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Effect of Weight and Maturation on Busulfan Clearance in Infants and Small Children Undergoing Hematopoietic Cell Transplantation

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A B S T R A C T

Little information is currently available regarding the pharmacokinetics (PK) of busulfan in infants and small children to help guide decisions for safe and efficacious drug therapy. The objective of this study was to develop an algorithm for individualized dosing of i.v. busulfan in infants and children weighing ≤12 kg, that would achieve targeted exposure with the first dose of busulfan. Population PK modeling was conducted using intensive time-concentration data collected through the routine therapeutic drug monitoring of busulfan in 149 patients from 8 centers. Busulfan PK was well described by a 1-compartment base model with linear elimination. The important clinical covariates affecting busulfan PK were actual body weight and age. Based on our model, the predicted clearance of busulfan increases approximately 1.7-fold between 6 weeks to 2 years of life. For infants age <5 months, the model-predicted doses (mg/kg) required to achieve a therapeutic concentration at steady state of 600-900 ng/mL (area under the curve range, 900-1350 μM·min) were much lower compared with standard busulfan doses of 1.1 mg/kg. These results could help guide clinicians and inform better dosing decisions for busulfan in young infants and small children undergoing hematopoietic cell transplantation.

INTRODUCTION

The pharmacokinetics (PK) and pharmacodynamics of drugs in infants can differ widely between children and adults [1-3]. Within the first year of life, age-related developmental changes in physiological and metabolic processes can lead to significantly altered drug disposition [1,4]. In addition, the relationships among dose, plasma concentration, and pharmacodynamic effect may be highly variable across different age groups and disease states. The value of understanding therapeutic differences in drug response because of developmental factors is dependent on the ability to define a dose–concentration relationship [5]. Unfortunately, barriers unique to the pediatric population can hinder PK studies, particularly in infants. Clinical therapeutic trials are often limited by ethical considerations, low study consent rates, limitations on blood volumes, and inadequate assay sensitivity [6].

Busulfan (Busulfex) is a bifunctional alkylating agent routinely used in conditioning regimens before hematopoietic cell transplantation (HCT) to treat various childhood malignant and nonmalignant disorders [7]. Despite the widespread use of busulfan, PK studies in infants remain inadequate to ensure safe and efficacious drug therapy. As defined by drug manufacturers' guidelines, initial doses of busulfan are based on actual body weight, with the aim of achieving an area under the curve (AUC) of 900-1350 μM·min (equivalent to a concentration at steady state [Css] of 600-900 ng/mL) (Table 1) [7]. However, this dose was based on the results of a single clinical trial of only 24 children undergoing HCT who received busulfan in combination with cyclophosphamide [7,8]. The patients ranged in age from 3 months to 16 years (mean age, 6.3 years) and included only 14 children age ≤4 years [7]. More recently, several population PK studies in children have shown that individualized (eg, personalized) model-based algorithms for busulfan clearance that incorporate body size and/or age provide improved targeted therapy compared with stratified weight- or age-based regimens alone [9-13]. Unfortunately,

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Table 1
PK Parameters Used in the Therapeutic Dose Monitoring of Busulfan, Expressed in Unit Equivalents

PK Parameter	Equivalent Value of the Therapeutic Range*
Css, ng/mL	600-900
AUC, μM·min	900-1350
AUC, mg·h/L	3.6-5.4

* Equivalent values reflect the therapeutic range for a 6-hour dosing interval.

most published covariate models have included limited data in infants and small children weighing <12 kg, and thus the appropriateness of extrapolating these dosing algorithms to infants or very young children remains unclear.

