara PK data were collected between 2012 and 2018 at the University of California San Francisco Benioff Children's Hospital and the University of Minnesota Masonic Children's Hospital. Table 1 presents patient demographic data and information on preparative regimens and PK assessment strategies for both sites. All local Institutional Review Boards approved this study, and written informed consent/assent to undergo PK studies was obtained from all patients and/or guardians. This study was registered at ClinicalTrials.gov as NCT03609814.

Pretransplantation Conditioning Regimens

For subjects enrolled at the University of Minnesota (MN-Clo40), pretransplantation conditioning for high-risk metabolic disorders consisted of alemtuzumab .3 mg/kg/day i.v. on days -12 to -8, clo-fara 40 mg/m^2/day i.v. on days -7 to -3, melphalan 140 mg/m^2 i.v. on day -2, and TBI 200 cGy in a single fraction on day -1 [5]. Two protocols using dual nucleoside analog therapy with clo-fara and fludarabine were evaluated at the University of California. From 2012 to 2013 (SF-Flu40/Clo10), patients undergoing HCT for a variety of nonmalignant and high-risk malignancies were conditioned with exposure-targeted model-based dosing of i.v. busulfan on days -5 to -2, clo-fara 10 mg/m^2/day i.v. on days -5 to -2, fludarabine 40 mg/m^2 i.v. on days -5 to -2, and serotherapy. Subjects undergoing PK assessment from 2014 to 2018 (SF-Flu10/Clo30) received pretransplantation conditioning for high-risk malignancies with exposure-targeted model-based dosing of i.v. busulfan on days -5 to -2, clo-fara 30 mg/m^2/day i.v. on days -5 to -2, and fludarabine 10 mg/m^2 i.v. on days -5 to -2 with or without rabbit antithymocyte globulin mg/kg on days -5 to -2. In the SF-Flu10/Clo30 group, to account for potential differences in drug clearance with age, children weighing <12 kg or younger than 1 year of age, clo-fara was administered at 1 mg/m^2 per protocol.

Bioanalysis

For MN-Clo40, plasma samples were analyzed for clo-fara by MicroConstants (San Diego, CA) using validated reverse-phase high-performance liquid chromatography with mass spectrometry as described previously [5]. The assay was linear in the range of 1 to 500 ng/mL. Samples with clo-fara levels reported below the lower limit of quantification (1 ng/mL) were entered into PK analysis as having a concentration of .5 ng/mL (one-half the lower limit of quantification) [7]. Assay accuracy and intraday, and interday variability were 95% to 96.2%, 5.1% to 7.4%, and 6.7% to 14.4%, respectively.

Plasma samples for the SF-Flu40/Clo10 and SF-Flu10/Clo30 studies were analyzed for clo-fara by the University of California San Francisco, Department of Clinical Pharmacy Drug Research Unit Laboratory using a validated reverse-phase high-performance LC-MS/MS methods as described previously [8]. Clo-fara plasma concentrations that fell below the lower limit of quantification (.5 ng/mL) were reported by the laboratory and entered into the model as the true value. The assay was linear in the range of .5 to 80 ng/mL.

Table 1
Overview of the Clinical Studies Included in the Population PK Analysis

Study Identifier	Number of Subjects	Pretransplantation Combination Therapy	PK Sampling Strategy
MN-Clo40 [5]	15	Alemtuzumab 0.3 mg/kg/d i.v. on days -12 to -8; clo-fara 40 mg/m^2/d i.v. on days -7 to -3; melphalan 140 mg/m^2 i.v. on day -2; and TBI 200 cGy single fraction on day -1	Day 1: 0 (preinfusion), then 2, 3, 4, 6, 8, and 24 h after start of infusion; day 5: 0 (preinfusion), then 4, 8, and 72 h after start of infusion
SF-Flu40/Clo10	18	Model-based busulfan i.v. infusion over 2 h administered every 6 h on days -5 to -2; clo-fara 10 mg/m^2/d i.v. over 2 h on days -5 to -2; fludarabine 40 mg/m^2/d i.v. on days -5 to -2; and serotherapy	Limited sampling following any of 4 single daily doses of clo-fara (doses 1-4), at 2, 3, 6, and 24 h after start of infusion
SF-Clo30/Flu10	18	Model-based busulfan once daily i.v. infusion over 3 h on days -9 to -6; clo-fara 30 mg/m^2/day i.v. over 2 h on days -5 to -2; fludarabine 10 mg/m^{2}i.v. over 1 h on days -5 to -2; with or without rabbit antithymocyte globulin mg/kg on days -5 to -2	Limited sampling following a single dose of clo-fara (doses 1- 4), at 2, 3, 6, and 24 h after start of infusion

The mean accuracy (mean ± coefficient of variation) of the assay was 98.5 ± 7.0% at low, 101.7 ± 6.6% at medium, and 92.8 ± 7.8% at high quality control levels.

