

Population Pharmacokinetics of Busulfan in Children: Increased Evidence for Body Surface Area and Allometric Body Weight Dosing of Busulfan in Children

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Abstract

Purpose: To evaluate the best method for dosing busulfan in children, we retrospectively analyzed two different data sets from three different dosing regimens by means of population pharmacokinetics using NONMEM.

Experimental Design: The development data set consisted of plasma samples from 94 children, in the age range of 0.4 to 18.8 years, receiving either oral or intravenous busulfan. The external model evaluation data set comprised 24 children, in the age range of 0.1 to 18.9 years, who belonged to the once-daily intravenous busulfan dosing regimen. A one-compartment model with first-order absorption using body surface area (BSA) or allometric body weight (BW) as covariate on clearance (CL) and BW as covariate on volume of distribution (V) were used to describe the results sufficiently. In addition to interindividual variability on all pharmacokinetic parameters, interoccasion variability was included for CL and V.

Results: CL values in the present study did not reflect the shape of the CL versus weight curve reported in previous investigations. By external model evaluation, we were able to confirm these findings. Furthermore, bioavailability was calculated to be between 93% and 99% for the development data set. On the basis of the final models, we simulated two dosing schemes according to allometric BW and BSA showing that we estimated to include about 30% more patients into the proposed therapeutic area under the curve (AUC) range of 900 to 1,500 $\mu\text{M} \cdot \text{min}$ and could, furthermore, achieve a reduction in the AUC variability when dosed according to the labeled European Medicines Agency (EMA) dosing recommendation.

Conclusion: We recommend a BSA or an allometric BW dosing regimen for individualizing busulfan therapy in children to reduce variability in busulfan exposure and to improve safety and efficacy of busulfan treatment. *Clin Cancer Res*; 17(21); 6867–77. ©2011 AACR.

Introduction

Busulfan, a DNA-alkylating agent, is used in high-dose conditioning regimens before bone marrow transplantation (BMT) in children and adults for hematologic malignancies and nonmalignancies as an alternative to total body irradiation (1). Busulfan has a very narrow therapeutic index with side effects such as sinusoidal obstruction syn-

drome (SOS) of the liver and mucositis as a result of high busulfan exposure or increased incidence of graft failure due to low exposure to busulfan (2–9). On the basis of these findings, an area under the curve (AUC) of 900 to 1,500 $\mu\text{M} \cdot \text{min}$ after the first dose has been documented to be a suitable target exposure range in adults (4, 10). Therefore, controlling the patient's AUC by therapeutic drug monitoring (TDM) became mandatory in many transplant centers, especially in oral therapy with busulfan (11).

Busulfan is administered in various combinations according to different regimens with 1 or 2 other drugs such as cyclophosphamide, thiopeta, melphalan, or fludarabine according to tumor type or the underlying hematologic disorder of the patient. These combinations can cause increased toxicity, which until now is not exactly defined in its appearance (12). Oral busulfan is administered over 4 days, every 6 hours, with a dose of 1.0 mg/kg. In most of the protocols, the intravenous formulation of busulfan is given in a similar regimen, with 80% of the oral dose being administered to achieve equivalent AUC values (13–15). The European Medicines Agency (EMA) recommendation for intravenous busulfan (Busilvex[®]) dosing is a

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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doi: 10.1158/1078-0432.CCR-11-0074

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Translational Relevance

In this article, we propose novel individualized busulfan dosing regimens to increase safety and efficacy during busulfan therapy, especially in very small children. Busulfan has a very narrow therapeutic range with higher AUC values causing a high risk of sinusoidal obstruction syndrome of the liver and mucositis as a consequence of high busulfan exposure. Lower AUC values can result in an increased incidence of graft failure due to low exposure to busulfan. The labeled EMA dosing recommendation is weight based with 5 dosing groups. In our population, we could not confirm the shape of the clearance-versus-weight relationship on which the EMA dosing recommendation is based. Two newly developed dosing regimens, tailored according to each individual's BSA or allometric BW, are expected to provide AUCs that are closer to the therapeutic target and consequently increase efficacy and safety of therapy.

weight-based regimen, with 5 dosing groups defined according to Nguyen, and includes: (i) 1.0 mg/kg for <9 kg; (ii) 1.2 mg/kg for 9 to <16 kg; (iii) 1.1 mg/kg for 16 to 23 kg; (iv) 0.95 mg/kg for >23 to 34 kg; and (v) 0.80 mg/kg for >34 kg (13, 14). In contrast, studies in children have shown that similar AUC values to those observed in adults can be achieved when dosing busulfan according to BSA (16–19).

Oral busulfan displays high interindividual variability (IOV) and interoccasion variability (IOV), especially in children and a higher total CL and, thus, lower AUC in children than in adults (20). In addition, differences in the cytochrome P-450, variability in drug absorption (4, 16), disease status (21–23), circadian rhythmicity (24), drug interactions (25–27), and hepatic function (20, 28) play a role in the high pharmacokinetic (PK) variability of busulfan (19). Busulfan is mainly metabolized by glutathione conjugation, catalyzed by glutathione S-transferase (GST), in the liver (29). It remains controversial whether GST polymorphisms influence busulfan PK (30–32). Furthermore, it needs to be examined whether the outcome and the incidence of side effects such as acute graft-versus-host disease (aGvHD) and SOS correlate with the existence of GST polymorphisms. In addition, especially when giving busulfan orally, co-medications like phenytoin, metronidazole, and azole antifungals, which are prescribed for seizure and infection prophylaxis (33), have been shown to influence busulfan clearance although they do not play a major role when busulfan is administered intravenously. Intravenous formulations of busulfan are given with more priority to reduce the high PK variability (34).