Intervention with HCT very early in life is often considered critical to the effective treatment of several childhood diseases, including many immunodeficiencies and genetic metabolic disorders [14-17]. For example, children with severe combined immunodeficiency disease (SCID) usually require definitive therapy with HCT soon after diagnosis. These children typically present early in life (age <6 months). With newborn screening for SCID becoming increasingly available, children are now being diagnosed in the first 4 weeks of life, allowing the use of HCT at a young age when outcomes are superior [18]. For children with Hurler's disease, a mucopolysaccharidosis disorder, the younger that a child is treated with HCT (the only effective treatment currently available), the better the overall outcome [17]. The inclusion of busulfan in the conditioning regimens of very young children is often desirable to promote stem cell engraftment, correct B cell functionality, and avoid long-term consequences of total body irradiation, including growth and developmental delay, poor jaw and tooth development, cataracts, and increased risk of malignancy later in life [19]. Unfortunately, limited busulfan PK data are available in infants and very small children to guide dosing and ensure optimal drug therapy. The objective of the present study was to develop an algorithm for individualized dosing of busulfan in children weighing <12 kg that would achieve targeted exposure with the first dose of busulfan.

PATIENTS AND METHODS

Study Population

This retrospective study used PK data available from routine therapeutic drug monitoring of busulfan levels in 149 pediatric patients treated with HCT from multiple study centers. Eligibility criteria for busulfan PK analysis in this study included (1) an actual body weight ≤12 kg, (2) related or unrelated HCT that included i.v. busulfan therapy, and (3) busulfan plasma time–concentration data available for analysis. Busulfan PK data were provided by 3 different study groups in the United States, Canada, Europe, and the United Kingdom (Table 2). Specific centers (n = 8) within the 3 study groups contributing data for analysis included the University of California San Francisco Benioff Children's Hospital, Boston Children's Hospital, University Medical Center Utrecht, Leiden University Medical Center, Royal Manchester Children's Hospital, University of Manitoba, and Children's Hospital of Westmead, Sydney. Local Institutional Review Boards approved this study, and written informed consent to undergo therapy and PK studies was obtained from all patients and guardians.

The preparative regimen, diagnoses, timing of PK sampling, and bioanalytical analysis have been described in detail previously [9,20-22]. In brief, patients underwent HCT for a wide variety of malignant and nonmalignant pediatric disorders. HCT preparative regimens included busulfan along with different chemotherapeutic agents varying according to the study sites and diagnoses. Busulfan was administered i.v. in all subjects. In the majority of patients, busulfan was administered every 6 hours or every 24 hours (once daily) over a period of 3-4 days. As part of routine clinical care, busulfan plasma concentrations were therapeutically monitored and dose adjustments made to achieve individual protocol-specific targets.

Table 2
Patient Demographic and Baseline Characteristics by Study Group

	Study Group A*	Study Group B†	Study Group C‡
Number	24	24	101
Age, y, median (range)	0.8 (0.1-3.0)	0.7 (0.08-1.8)	1 (0.1-3.3)
Age ≤6 mo, n	4	6	10
Age >6 mo, n	20	18	91
Weight, kg, median (range)	8.3 (3-12)	6.9 (3.3-10.3)	9.2 (3.5-12)
Weight ≤6 kg, n	1	6	14
Weight >6-12 kg, n	23	18	87
Males/females, n§	11/13	16/8	43/41
Height, cm, median (range)	70 (51-88)	65 (51-78)	74 (51-123)
BSA, m^2, median (range)	0.4 (0.2-0.6)	0.4 (0.2-0.5)	0.4 (0.2-0.6)
Dose, mg, median (range)	8.4 (2-17)	7.35 (3.3-11)	20 (3.5-70)
Dose, mg/kg, median (range)	0.97 (0.67-1.5)	1.1 (0.6-1.1)	4.1 (0.8-7.5)
Dosing interval	Every 6 h	Every 6 h	Every 6 hr or 24 h

* University of San Francisco Benioff Children's Hospital.
† Boston Children's Hospital.
‡ Combined data from 6 collaborative centers [9]: University Medical Center Utrecht, Leiden University Medical Center, Royal Manchester Children's Hospital, Newcastle upon Tyne Hospital, University of Manitoba, and the Children's Hospital at Westmead, Sydney.
§ Sex was not reported in 17 subjects.