Population PK Analysis

A nonlinear mixed-effects modeling approach to describe the time course of clo-fara plasma concentrations was implemented using Phoenix v8 (Certara, Princeton, NJ). The freely available software R version 3.4.4 was used for graphical inspection, to aid selection of the most appropriate model and display the model-predicted results in visual form [9]. Model selection was based on comparison of the objective function value (χ^2, df = 1, α = .05) and the goodness-of-fit plots.

Between-subject variability (BSV) [10,11] was modeled based on the assumption of a log-normal distribution as

$$P_i = tvP * e^{\eta_i},$$

where P_i is the individual PK parameter for patient i, such as CL_i; tvP is the typical value of that PK parameter, such as $tvCL$; and η_i is the corresponding BSV for patient i, which is assumed to follow a normal distribution with mean 0 and variance of ω^2_{BSV}.

Different residual error models [10,11] were tested (eg, combined error model: proportional + additive) as shown below:

$$OBS_{ij} = IPRED_{ij} * (1 + \varepsilon_{1\,ij}) + \varepsilon_{2\,ij},$$

where OBS_{ij} and $IPRED_{ij}$ are the observed and individual predicted concentration in the central compartment for patient i at time j, respectively; $\varepsilon_{1\,ij}$ is the corresponding proportional error term, which is assumed to follow a normal distribution with mean 0 and variance of σ^2_1; and $\varepsilon_{2\,ij}$ is the corresponding additive error term for patient i at time j, which is assumed to follow a normal distribution with mean 0 and variance σ^2_2.

Covariate Modeling

Clinical data were collected on each day of PK sampling before clo-fara administration to assess the influence of patient-specific factors on clo-fara CL. Covariate analysis was performed using a stepwise forward additive approach followed by a stepwise backward elimination approach [12] with an α value of .05 and .001, respectively. An improvement in the precision of the parameter estimates (relative standard error), along with decreases in between-subject variability and residual variability, were used to determine the importance the tested covariate as a significant predictor of drug CL. Age, sex, height, body surface area, serum creatinine, creatinine clearance (CRCL), blood urea nitrogen, albumin, WBC count, absolute neutrophil count, diagnosis, and preparative regimen (coadministration of fludarabine) were evaluated.

Allometric scaling provides a mechanistic- and physiologic-based approach for describing the effects of organ size and blood flow on drug clearance [13,14]. A body weight-based allometric model was added to all clearance and volume parameters for clo-fara with an exponent of .75 and 1, respectively [15]. Although allometric scaling provided an improved model for describing drug clearance, it was insufficient for describing changes in CL and PK variability, particularly in subjects age <2 years. The maturation of kidney function, which occurs over the first year of life, was found to best explain variations in PK attributed to age, in addition to weight, evaluated as a continuous covariate.

In pediatric patients, CRCL was estimated by the Schwartz method and in young adults by the Cockcroft-Gault equation using ideal body weight and capping the maximum value at 150 mL/min/1.73 m^2 for covariate analysis [16,17]. Continuous covariates were tested as various relationships including a power function and centered on the median population value, an E_{max} function, and an exponential function. Dichotomous covariates were tested in the model via binary indicator variables.

Model Evaluation

Model evaluation was based on various methods of evaluating the predictive ability of the final model on individual data. Normalized prediction distribution errors (NPDEs) were generated using 1000 simulations for each observation in the original dataset and used to identify trends for model misspecification [12]. A nonparametric bootstrap resampling method was used to evaluate the robustness of the final PK model [18]. Resampling with replacement generated 1000 bootstrap datasets, and the final population PK model was fitted repeatedly to each dataset. The means and 95% confidence intervals (CIs) of parameters obtained from this step were compared with the final parameter estimates. In addition, the prediction-corrected visual predictive check (pcVPC) with 1000 simulated datasets was also performed [19]. Results from the pcVPC were assessed using graphical comparison of the appropriate 90% prediction intervals from simulated data and were visually explored in comparison with overlaid observed data from the original dataset.