The aim of the current analysis was to evaluate whether the existing dosing recommendation for intravenous busulfan according to BW is adequate for dosing busulfan in children or if more precise dosing recommendations can be developed using population pharmacokinetics (PopPK). Of particular interest was the comparison of the AUC of a

BW-based dosing regimen, as recommended in the labeling of Busilvex[®] according to Nguyen (13), with other dosing regimens such as a BSA-based dosing regimen previously described by Vassal and colleagues (16), Shaw and colleagues (18), as well as Yeager and colleagues (17). Therefore, it remains unclear and needs further evaluation with regard to which dosing regimen for busulfan in children would be most feasible.

Materials and Methods

Patients

During this PopPK analysis, 3 different data sets were used. Two data sets, 1 from oral and 1 from intravenous busulfan administrations, were used for model development and the third data set from once-daily intravenous busulfan administrations served as external model evaluation data set.

The development data set consisted of 94 children, with a median age of 9.2 years (range 0.4–18.8 years; Table 1A), who received busulfan before bone marrow transplantation for either malignant or nonmalignant conditions. Drug monitoring was used to retrospectively observe the AUC of the plasma concentrations of busulfan. Out of the 94 children, 54 children received oral busulfan every 6 hours. The PK of busulfan in these children were recently described by Schiltmeyer and colleagues (19).

The other 40 children received intravenous busulfan as an infusion. Results from 19 children were recently described by Oechtering and colleagues (35) during a prospective, open, multicenter trial conducted at the University Hospital Münster and additional transplant units. The other 21 children receiving intravenous busulfan were included into the study through routine drug monitoring at the University Hospital Münster. All 94 children received high-dose

Table 1. Patient characteristics of the (A) development data set and (B) evaluation data set

Demographics	Median	Min	Max
A. Developmental data set			
Age, y	9.2	0.4	18.8
BW, kg	27.2	4.2	80
Height, cm	130	56	189
BSA, m ²	1.015	0.26	2
BMI, kg/m ²	16.9	11.6	31.7
BW/BSA, kg/m ²	26.8	16.2	40
B. Evaluation data set			
Age, y	2.6	0.1	18.9
BW, kg	15.1	4	66.6
Height, cm	93	51	165
BSA, m ²	0.62	0.24	1.73
BMI, kg/m ²	17.6	13.2	25.7
BW/BSA, kg/m ²	24	16.7	38.5

Table 2. Patient distribution across 5 different weight strata

Patient body weight, kg	Development data set (n)	Evaluation data set (n)
<9	5	5
9 to <16	24	11
16 to <23	13	1
23–34	16	3
>34	36	4

busulfan in combination with 1 or 2 other chemotherapeutic agents such as cyclophosphamide, melphalan, thiopeta, etoposide, or fludarabine. To prevent seizures, children were treated with short-term infusions of clonazepam or phenobarbital starting a day before the first busulfan therapy.

The evaluation data set comprised 24 children, with a median age of 2.6 years (range 0.1–18.9 years; Table 1B), who were treated with intravenous busulfan. These children were previously described by Bartelink and colleagues (36).

The patient distribution of the development and the evaluation data set comprising the 5 different dosing groups in children is shown in Table 2.

Drug administration and dosage form

The patients treated with oral busulfan received it 4 times daily every 6 hours over 4 consecutive days for a total of 16 doses. The standard oral busulfan dose was 1 mg/kg or 37.5 mg/m². Forty-one children were administered a total dose of 13 to 20 mg/kg busulfan and 7 children were given a dose of 600 mg/m². The formulations of busulfan were either prepared by the pharmacies on individual request or comprising the licensed formulation Myleran®, a 2-mg tablet, was used. The patients receiving intravenous busulfan got 80% of the standard oral dose. This was calculated to a dose of 30 mg/m² or 0.8 mg/kg. In 18 patients out of the 40 patients receiving intravenous busulfan, the first dose was given as a loading dose, giving a double dose of 1.4 to 2.0 mg/kg over 4 hours. This dose was followed 12 hours later by 15 doses of 0.7 to 1.0 mg/kg every 6 hours. The other 22 patients received 16 doses of 0.7 to 1.0 mg/kg intravenous busulfan during 2-hours infusions every 6 hours. The whole regimen of intravenous busulfan treatment was carried out over 4 consecutive days.

Patients from the evaluation data set received busulfan as once-daily infusions administered over 3 hours for 4 consecutive days. Patients older than 1 year of age were started on a first dose of 120 mg/m² and patients younger than 1 year of age received a dose of 80 mg/m² (36).

The licensed product, which was administered to all patients receiving intravenous busulfan, was Busilvex®.