Population PK Analysis

PK model development using busulfan plasma concentration–time data was performed using the nonlinear mixed-effects modeling program NONMEM version 7 (ICON Development Solutions, Ellicott City, MD). Diagnostic graphics and postprocessing of NONMEM output and simulations were performed using R (R Core Team, Vienna, Australia, 2013, http://www.R-project.org.) and Xpose statistical software [23]. The first-order conditional estimation method with interaction was used throughout the model-building process to estimate PK parameters and variability. Model development was guided by exploratory analysis of the data, changes in the NONMEM objective function value (OFV), diagnostic plots, and the potential biological plausibility of a relationship between clinical covariates and PK parameters. Because many subjects underwent intensive sampling on multiple occasions, interoccasion variability was investigated. Residual unexplained variability was characterized by an additive and proportional error model. Using standard principles of allometric scaling, weight was built into the base model a priori and scaled to a reference patient with a median weight of 8 kg [24]. The model was parameterized in terms of clearance (CL) and volume of distribution in the central compartment (V_c).

Patient-specific factors considered for covariate testing included age, height, body surface area, and sex. Different covariate relationships on PK parameters were investigated with power, linear, and exponential functions. Correlations between covariates were investigated as well. The final PK model was built through the stepwise covariate model-building process of forward selection and backward elimination of clinical covariates. The likelihood ratio test was used to assess the significance of all covariates in the final model. During forward selection, covariates were univariately tested and deemed significant if the OFV decreased by at least 3.84 ($P \leq .05$, chi-squared test, degrees of freedom [df] = 1) with its inclusion in the model. During backward elimination, significance of the covariates were confirmed by removing one covariate at a time from the full model, with an increase in OFV of at least 5.99 ($P \leq .01$, chi-squared test, df = 1) required for retention in the model.

Model Evaluation

The precision of the final model parameter estimates was evaluated using a nonparametric bootstrap approach. A total of 1000 bootstrap datasets were generated by repeated sampling with replacement from the original data, and the final PK model was fitted to each of the bootstrap datasets. The median, 5th, and 95th percentiles were then obtained for each PK parameter and compared with the final model PK estimates. For a visual predictive check (VPC), 500 datasets using the covariate distributions from

the original dataset were simulated using the parameter estimates from the final model and the median, 5th, and 95th percentiles compared with observed concentrations. Individual VPCs were performed for every-6-hour dosing and once-daily administration.

Determination of Targeted Dose

Based on our final model, simulations were performed to achieve the conventional therapeutic targets for busulfan exposure. In addition, simulated Css values based on conventional dosing were compared with the model-based strategy for achieving conventional exposure. The following model-based equation was used for the simulations:

$$\text{Dose (mg)} = \text{AUC}_{\text{target}} \times CLi, \text{ where } CLi = f(\text{weight, age}).$$

Model-based doses were calculated to achieve the midpoint AUC corresponding to a targeted Css range for conventional exposure. Conventional exposure as proposed by the manufacturers' guidelines was defined as an $\text{AUC}_{\text{target}}$ of 4.5 mg·h/L (range, 3.6-5.4 mg·h/L) over a 6-hour dosing interval. This target is equivalent to a Css of 750 ng/mL (range, 600-900 ng/mL) and an AUC of 1098 μM·min (range, 900-1350 μM·min) or 4.5 mg·h/L (range, 3.6-5.4 mg·h/L). Css was calculated as AUC/dose.

RESULTS

Patient Demographics

Patient demographics and baseline characteristics by study group are presented in Table 2. In the 149 study subjects, the overall median age was 0.94 years (11 months; range, 0.1-3.3 years), with 14% of subjects age <6 months. Overall median actual body weight was 8 kg (range, 3-12 kg), and 14% of the children weighed ≤6 kg. Doses normalized to body weight were variable, with higher doses reflecting once-daily versus every-6-hour administration.

Population PK Model Building

A total of 1247 quantifiable concentrations were available for population PK modeling and were best described with a 1-compartment base model with linear elimination. The range of concentrations was 25-8778 ng/mL. Irrespective of each individual center-specific assay, <1% of busulfan plasma concentrations were below the level of quantification and were included in the analysis. A 1-compartment model provided an adequate fit to the data. Addition of a second disposition compartment and/or nonlinear elimination did not produce any further improvement. Interoccasion variability improved the model markedly (ΔOFV >222; $P < 10^{-50}$).