Simulations to Demonstrate Covariate Effects on Clo-fara Dose

Based on our final population PK model, individual clo-fara doses were estimated using the final covariate model and compared with a typical dosing regimen of 40 mg/m^2 administered daily for 4 days. Clinical covariates (age and weight) for a typical patient were based on the 50th percentile estimates of weight per age as provided by the Centers for Disease Control standard growth charts for infants and children [20]. First, the $AUC_{0\text{-}24}$ for each patient was derived from the empirical Bayes estimates of individual CL ($AUC_{0\text{-}24}$ = dose/CL). Then individual $AUC_{0\text{-}24}$ values were multiplied by the total number of doses of clo-fara received to derive the cumulative AUC. For dose simulation purposes, we selected our target $AUC_{0\text{-}24}$ to reflect the median value of patients receiving 40 mg/m^2 as either monotherapy or with coadminstration with fludarabine. The combination of clo-fara plus fludarabine is still relatively uncommon, and we felt that a 4-day regimen of 40 mg/m^2 alone was most reflective of current HCT clinical practice (clo-fara used alone in combination with an alkylator). Doses of clo-fara administered once daily over 4 days of therapy were simulated to achieve the median $AUC_{0\text{-}24}$ in patients receiving 40 mg/m^2 ($AUC_{40\ mg/m2}$) using the following equation:

$$\textbf{Dose of clo}-\textbf{fara}(\textbf{mg}) = AUC_{40mg/m2} X\ CL_{(individual)}$$

RESULTS

Patient Demographics

A total of 51 subjects completed PK assessments and were used for PK model building. Patient demographics for all subjects are presented in Table 2. Among the study subjects, the overall median age was 4.9 years (range, .25 to 14.9 years), with 4 subjects (7.8%) of subjects age ≤12 months. Median actual body weight was 15.6 kg (range, 6.23 to 97.5 kg) and included 8 children (16%) weighing ≤10 kg. All patients had normal renal function for age starting with the first dose of clo-fara, with a median CRCL of 150 mL/minute (range, 96 to 150 mL/minute). Fifteen patients received single nucleoside therapy with clo-fara, and the remaining patients received dual nucleoside analog therapy (fludarabine plus clo-fara).

Population PK Analysis

Following inspection of the data, a total of 311 quantifiable concentrations of clo-fara were available for population PK model building. Less than 5% (n = 10) of the plasma samples collected from MN-Clo40 were reported as below the lower limit of quantification. Figure 1 displays the dose-normalized observed clo-fara plasma concentrations (normalized to 10 mg/m^2). A 2-compartment model provided the most reasonable fit of the data and thus was retained for subsequent covariate model development. A body weight-based allometric

Table 2
Patient Demographics and Baseline Characteristics

Characteristic	Value
Number of subjects	51
Females/males, n (%)	28 (55)/23 (45)
Age, yr, median (range)	4.9 (.25-14.9)
Weight, kg, median (range)	15.6 (6.2-97.5)
Body surface area, m^2, median (range)	.68 (.31-1.89)
Serum creatinine, mg/dL, median (range)	.3 (.1-.5)
CRCL, mL/min/1.73 m^2*, median (range)	150 (96-150)
WBC count, × 10^9 cells/L, median (range)	2.9 (.1-11.7)
Combination pretransplantation conditioning regimen, n (%)	
Clo-fara 40 mg/m^2/melphalan/TBI	15 (29.4)
Clo-fara 10 mg/m^2/ fludarabine/busulfan	18 (35.3)
Clo-fara 30 mg/m^2/fludarabine/busulfan	18 (35.3)

Laboratory data were collected on the day of PK sampling, before drug administration.

* CRCL was estimated in children using the Schwartz method [16] and in young adults age >17 years by the Cockcroft-Gault equation [17] using ideal body weight.

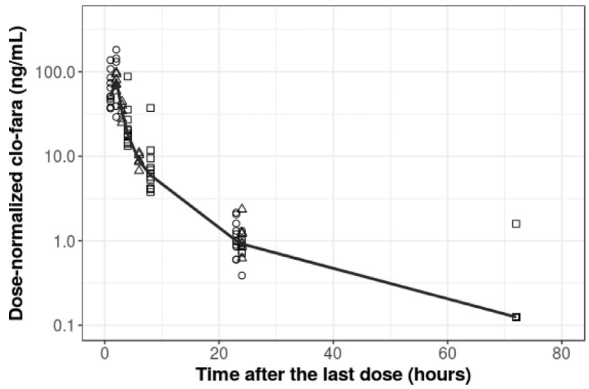

Figure 1. Scatterplot of dose-normalized (by 10 mg/m^2) plasma clo-fara concentration-time profiles. Black points represent the observed dose-normalized clo-fara concentrations from the different dose regimens: 10 mg/m^2(open circles), 30 mg/m^2(open triangles), and 40 mg/m^2(open squares). The black solid line represents the median values of the observed concentrations.

model was added to all clearance and volume parameters for clo-fara with exponents of .75 and 1, respectively [15]. The model describes an increase in clo-fara CL with increasing body weight in children. For children age <2 years, age-related developmental changes in physiological and elimination processes independent of body weight can lead to significantly altered drug disposition. Thus, we found that body weight alone was not sufficient to describe drug clearance in children, particularly in subjects age <1 year. When we implemented an inverse exponential function, age was found to be significant covariate affecting clo-fara CL (independent of weight) and it was retained in the final model to best describe the effects of renal maturation ($P < .001$) and body weight on drug exposure and disposition. No other laboratory parameters or patient-specific clinical factors evaluated were found to significantly impact clo-fara CL, including markers for renal function, diagnosis, or preparative regimen coadministration with fludarabine ($P = .20$).