Blood sampling and busulfan analysis

Plasma samples of the development data set were collected between December 1997 and April 2008. Samples

from the oral busulfan patients were collected between 1 and 8 times per administration. Overall, on average, 9 samples per patient and a total of 508 plasma samples from 250 administrations were collected. The samples were analyzed either by a modified validated high-pressure liquid chromatography with UV detector (HPLC-UV) method, based on a method reported by Blanz and colleagues (37) or by a validated liquid chromatography mass spectrometry (LC/MS) method with a limit of quantification of 5 µg/L and a linearity over the range of 5 to 2,000 µg/L (38). The HPLC-UV assay had a quantification limit of 50 µg/L and linearity could be shown over the range of 50 to 10,000 µg/L.

Samples collection after administration of intravenous busulfan, which were previously described in a study conducted by Oechtering and colleagues (35), was done from the central venous line at 3, 4 (end of infusion), 4.5, 5, 6, 8, or 12 hours after beginning the first infusion on day 1 of treatment. Optional sampling was designated before the 4th dose and 2, 3, 4, and 6 hours after starting infusion of the 13th, 14th, or 15th dose. In very small children, these samples were occasionally omitted. In the other 22 patients receiving intravenous busulfan at the University Hospital of Münster, occasional blood sampling for drug monitoring was done. In total, 348 plasma samples (mean total of 8 samples per patient) were collected from the intravenous administration group. These samples were analyzed by the validated LC/MS method as described above (38).

Plasma samples of the evaluation data set were collected during routine TDM. All patients underwent TDM before their second dose. If dose adjustments were necessary, further plasma samples were collected. This resulted in a total of 94 plasma concentration samples. All samples were measured by LC/MS method at the laboratory of the University Medical Center Utrecht (36).

All patients or their parents gave written informed consent to the blood sampling according to the respective protocols. All protocols were approved by the local ethics committee.

PopPK analysis

The plasma concentration data of the model development data set were analyzed using Nonlinear Mixed Effect Modelling with NONMEM (version VI, ICON Development Solutions; ref. 39). Determination of the best PopPK model for our population was achieved by investigating 1- and 2-compartment structure models. Parameter estimation was done using first-order conditional estimation method with interaction (FOCE-I). Parameter variability in the form of IIV and IOV were exponentially scaled on each population parameter and were assumed to be log-normally distributed for all PK parameters, except for bio-availability (F) for which a logit transformation was used to maintain all individual parameters between 0 and 1. Residual unexplained variability (RUV) was described by a proportional error model.

Candidate covariates for the PK parameters were initially selected on the basis of clinical plausibility and scatter-plots

of individual parameters versus covariates (40). The selected candidate covariates were tested for inclusion by stepwise forward inclusion and backward elimination until a change in the objective function value (OFV) as a goodness-of-fit (GOF) parameter was achieved. Different covariate relationship parameterizations were considered. Power, linear, and exponential covariate effects on PK parameters were investigated (41). For BW, traditional allometric scaling was also studied (42).

Model evaluation

Internal model evaluation during the model-finding steps included assessments of decrease in OFV, inspection of GOF plots from the basic model without inclusion of covariates and of the different covariate models, as well as graphical observation of the relative standard error of the mean by plotting the conditional weighted residuals (CWRES) over time after dose (TAD) and time. In addition, a simulation-based advanced internal evaluation was done with prediction-corrected Visual Predictive Checks (pcVPC; ref. 43). The pcVPCs were created with the Pearl-speaks-NONMEM (PsN; ref. 44) toolkit and Xpose (Version 4.2.1; ref. 45) for graphical analysis.

External model evaluation was carried out with the evaluation data set (Table 1B), which was not used during the model-development phase. The evaluation data set was applied to the final development covariate model and PK parameters of the final development model were reestimated on the basis of the evaluation data set, with the exception of F , absorption rate constant [k_a], IIV k_a , and IIV F because the evaluation data set contained no oral busulfan concentrations. In addition, simulations by means of a pcVPC were done with the evaluation data set.

Comparison of dosing strategies

On the basis of our 2 final models, we simulated new dosing regimens according to BSA or allometric BW. The 2 new dosing formulas used for the simulations were:

$$\text{Dose in milligrams} = \text{AUC}_{\text{target}} \times \text{CL}_{\text{pop}} \times \text{BSA}_{\text{individual}} \quad (\text{A})$$

$$\begin{aligned} \text{Dose in milligrams} = & \text{AUC}_{\text{target}} \times \text{CL}_{\text{pop}} \\ & \times (\text{BW}_{\text{individual}})^{0.75} \end{aligned} \quad (\text{B})$$

with $\text{AUC}_{\text{target}}$ as desired AUC, CL_{pop} as the population value for CL from either the BSA or the allometric BW model, and either the individual BSA or the individual allometric BW of the patient.