Separate models were developed for different dosing schedules to examine potential differences between once-daily and every-6-hour dosing, however, no differences were detected. Different residual error models were allowed for different centers, to accommodate potential between-center differences in assay errors.

Effects of Covariates on Busulfan Clearance

Important patient-specific covariates found to significantly impact busulfan CL were actual body weight and age. All covariates identified were supported by individual Bayesian PK parameter estimates versus covariate plots. Implementing allometric scaling of PK parameters greatly improved the model, however, it was not sufficient to describe the observed growth-dependent changes in CL in very young children (Figure 1). Estimated CL values suggested that younger children have lower CL compared with the values anticipated by allometric scaling only. The addition of a nonlinear function of CL versus age was implemented to describe maturation of CL, and this function was significant ($P < .001$) and beneficial for the model. No significant impact of any other covariates on busulfan CL was identified.

Final Population PK Model

The population PK parameters estimates and their relative standard errors (SEs; %) from the final model are presented in Table 3. The final model for busulfan CL incorporating both a weight and maturation effect was as follows:

$$\text{CL} = 2.3L/h \times \left(Mat_{mag} + (1 - Mat_{mag}) \times \left[1 - e^{(-Age \times K_{mat})}\right]\right) \times (Weight/8\ kg)^{0.75},$$

where 2.3 L/h is the typical value of busulfan CL, Mat_{mag} is the estimated maturation magnitude effect for age on CL, and K_{mat} is the maturation rate constant for the effect of age on CL. The goodness-of-fit plots for the base and final model

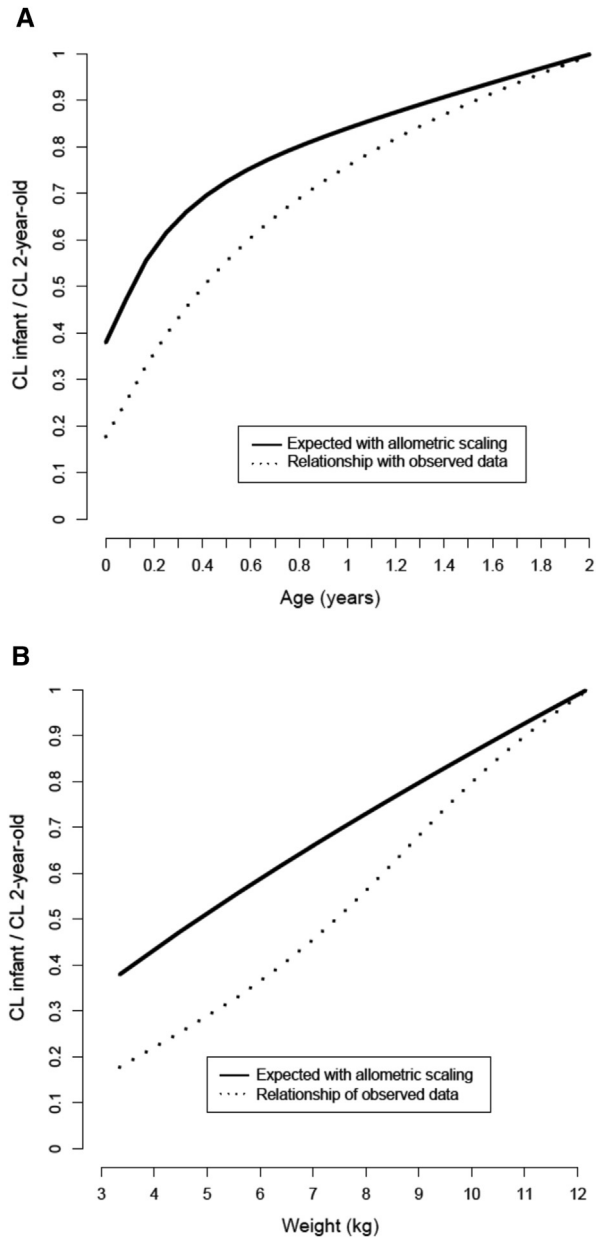

Figure 1. Fraction of busulfan clearance for infants compared to an average 2-year-old child by change in busulfan clearance versus weight (A) and change in busulfan clearance versus age (B).