The population PK parameters estimates and their relative standard errors (%) from the final model are presented in Table 3. The final model for clo-fara CL incorporating age and weight was

$$CL = 23.4 \cdot \left(\frac{Bodyweight}{15\ kg}\right)^{0.75} \cdot \left(1 - e^{\left(-AGE \cdot \frac{\ln(2)}{T_{half}}\right)}\right) \cdot \exp(\eta_{CL}),$$

where 23.4 L/hour is the typical value of clo-fara for a child weighing 15 kg, *AGE* equals 5 years, and T_{half} is the renal maturation half-life of age effect on CL, which was estimated as .41 year. The between-subject variability for clo-fara CL and volume of central compartment were 26% and 40%, respectively. A combination residual error model best described residual unexplained variability with proportional residual and additive residual error values of 33.2% and .36 ng/mL, respectively. The inclusion of interoccasion variability did not improve the model, and thus it was not included.

The goodness-of-fit plots for the base and final model demonstrated good improvement, with adequate distribution of population-predicted concentrations around the line of unity and no obvious trends for model misspecification or bias (Figure 2). Standard diagnostic plots of the normalized standard predication error (Figure 2) and representative model fits (Figure 3) for individual PK profiles for clo-fara indicate that the model captured the data very well. The mean PK parameter estimates and 95% CIs from the bootstrap analysis are presented in Table 3. Estimates of PK parameters, between-subject variability, and residual unexplained variability derived from the bootstrap analysis were comparable with the typical values derived from the original dataset. In addition, the pcVPC for the final covariate model shows that the variability in the data (quantified by the 5th and 95th percentiles) was well predicted (Supplementary Figure S1).

Table 4 displays the derived clo-fara $AUC_{0\text{-}24}$ values from the observed data at different dose regimens for a single 24-hour dosing interval. The median daily clo-fara $AUC_{0\text{-}24}$ for all patients included in the analysis, irrespective of the protocol-specific dose (e.g. 10, 30, or 40 mg/m^2), was .78 mg·hr/L (range, 0.22 to 4.0 mg·hr/L). For those patients receiving a daily dose of 40 mg/m^2 (n = 15), the median $AUC_{0\text{-}24}$ was 1.04 mg·hr/L (range, 0.87 to 4.0 mg·hr/L), which is equal to a cumulative AUC of 4.2 mg·hr/L for 4 doses. One subject, a 6-month-old with a diagnosis of osteopetrosis receiving 40 mg/m^2 had an outlier value of $AUC_{0\text{-}24}$ (4 mg·hr/L), as shown in Table 4. Notably, this child developed acute renal failure with dose 2 of clo-fara, required the initiation of peritoneal dialysis on day 0, and unfortunately died of multiorgan failure shortly after transplantation. This was the only subject receiving a daily dose of 40 mg/m^2 with an $AUC_{0\text{-}24}$ >1.2 mg·hr/L. The other subjects had an $AUC_{0\text{-}24}$ of ≤1.17 mg·hr/L. Important patient-specific covariates found to significantly impact clo-fara CL were actual body weight and age. Figure 4 shows the change in the model-predicted dose of clo-fara with age compared with the common dosing strategies of 40 mg/m^2 and 1.33 mg/kg. The model demonstrates that for subjects age <2 years, dose modifications are needed to avoid elevated drug exposure. The model-predicted dose increases

Table 3
Final Population PK Model Parameter Estimates and Bootstrap Results

Population PK Parameters	Final Model Results		Bootstrap Results	
	Parameter Estimate	RSE (% mean)	Mean	95% CI
Typical value for clo-fara CL, L/h/15 kg	23.4	6.30	23.4	20.7-26.2
Volume of the central compartment, V_c, L/15 kg	42.3	11.6	42.3	32.5-52.2
Inter-compartmental CL, L//h/kg*	9.82	9.88	9.82	7.91-11.8
Volume of the peripheral compartment, V_p, L/15 kg	47.4	6.14	47.3	42.0-53.8
Maturation half-time, T_{half}, yr	.41	42.2	.43	.17-.81
Interindividual variability on CL, %CV	25.7	39.0	24.2	10.2-32.7
Interindividual variability on V_c^4, %CV	40.3	26.5	40.1	22.7-52.0
Proportional residual unexplained variability, %	33.2	8.89	32.7	
Additive error, ng/mL	.36	6.43	.36	

RSE indicates relative standard error.
* First-order rate constant for drug moving from the central compartment to the intracellular compartment.