We first set the target AUC ($\text{AUC}_{\text{target}}$) to 1,125 $\mu\text{M} \cdot \text{min}$ as proposed in the literature (13) and simulated individual steady-state AUCs, CL values, and busulfan concentrations for our simulation data set, which comprised 120 individuals from our development and evaluation data set. The distribution of the BW and BSA were kept the same as in the original data set. The same procedure was repeated for an $\text{AUC}_{\text{target}}$ of 1,150 $\mu\text{M} \cdot \text{min}$ to test whether the number

of individuals reaching the therapeutic AUC range would possibly increase. We further simulated 5 weight-strata dosing according to the EMA dosing recommendation and a dosing of 0.8 mg/kg for each individual as this was the dose that was mainly administered to the patients in our data set.

The median-simulated AUCs and the percentage of patients within the AUC range of 900 to 1,500 $\mu\text{M} \cdot \text{min}$ were compared and evaluated. Results favored the dosing regimen causing the lowest variability in AUC values and having a highest percentage of patients within the therapeutic AUC range of 900 to 1,500 $\mu\text{M} \cdot \text{min}$. Furthermore, a TDM scenario based on a single plasma sample taken 0.5 or 8 hours after the end of the first infusion was investigated by simulation and reestimation with the final model and the 2 suggested dosing algorithms.

Results

Patients

During routine drug monitoring, we assessed the busulfan kinetics of 94 children included in the development data set. Of these, 54 patients received oral busulfan and 40 patients received intravenous busulfan. According to the BW/BSA index (Table 1A), our population does not show a linear relationship between BW and BSA. All 94 patients underwent the drug monitoring during different myeloablative regimens either at the University Hospital Münster or other transplant units.

The busulfan kinetics of the evaluation data set, consisting of 24 children receiving once-daily intravenous busulfan, was assessed during routine TDM done for dose adjustments at the University Medical Center Utrecht. All patient characteristics are described in Table 1.

PopPK analysis

A one-compartment model with first-order absorption was found to adequately describe the busulfan kinetics of the development data set. In addition to IIV on all PK parameters (CL, V , k_a , and F), IOV was included for CL and V . RUV of the model was best described through a proportional error model with similar magnitude for both the oral and intravenous data. BSA, BW, and age were identified as candidate covariates for effects on CL, V , and F by scatter-plots (40). V was found to be linearly related to BW. A slope and intercept model was superior to a strictly allometric model (e.g., V estimated in L/kg).

BSA and allometric BW were both good predictors of CL and both were superior to a linear relationship between BW and CL. In the allometric model, CL was modeled as a function of BW raised to the power of $3/4$ (42,46). CL and V values for the development data set are: BSA model: CL 4.2 L/h/m²; V 18.4 L/kg¹; allometric BW model: CL 4.1 L/h/kg^{0.75}; V 18.3 L/kg¹. Evaluation of the accuracy of the scaling factor 0.75 in the allometric model was done by estimating the allometric factor on BW. The parameter was estimated to be 0.754, confirming the $3/4$ power law to be a good predictor of body function for our patient population.

Scatter-plots of individual parameters from the base model suggested a possible increase in F with increasing age. However, in the final models, no tendency towards an age-dependent effect on F could be detected. In all our models, the estimated value of F was between 93% and 99%, and not around 80% as reported by Leger and colleagues (15). Removing the logit transformation on F did not result in a relevant change in the typical value of F or an improved fit to the data. All PopPK parameters from the different models are shown in Supplementary Table S1.

A distinct difference can be seen with the base model when plotting CL per BW as if dosed according to the EMA dosing recommendation, per allometric BW or BSA in different weight strata (Fig. 1). CL values did not reflect the shape of the CL-versus-weight curve reported in previous investigations (14). Instead, our analysis shows a 22%-higher CL for children of less than 9 kg of BW and lower CL values (range 33% to 58%) for children of more than 9 kg of BW (Fig. 1A). Comparing the CL per

allometric BW (Fig. 1C) or BSA (Fig. 1D), for all 5 weight strata, revealed no differences in the scaled CL among the 5 weight groups.

Model evaluation

GOF plots were used to compare the fit of the model. Best correlation for our final model was found with allometric BW or BSA as covariate on CL and BW as covariate on V (Fig. 2). The CWRES are plotted versus TAD (Supplementary Fig. S1A and S1B). The residuals are regularly spread around the line of identity without any obvious indication of a model misspecification.

The pcVPCs for the development models indicate a slight underprediction for the median busulfan observations that is not observed for the 5th and 95th percentiles (Supplementary Fig. S2A). By conducting 2 separate pcVPCs, one for the oral data and one for the intravenous data, the underpredictions were only observed within the oral busulfan data (Supplementary Fig. S2B and S2C). These