Table 3
Final Population Pk Model Parameter Estimates and Bootstrap Results

Population PK Parameter	Unit	Final Model Results		Bootstrap Results	
		Typical Value Estimate*	Relative SE, %	Median	95% CI
Clearance (CL)	L/h	2.3	6	2.3	2.1-2.6
Exponent for effect of weight on CL		0.75 (fixed)	—	0.75 (fixed)	—
Volume of central compartment (V_c)	L/kg	6.4	2	6.4	6.1-6.7
Exponent for effect of weight on V_c		1 (fixed)	—	1 (fixed)	—
Maturation magnitude (Mat_{mag})	Fraction	0.46	9	0.45	0.36-0.55
Maturation rate constant (k_{mat})	1/y	1.4	27	1.4	0.8-2.3
Interindividual variability (IIV)					
IIV for CL, %CV†		25	14	26	23-29
IIV for V_c, %CV		25	20	24	18-29
Correlation of CL and V_c		0.74	20	0.64	0.58-0.69
Residual variability, %CV					
Proportional, study group A		8	20	8	5-12
Proportional, study group B		12	17	11	7-16
Proportional, study group C		16	12	16	14-18
Additive, study group A	ng/mL	46	9	61	33-84
Additive, study group B	ng/mL	63	15	62	39-87
Additive, study group C	ng/mL	17	7	17	11-23

* Mean typical value of the PK parameter in the final model.
† %CV, ratio of the SD to the mean.

showed clear improvement, with good distribution of population-predicted concentrations around the line of unity, indicating that the data are adequately described by the final model (data not shown). The vast majority (95%) of conditional weighted residuals fell within 2 standard deviations, demonstrating good predictability of the model. No trend in the residuals was observed.

Model Evaluation

The median PK parameter estimates and 95% confidence intervals from the bootstrap analysis are presented in Table 3. Median estimates of PK parameters, interpatient variability, and residual unexplained variability derived from the bootstrap analysis were comparable with the typical values derived from the original population PK analysis. The VPC showed that the median and percentiles of 500 simulated datasets captured the median and percentiles of the original observed PK data well for both the every-6-hours and once-daily dosing schedules (Figure 2).

Model-Based Dosing to Achieve Conventional Therapeutic Target

Doses (per actual body weight) to achieve conventional exposure (600-900 ng/mL) were simulated and estimated using the model-based algorithm. In particular, children weighing <10 kg required lower doses with the model-based algorithm compared with conventional dosing recommending 1.1 mg/kg (Figure 3). Table 4 provides the estimated doses (mg/kg) required for typical 6-week-old, 3-month-old, and 6-month-old individuals to achieve therapeutic exposure at steady-state using the model-based algorithm. For example, the model-predicted dose needed to achieve a Css of 750 ng/mL for 6-week-old infant weighing 4.5 kg would be 3.6 mg (0.79 mg/kg), representing a decrease of approximately 28% compared with conventional dosing. Figure 4 shows conventional dosing versus the model-based algorithm for achieving a Css of 600-900 ng/mL by age. Compared with model-based dosing, when using the conventional dosing algorithm, children under approximately 5 months of age are more likely to achieve Css values above the recommended threshold of toxicity (900 ng/mL).

DISCUSSION

To the best of our knowledge, this is the largest PK analysis reported to date composed exclusively of children weighing ≤12 kg who received busulfan as part of a conditioning regimen for HCT. Before our results, very little information was available regarding the PK of busulfan in young infants and small children to help determine initial doses for drug therapy. In this study, we developed a population PK model for busulfan CL using time–concentration data collected through routine therapeutic drug monitoring from multiple study centers. Plasma concentrations of busulfan were well described by a 1-compartment model with linear elimination. Our covariate analysis identified both weight and age as significant patient-specific factors impacting busulfan CL.