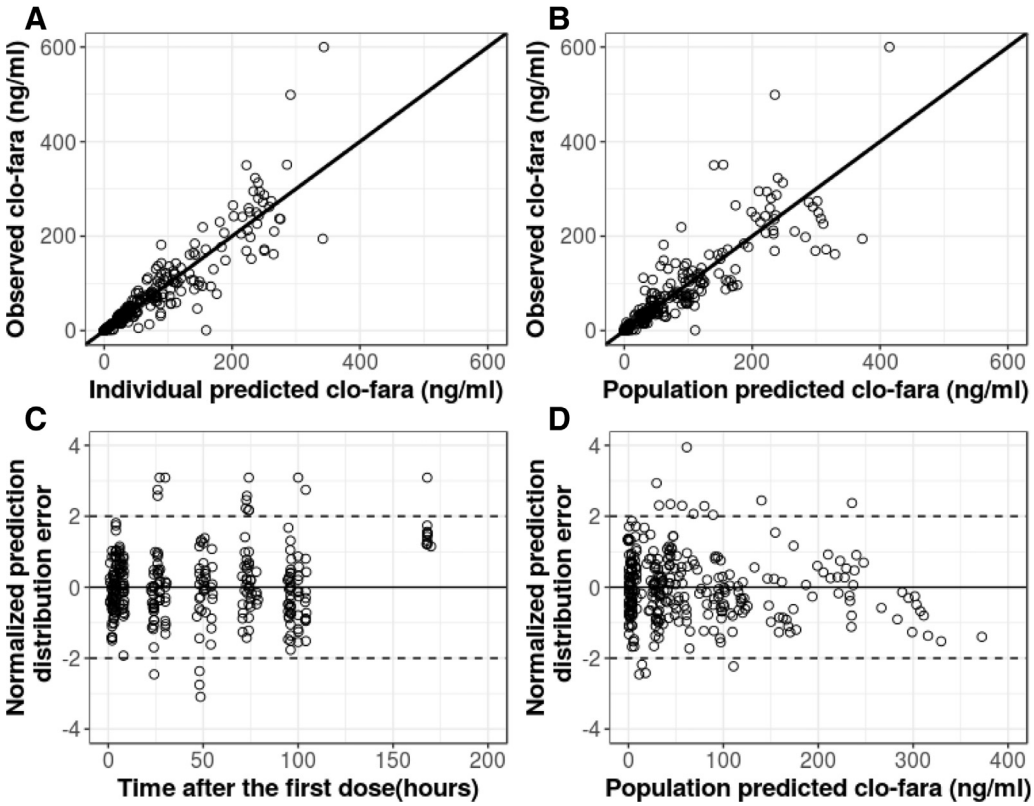

Figure 2. (A) Observed versus individual-predicted clo-fara plasma concentrations. (B) Observed versus population-predicted clo-fara plasma concentrations. (C) Normalized prediction distribution error versus time after first dose. (D) Normalized prediction distribution error versus population-predicted clo-fara plasma concentrations.