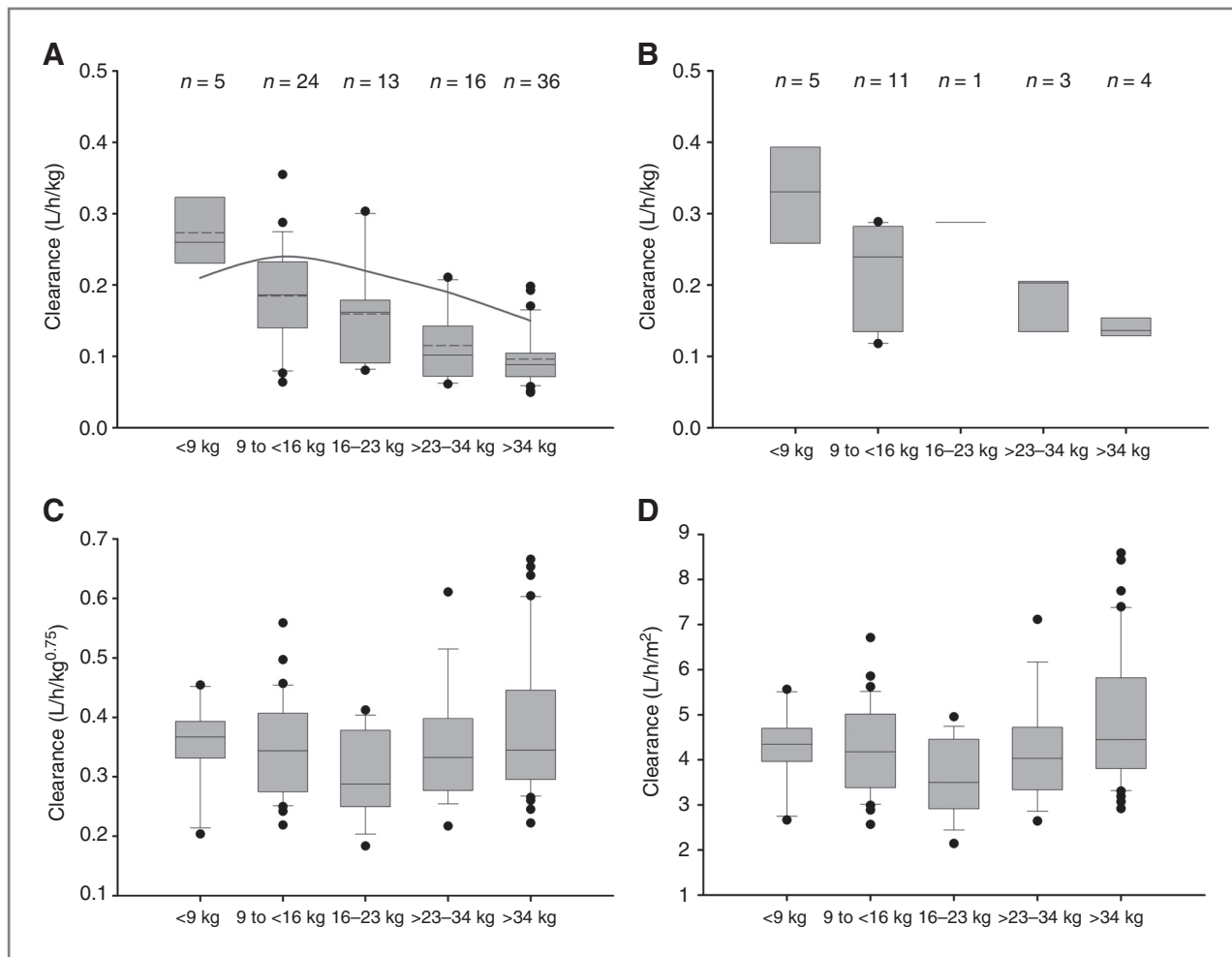


Figure 1. Individual clearance estimates for different weight strata. A, per body weight for the development data set; dashed black lines, mean clearance values and black solid curve, mean clearance values from a previous investigation (14). B, per body weight for the evaluation data set. C, per allometric body weight. D, per BSA; in (C and D), data from the development and the evaluation data set were used. Box plots show the median, 10th, 25th, and 90th percentiles as vertical boxes with error bars.