Investigating age-related changes resulting from physiological and enzymatic processes can make the PK modeling of drugs in infants and small children particularly challenging [5]. Precise quantification of maturation processes requires a large sample size of children with a wide range of weight and age combinations. The allometric scaling of weight provides a mechanistic and physiological-based approach that if used a priori allows for the delineation of the effect of size from other covariates that demonstrate a high degree of correlation [25]. By choosing weight as our primary covariate and by assuming an exponent of 0.75, we were able to identify the impact of maturation on busulfan CL. Other investigators may chose to estimate the exponent for the effect of weight on CL instead of fixing it to the physiological value of 0.75 [8,9,13,26]. Even though this approach may provide a good fit to the model and may result in the most parsimonious model, mechanistic interpretation of such parameters is limited. By Including both weight and age in our model accounts not only for changes in drug CL owing to body size and liver blood flow, but also for the maturation of enzymes, which is best described as a function of age. In addition, the use of body surface area has previously been suggested as a predictor of busulfan CL [10,27]. Body surface area is a derived parameter from height and weight, and as such limits the physiological interpretation of such models.

Based on our model, the predicted CL of busulfan increases by approximately 1.7-fold between 6 weeks and

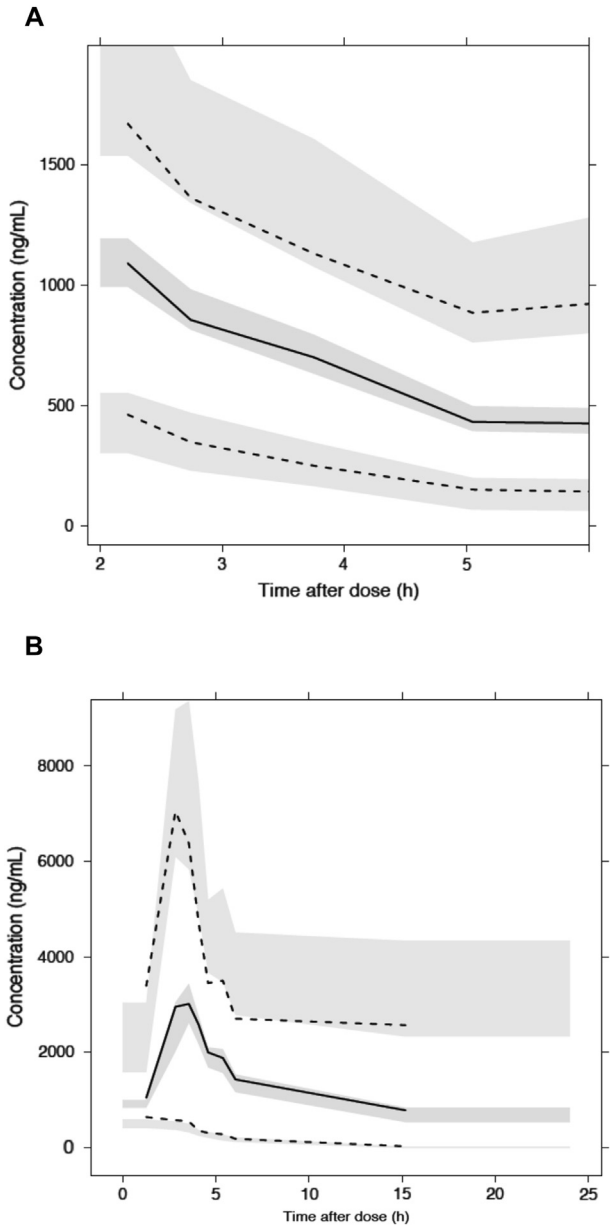

Figure 2. Visual predictive check for every 6-hour dosing (A) and once-daily administration (B). The solid line represents the data median. The upper and lower dashed lines are 97.5th and 2.5th data percentiles, respectively. The upper, middle, and lower shaded areas are simulated 97.5th percentile, median, and 2.5th percentile with uncertainty, respectively. An appropriate model fit is indicated if lines are contained within shaded areas.