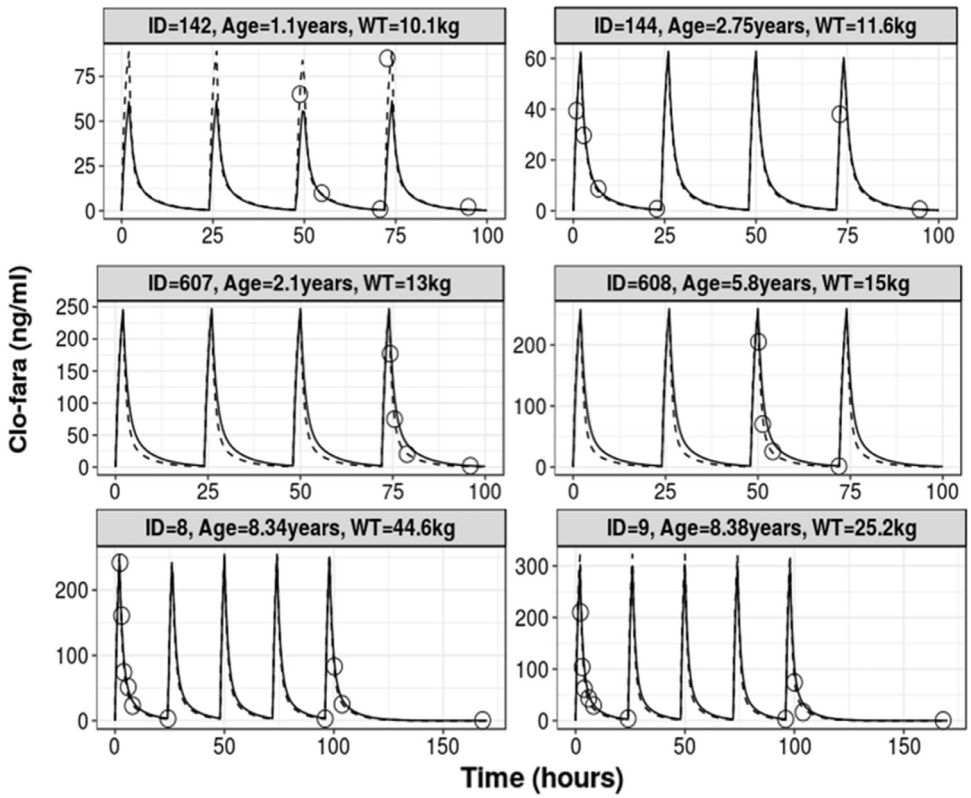

Figure 3. Representative individual fit plots of observed and predicted time-concentration data for the different dosing regimens: 10 mg/m^2 (top row), 30 mg/m^2 (middle row), and 40 mg/m^2 (bottom row). Open circles represent the observed concentrations, the black solid line is the population prediction, and the dashed line is the individual prediction.

Table 4
Comparison of Daily and Cumulative Exposure of Clo-Fara in the Observed Data Presented by Clo-fara Daily Regimen

Clo-fara Regimen	Number of Subjects	Total Doses	$AUC_{0\text{-}24}$, mg·h/L, median (range)	Cumulative AUC, mg·h/L, median (range)
All dose regimens			.78 (.22-4.0)	3.3 (.87-20.0)
10 mg/m^2/dose	18	4	.30 (.22-.36)	1.2 (.87-1.44)
30 mg/m^2/dose	18	4	.82 (.37-1.39)	3.3 (1.5-5.5)
40 mg/m^2/dose	15	5	1.04 (.87- 4.0)	5.2 (4.4-20.0)

from birth (.011 mg/kg) to age 2 years (1.7 mg/kg) and then decreases until around age 18 years (1.2 mg/kg).

Simulated CL values suggest that for young children, particularly those age <6 months, a decrease in the dose is required to provide exposure comparable to that of an older child (Figure 4). For example, the model-predicted dose for a 6-month-old child weighing 6.3 kg would be 7.2 mg per dose, representing a 45% dose decrease compared with a conventional dose of 40 mg/m^2 (13 mg) and a 14% decrease compared with the modified regimen of 1.33 mg/kg (8.4 mg) which is often performed in young children to prospectively prevent drug-related toxicity. In contrast, the model-predicted dose for a 6-month-old weighing 10 kg would be 10.2 mg per dose, which reflects a dose decrease of 44% and 23%, respectively, compared with a conventional dose of 40 mg/m^2 (18.4 mg) or a modified regimen of 1.33 mg/kg (13.3 mg).

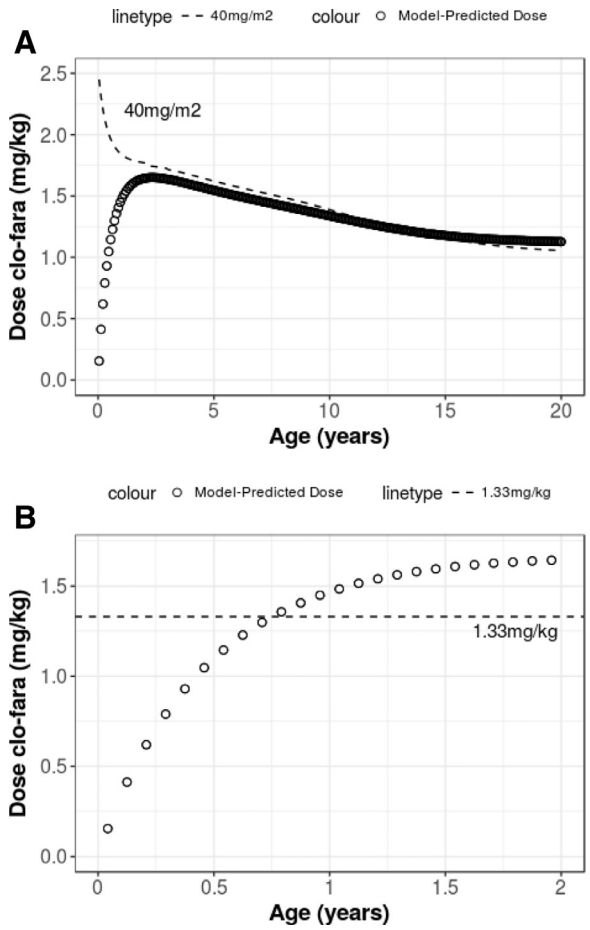

Figure 4. Model-predicted dose of clo-fara (in mg/kg) to achieve a daily $AUC_{0\text{-}24}$ of 1.04 mg·hr/L for age 0 to 20 years (A) and 0 to 2 years (B). The dashed line in (A) represents the doses at corresponding ages based on the conventional dose of 40 mg/m^2. The dashed line in (B) represents the modified regimen of 1.33 kg in young children.