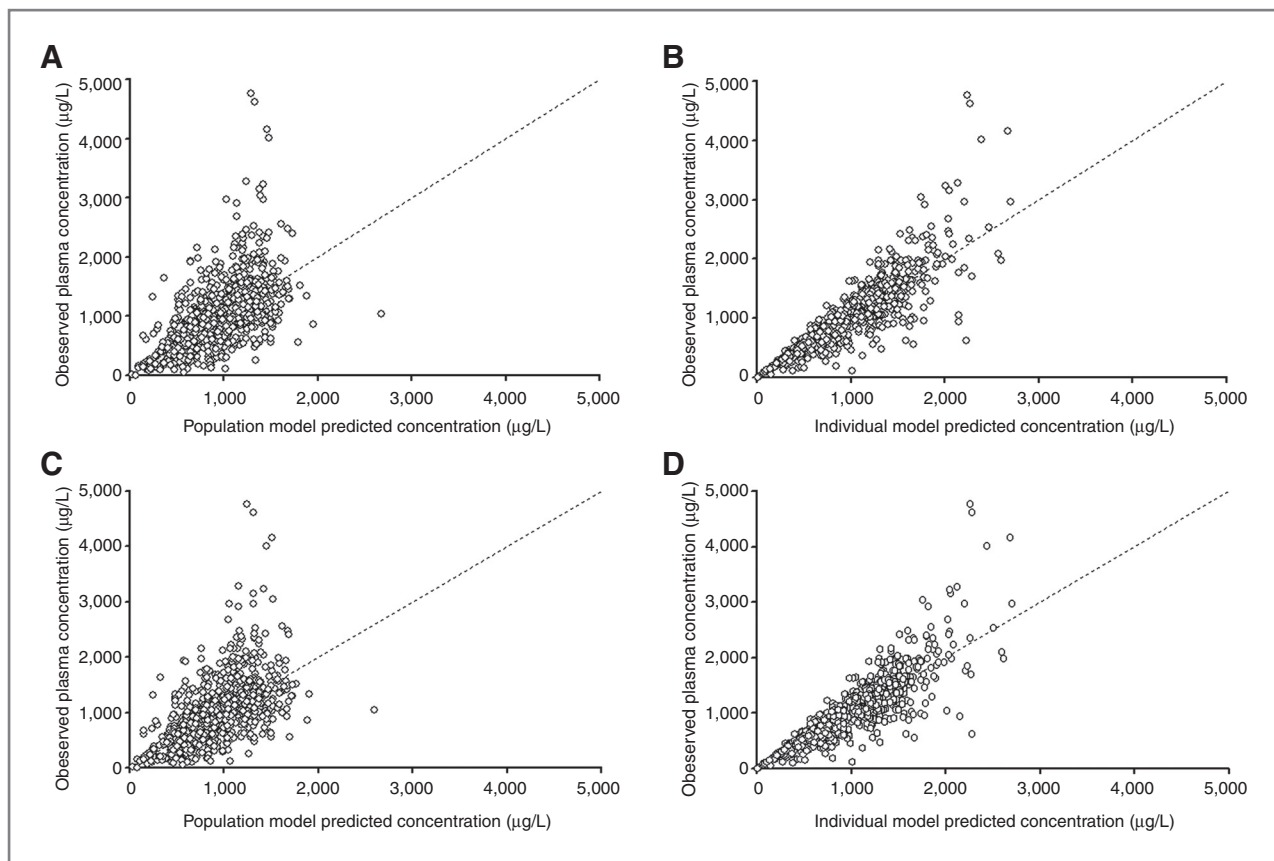


Figure 2. Goodness-of-fit plots. A and B, development data set with allometric body weight as covariate on CL. C and D, development data set with BSA as covariate on CL. For each model, the observed plasma concentrations are plotted against the population model-predicted concentrations and against the individual model-predicted concentrations. The line of identity is shown.

findings could be confirmed by pcVPCs of the evaluation data set, which consisted of only intravenous data and did not show any underprediction of the median busulfan observations (Supplementary Fig. S2D). As the underprediction was not very pronounced and did not affect the predictions for the intravenous busulfan data, we decided that both final models were valid for predicting busulfan PK after intravenous administration.

The evaluation data set was further used for external model evaluation by estimating the PK parameters for the evaluation data set on the basis of the developed PopPK models. No distinct discrepancies are seen between the fixed effects of the 2 data sets (CL and V values for the evaluation data set: BSA model: CL 5.0 L/h/m²; V 15.2 L/kg¹; allometric BW model: CL 4.8 L/h/kg^{0.75}; V 15.2 L/kg¹). Assessing the GOF plots for the evaluation data set (Supplementary Fig. S3), a regular distribution could be observed around the line of identity that did not indicate a distinct bias or show discrepancies towards the development data set. These results confirmed our previous findings through external model evaluation and simulation. In addition, we could confirm our findings with regard to the busulfan CL-versus-weight relationship using the evaluation data set (Fig. 1B). Thus, by this external model

evaluation, we can confirm our findings that, in the pediatric population, BSA and allometric BW are good predictors for CL and should be considered for dose adjustments.

New dosage suggestions

According to our 2 final models, we simulated AUC and CL values according to BSA or allometric BW by implementing 2 new dosing formulas [Equations (A) and (B)].

Comparing the median simulated AUCs and the percentage of patients within the AUC range of 900 to 1,500 µM*min, best results were achieved with the new allometric BW and the new BSA dosing regimen. With these 2 new dosing regimens, we were able to have 70% to 71% of the simulated patients within the therapeutic AUC range of 900 to 1,500 µM*min when the AUC_{target} was set to 1,125 µM*min (Table 3). In contrast, with the EMA dosing regimen only 44%, and with a dosing of 0.8 mg/kg for each individual only 59%, were within the therapeutic AUC range (Table 3). More patients were found to be below the therapeutic AUC range of 900 to 1,500 µM*min than above (Table 3). Further simulations with an AUC_{target} of 1,150 µM*min did not increase the percentage of patients being within the therapeutic AUC range of 900 to 1,500 µM*min but resulted in an even distribution of

Table 3. Comparison of different dosing regimens

	Dosing in data set	EMA dosing	Allometric BW dosing	BSA dosing	Allometric BW Dosing	BSA Dosing
Target AUC, $\mu\text{M} \cdot \text{min}$	1,125	1,125	1,125	1,125	1,150	1,150
Median simulated AUC, $\mu\text{M} \cdot \text{min}$	1,248	1,533	1,127	1,110	1,152	1,134
Root mean square error	428	610	283	283	289	289
% Patients within AUC range 900–1,500 $\mu\text{M} \cdot \text{min}$	59	44	71	70	72	70
% Patients with an AUC <900 $\mu\text{M} \cdot \text{min}$	15	3	17	20	15	17
% Patients with an AUC >1,500 $\mu\text{M} \cdot \text{min}$	26	53	12	10	13	13
Dosing regimen	0.8 mg/kg	5 dosing regimen	$\text{TAUC} \times 4.11 \times \text{BW}^{0.75}$	$\text{TAUC} \times 4.16 \times \text{BSA}$	$\text{TAUC} \times 4.11 \times \text{BW}^{0.75}$	$\text{TAUC} \times 4.16 \times \text{BSA}$