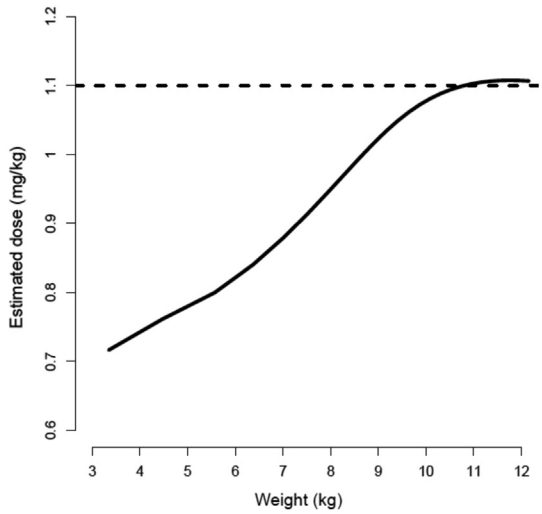

Figure 3. Model-based estimated dose (mg/kg) required to achieve a therapeutic Css of 600-900 ng/mL (solid line). For comparison, the dashed line represents conventional dosing of 1.1 mg/kg in children weighing <12 kg.

2 years of life. Busulfan undergoes extensive metabolic conversion in the liver through conjugation with glutathione by glutathione S-transferase (GST) enzymes, predominantly via GSTA1, and minor contributions from GSTM1 and GSTP1 [28,29]. The GST enzymes involved in busulfan metabolism can undergo significant changes in activity and/or expression, increasing gradually over the first 2 years of life [1,28,30]. It is plausible that the variability in PK among infants, children, and adolescents/adults is related to differences in GST activity and/or expression with age. No formal studies investigating the relationship between busulfan drug levels and the ontogeny of hepatic GSTs in the very young have been reported to date. Moreover, given that busulfan undergoes extensive metabolic conversion in the liver, it is plausible that prematurity may have a significant effect on busulfan exposure. Unfortunately, we were unable to obtain individual information on gestational age, and thus the appropriateness of our model for preterm infants is unknown, and it should be applied with caution in this population.

Strategies for the therapeutic drug monitoring of busulfan, such as Css or AUC accumulative exposure over the entire course of therapy, differ among treatment centers [7,31]. The incorporation of age and weight relationships into our model for busulfan CL ensures the same likelihood of reaching the desired therapeutic exposure in all patients, irrespective of the selected target goal. Particularly for children age <5 months, the model-based algorithm demonstrates significant improvement over conventional dosing, given that these children are more likely to experience drug concentrations above the therapeutic threshold for toxicity with standard dosing. The model-predicted doses required to achieve the therapeutic range of 600-900 ng/mL in children age <5 months are much lower than the standard dose of 1.1 mg/kg. Improved dosing strategies for busulfan in infants can be expected to reduce morbidity and mortality through improved rates of stem cell engraftment and less drug-related toxicity (eg, hepatic sinusoidal obstructive syndrome). Furthermore, infant survivors of HCT can experience

Table 4
Estimated Doses for A Typical 6-Week-Old, 3-Month-Old, and 6-Month-Old Individual to Achieve Therapeutic Exposure at Steady-State Using the Model-Based Algorithm*

Therapeutic Target (Css 600-900 ng/mL)†	Age of Individual		
	6 Weeks	3 Months	6 Months
Weight, kg	4.5	6.4	8.4
Dose, mg/kg	0.79	0.84	0.97
Dose, mg	3.6	5.4	8.1

* Based on an "average" estimate of weight per age, according to the World Health Organization growth standards for infants and children age 0-2 years.

† Doses are based on achievement of the desired therapeutic Css over a 6-hour dosing interval. An estimated Css of 750 ng/mL (range, 600-900 ng/mL) is equivalent to an AUC of 1098 uM·min (range, 900-1350 uM·min) or 4.5 mg·hr/L (range, 3.6-5.4 mg·hr/L).