DISCUSSION

The primary objective of this work was to characterize the PK of systemic clo-fara in pediatric allogeneic HCT recipients receiving either nucleoside monotherapy or a dual nucleoside analog preparative regimen. Before these results, there were no PK data available for a combination nucleoside analog regimen consisting of both clo-fara and fludarabine and limited data characterizing the PK of clo-fara alone in a pediatric HCT population. Given that clo-fara and fludarabine share a similar metabolic pathway for drug disposition, we hypothesized that fludarabine may alter the PK of clo-fara with coadministration and provide insight into the potential contribution of enhanced synergy of the combination reported in vitro. Clo-fara and fludarabine are excreted primarily unchanged via the kidneys through a combination of glomerular filtration and active tubular secretion via transporters [2,21]. On both the basolateral and brush border membrane, the proximal tubule contains numerous drug transport systems, including equilibrative nucleoside transporters and concentrative nucleoside transporters [22,23]. These transporters have been shown to influence the distribution and accumulation of fludarabine and clofara in vitro and are potential sites for significant drug-drug interaction in vivo [21,24-26]. Coadministration of these 2 drugs could potentially lead to significantly altered drug clearance and systemic exposure via competitive inhibition of transporters. However, based on our model, coadministration of fludarabine in the presence of clo-fara was not found to have a significant impact on clo-fara CL. Subtle differences in the transporters required for intracellular influx, efflux, or conversion to the active triphosphate species or timing of the infusion may contribute to such findings. Going forward, studies evaluating the intracellular concentrations of clo-fara may provide more insight and should be pursued.

Organ function maturation and allometric scaling play important roles in predicting the PK of many drugs in infants and young children, whereas the latter is often sufficient in children age >2 years with normal renal function. Before our present results, very little information was available regarding the PK of clo-fara in children age <2 years to guide dose selection in clinical trials and individualized dose regimens in clinical therapy. Our analysis identified both weight and age as significant independent patient-specific factors impacting clofara CL. Clo-fara CL increased with age in our analysis; however, Bonate et al [27] reported that age has a decreasing relationship with clo-fara CL in pediatric and adult patients. The decreasing relationship between CL and age in Bonate et al was driven by an older population (>40 years). Whereas an increasing relationship between CL and age was observed in the population age <20 years (2.8 to 21 years), the age range in our study was .25 to 15 years, which makes the comparison difficult. For each drug, one model across the continuum of physiological changes (age) should be the goal of population PK modeling; however, seldom do we get access to such comprehensive datasets. The inclusion of both allometric scaling and a maturation function in our model enhances the ability to estimate drug CL with relative accuracy in a pediatric

population of various ages. Particularly for children age <6 months, the model suggests that significant dose reductions are necessary to achieve comparable exposure to older children when using the traditional dosing strategy based on body surface area (mg/m^2). In addition, clinicians often struggle with the decision to prospectively modify the dosing strategy in children weighing <12 kg or age <1 year in their attempts to limit the potential for drug-related toxicity. By accounting for both age and weight, our model provides an improved strategy compared with the empiric 1.33 mg/kg conversion often applied in the clinical setting for young children. Based on our model, the predicted CL of clo-fara in a child age 6 months and a child age 1 year was predicted to be was approximately 57% and 27% lower, respectively, than the expected CL of a 2-year-old child.

In the study reported by Long-Boyle et al [5], clo-fara PK parameters were estimated by noncompartmental analysis, compared with the nonlinear mixed-effects modeling in our study. Noncompartment methods are much less sensitive for evaluating clinical covariates that may impact drug exposure, particularly in children. In addition, the sample size of 16 was very limited. In the previous analysis, clo-fara clearance was weakly correlated with weight (R^2 = .33) and body surface area (R^2 = .26). Given that the present analysis was conducted using nonlinear mixed-effects modeling and has a much larger sample size, including a wider range of ages and weights, it would be statistically better powered to determine a significant weight effect on clearance.