NOTE: Root mean square error is calculated by the deviation in comparison to the target AUC.
Abbreviation: TAUC, target area under the curve.

patients being outside the therapeutic AUC range (Table 3). Therefore, we compared the median simulated AUCs of the simulations with the $\text{AUC}_{\text{target}}$ set to 1,150 $\mu\text{M} \cdot \text{min}$ with the simulated AUCs of the EMA regimen and the simulated AUCs with a 0.8 mg/kg dosing. Compared with the EMA dosing regimen, we achieved lower median AUCs and decreased variability in AUC values with both our new dosing regimens (Fig. 3A). Comparing the AUC values by weight strata according to the EMA dosing regimen resulted in higher median AUC values than 1,500 $\mu\text{M} \cdot \text{min}$ in children with more than 9 kg of BW (Fig. 3B). In contrast, a higher percentage of patients (69% to 73%) within the therapeutic AUC range is seen using our new dosing regimens (Supplementary Fig. S4). Therefore, we recommend 2 new dosing regimens for dosing busulfan according to the following formulas:

BSA dosing regimen:

$$\begin{aligned} \text{Dose in milligrams} &= 4.72 \text{ mg/h/L} \times 4.16 \text{ L/h/m}^2 \\ &\times \text{BSA m}^2 = 19.6 \text{ mg/m}^2 \quad (\text{C}) \\ &\times \text{BSA m}^2 \end{aligned}$$

Allometric BW dosing regimen:

$$\begin{aligned} \text{Dose in milligrams} &= 4.72 \text{ mg/h/L} \times 4.11 \text{ L/h/kg}^{0.75} \\ &\times (\text{BW}/27.2) \text{kg}^{0.75} \\ &= 19.4 \text{ mg/kg}^{0.75} \quad (\text{D}) \\ &\times (\text{BW}/27.2) \text{kg}^{0.75} \end{aligned}$$

In Equations (C) and (D), the $\text{AUC}_{\text{target}}$ is 1,150 $\mu\text{M} \cdot \text{min}$ and is equal to 4.72 mg/h/L.

A TDM scenario was investigated by simulation and reestimation with the final model and the 2 recommended

dosing algorithms. Estimates of individual CL were made on the basis of a single plasma sample taken 0.5 or 8 hours after the end of the first infusion. The estimated CL was used to adjust the dose according to the new formulas [Equations (C) and (D)]. This resulted in a higher percentage of subjects within the therapeutic AUC range of 900 to 1,500 $\mu\text{M} \cdot \text{min}$ as seen in comparison with only the *a priori* dose (75%–81% patients; Table 4). Furthermore, to explore the maximum theoretical benefit with TDM, another simulation was carried out assuming that the true CL for each individual could be assessed during the first treatment cycle. Adjusting the dose according to these individual CL values resulted in 90% of the patients being comprised within the desired AUC range.

Discussion

This busulfan PopPK analysis was conducted for comparison of different novel dosing regimens with the recommended BW-based dosing regimen in accordance with the EMA recommendations. Earlier, BSA dosing regimens were described, with Vassal and colleagues (16) proposing a total dose of 600 mg/m², Shaw and colleagues (18) reporting a single dose of 150 mg/m²/d, and Yeager and colleagues (17) recommending a dose of 4 × 38.9 mg/m² as being more appropriate when dosing busulfan in children. Busulfan has a very narrow therapeutic range. Achieving higher AUC values than 1,500 $\mu\text{M} \cdot \text{min}$ can cause greater toxicity resulting in SOS or mucositis whereas AUC values less than 900 $\mu\text{M} \cdot \text{min}$ can lead to increased incidence of graft failure (2–5,9,10). In view of these severe side effects of busulfan seen in adults, a therapeutic AUC of 900 to 1,500 $\mu\text{M} \cdot \text{min}$ has been defined as valid for dosing intravenous busulfan. In children, the therapeutic AUC range has not yet been defined. Some studies have shown graft rejections with an AUC < 900 $\mu\text{M} \cdot \text{min}$ in children, but no studies have found a threshold limit for toxicities in