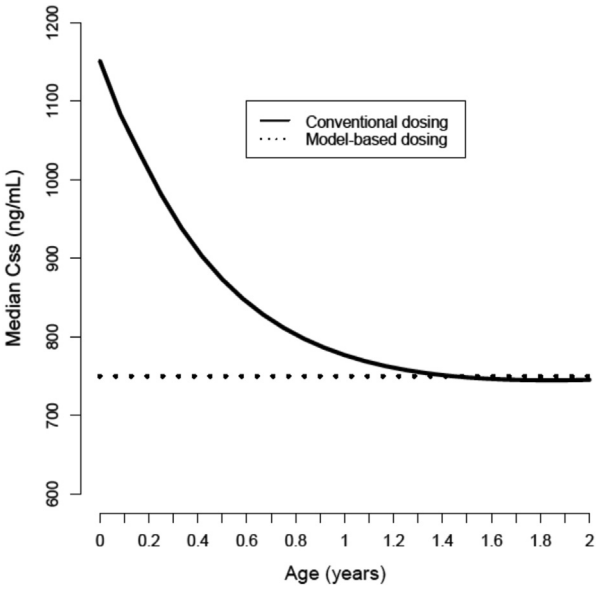

Figure 4. Plot demonstrating estimated Css with conventional dosing versus the model-based algorithm for achieving a therapeutic target of 600-900 ng/mL by age. The currently recommended dose (1.1 mg/kg) is shown for reference (horizontal dashed line).

significant life-long consequences associated with high-dose chemotherapy, including impaired pulmonary function, hypothyroidism, metabolic syndrome, and cognitive impairment [32]. Individualized busulfan therapy has the potential to minimize the risk of severe long-term complications attributable to chemotherapy in survivors of pediatric HCT and thereby improve overall quality of life. This is particularly true for patients with nonmalignant disorders in which, depending on the disease and goal for degree of stem cell chimerism, lower doses of busulfan may be feasible.

Although the PK parameters were well estimated by our final model, the between-subject variability remained modest, at approximately 25%. This finding suggests this other covariates not evaluated in this analysis also may be important determinants of busulfan CL. Physiological changes induced by specific disease states, including inborn errors of metabolism and thalassemia, have been demonstrated to alter busulfan CL [33-35]. Given the heterogeneity of diseases included in our study population, covariate analysis of different disease groups was not feasible. Similarly, the impact of medications previously shown to alter busulfan PK through the induction or inhibition of GSTs could not be adequately investigated. Information on the coadministration of the known enzyme-inducer phenytoin was not available for a majority of subjects. Drug–drug interactions among busulfan, azole antifungal agents, and metronidazole have been shown to alter busulfan CL through the inhibition of metabolic enzymes [36,37], but this could not be evaluated in the present study.

Other potential factors not explored in this study include genetic variants of genes involved in busulfan metabolism and disposition. In vitro studies have shown variants in GSTP1 result in functional alterations in activity leading to decreased enzymatic activity [38]. Clinically, the impact of several GST genetic variants on busulfan exposure has been investigated, with variable results reported [39-42]. Finally, genomic studies in children can be difficult, given the effects of ontogeny on drug-metabolizing enzymes, and require careful consideration [43].

Model validation, including prospective evaluation, is critical to ensure the predictability of any model. A large number of subjects spanning a wide range of ages is required to adequately investigate the effects of maturation on metabolic pathways. Thus, for this analysis, we elected to build the model using all data available (versus data splitting) and to perform model evaluation using a nonparametric bootstrap approach. We are currently collecting additional retrospective data, which will serve as an external validation dataset. In addition, we are in the early stages of planning a formal prospective evaluation of the busulfan algorithm in very young children undergoing HCT early in life for the treatment of SCID. This work will be presented in future reports.

In summary, we have developed a model for busulfan CL based on age and weight that can be used to determine initial doses in infants and young children weighing $\leq$12 kg. Compared with the conventional dosing guidelines, our individualized model-based algorithm may provide an improved dosing strategy for achieving targeted busulfan exposure in infants.

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