Additional other significant covariates found to affect clo-fara CL have been identified in previous reports based primarily in the pediatric leukemic population. Bonate et al [27] reported CRCL (range, 33.1 to 200 mL/min/1.73 m^2) as a significant covariate on clo-fara CL. This is to be expected considering clo-fara undergoes extensive renal elimination through a combination of both glomerular filtration and active tubular secretion. This is further supported by the fact that clo-fara undergoes limited hepatic and nonhepatic metabolic conversion (.2%) and 24-hour urine collections in pediatric studies describe that approximately 49% to 60% of the dose is excreted in the urine unchanged in accordance with the drug label [28]. Time-dependent maturation of transporters in the kidneys likely contributes to an increase in drug exposure in children age <2 years. However, the range of CRCL observed in the present study did not support CRCL as predictor for clo-fara CL. This is likely the result of all subjects having normal renal function for age in this study. Further, there was no trend observed between empirical Bayes estimates of individual clo-fara CL and other relevant biomarkers for renal function, such as serum creatinine and blood urea nitrogen, in our analysis. This relationship of CRCL on CL warrants further investigations in a more heterogenous population, particularly in older children with preexisting renal impairment. However, the evaluation of such a population may prove challenging, given the criteria for adequate renal function as part of the pre-HCT assessment process.

Similarly, in the previous study, WBC count was found to be a significant covariate, with the predicted volume of the central compartment of clo-fara increasing 2.38-fold between a WBC count of 0.3×10^3 /μL and 259×10^3 /μL [29]. The narrow range of WBC counts in this study (0.1×10^3 to 11.7×10^3 /μL), along with inherent differences between patient populations, may account for the lack of a relationship between absolute neutrophil count and clo-fara exposure in our study. This study includes a mix of indications for transplantation (malignant and nonmalignant), and in general, patients present to transplantation with normal to low WBC counts. This is significantly different from the leukemic population that routinely initiate clo-fara therapy with an expected elevated WBC count. In the covariate analysis preparative regimen (including busulfan) or underlying disease were not found to be significant predictors of clo-fara drug clearance. These factors may be important for clinical outcomes and will be evaluated again in future planned analyses, once a larger sample size is achieved to adequately evaluate them.

The primary purpose of this study was to better understand the PK of systemic clo-fara in pediatric allogeneic HCT recipients receiving either nucleoside monotherapy or a dual nucleoside analog preparative regimen. This analysis was not designed to determine whether the combination of Bu/Flu/clo-fara is more safe or efficacious than Bu/Flu or Bu/clo-fara. Subjects included in this dataset varied widely in several respects, including indication for transplantation, comorbidities, combination pretransplantation conditioning (cPTC), and immunosuppressive strategies. Thus, due to the sample size of the current analysis, no exposure-response analysis was performed. We continue to collect additional PK and outcomes data at both centers such that sufficient numbers for subjects to adequately inform drug-response relationships will be available for analysis. In addition, at our center we are now able to quantify drug levels for all agents used in a single individual as part of cPTC. Univariate PK-pharmacodynamic associations have been identified for several commonly used agents in pediatric HCT, including busulfan, fludarabine, and antithymocyte globulin. However, the analysis of single agents is likely insufficient to describe the overall effect of cPTC on clinical outcomes, and to date, no comprehensive evaluation of PK-pharmacodynamic relationships describing the relative contributions of individual agents when used in combination for cPTC has been performed. Therefore, our ongoing studies are moving to a more comprehensive investigation of cPTC and exposure-response relationships in pediatric HCT, with the goal of identifying optimal immunosuppression and prevention of severe toxicity.

CONCLUSION

This work represents the largest and most comprehensive study of clo-fara pharmacology in pediatric patients undergoing HCT reported to date. We found no significant impact on clo-fara PK when coadministered with fludarabine. Our covariate analysis identified actual body weight and age as significant patient-specific factors affecting clo-fara CL, demonstrating the application of model-based dosing can ensure equivalent exposure across different ages and weights for children requiring clo-fara as part of cPTC in HCT. We suggest that each individual child, especially very young or small children, should be administered a personalized dose based on specific age and body weight. Dose reductions of clo-fara based on our model suggested the current dose alternative dose 1.33 mg/kg may be sufficient for some children age <6 months and largely insufficient for many children weighing <12 kg or age <2 years, where it is often applied. Finally, moving away from the traditional dosing intensity strategies to model-based dosing in this setting has the potential to limit drug-related toxicity while maintaining efficacy. Future work will involve the identification of exposure-response relationships between clo-fara and clinical outcomes to enhance drug efficacy when applied with a combination of model-based dosing and Bayesian-driven therapeutic drug monitoring.

ACKNOWLEDGMENTS

The authors thank all the patients, families, nurses, and support staff for their contributions to this study.

Financial disclosure: This work was supported by the Thrasher Research Fund and the National Center for Advancing Translational Sciences, National Institutes of Health (UCSF-CTSI Grant KL2 TR000143, to J.L.B.).

Conflict of interest statement: There are no conflicts of interest to report.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2019.04.017.

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