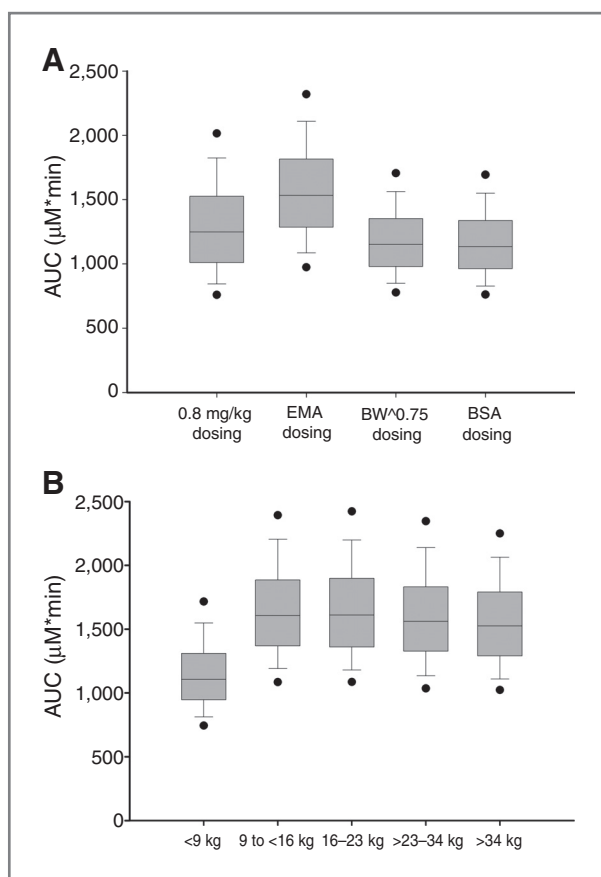


Figure 3. A, AUC simulations for the different dosing regimens with an AUC_{target} of $1,150 \mu M \cdot min$. B, AUC simulations for the EMA dosing regimen shown over the different weight strata; plots the 10th, 25th, 50th (median), 75th, and 90th percentiles as vertical boxes with error bars.

children as yet (6–8). Therefore, until that time when further studies define a different threshold limit in children, an AUC range of 900 to $1,500 \mu M \cdot min$, based on the findings in adults, will be used in children (13).

In this PopPK analysis, data from 94 children who received either oral or intravenous busulfan were collected. Using one-compartment model with first-order absorption, we were able to describe the PK of our population sufficiently. By using BSA or allometric BW as a covariate on CL and BW as covariate linear on V , the best results were obtained. No difference in PK parameter estimates or OFV could be observed between these 2 models. In both models, IIV is exponentially scaled on CL , V , k_{av} , and F and IOV is included for CL and V . All population estimates are shown in Supplementary Table S1.

By using an allometric scaling model, it is assumed that the value of 0.75 as a power function on BW is a good predictor of body size, physiologic functions, and maturation effects on CL (42, 46, 47). Estimating the allometric exponent for our data set resulted in a value of 0.754 and thus confirmed the $\frac{3}{4}$ power function. As we could not see any difference between the allometric BW and the BSA model, we decided that both models were valid for describing our development data set. This close agreement between a BSA model and an allometric $\frac{3}{4}$ power model, when used for scaling of metabolic processes such as CL , has already been described by Anderson and Holford (47). Therefore, allometric BW is recommended as the preferred choice together with BSA dosing for busulfan dosing in children. Instead, on a linear weight scale, CL is shown to be larger in young children than adults and would, therefore, not be recommended for drug dosing in children. This theory was confirmed in our results and is seen when comparing the CL values shown per BW between the different weight strata as no assumed hyperbolic shape is seen (Fig. 1A). In addition, the higher CL in children of less than 9 kg of BW compared with CL values reported by Vassal and colleagues (14) might be due to the combination of oral and intravenous administrations in the data set as 2 of the 5 children in this weight group received oral busulfan, which is known to have a higher CL in children (20). The difference in CL seen in children of more than 9 kg of BW is in line with the overexposure seen in AUC values by simulating the EMA

Table 4. TDM dosing

	Allometric BW dosing		BSA dosing	
	0.5 h	8 h	0.5 h	8 h
Time of TDM samples after end of first infusion (infusion time, 4 h)	0.5 h	8 h	0.5 h	8 h
Target AUC, $\mu M \cdot min$	1,150	1,150	1,150	1,150
Median simulated AUC, $\mu M \cdot min$	1,204	1,164	1,195	1,160
Root mean square error	287	245	291	246
% Patients within AUC range 900– $1,500 \mu M \cdot min$	75	81	75	80
% Patients with an AUC $<900 \mu M \cdot min$	8	9	9	9
% Patients with an AUC $>1,500 \mu M \cdot min$	17	10	16	11
Dosing regimen	$TAUC \times 4.11 \times BW^{0.75}$		$TAUC \times 4.16 \times BSA$	

NOTE: Root mean square error is calculated by the deviation in comparison to the target AUC. Abbreviation: TAUC, target area under the curve.

dosing regimen (Fig. 3B). In addition, a recent study evaluating the EMA dosing recommendation for Busilvex[®] published by Michel and colleagues (48) found a high PK variability in the studied children. Of 67 children, 11 children developed overexposure (AUC > 1,500 $\mu\text{M}\cdot\text{min}$) and 4 children underexposure (AUC < 900 $\mu\text{M}\cdot\text{min}$).

A second finding which differed from earlier studies was the high F value. In all our models, F was calculated to be between 93% and 99%. The adult intravenous busulfan dose of 0.8 mg/kg was defined on the basis of an F value of 80% and should yield in the same AUC as achieved with the oral dose of 1.0 mg/kg busulfan (15). Hassan and colleagues (20) observed a mean F of 80% for adults and 68% for children and reported a high variability in F of about 6-fold in children and 2-fold in adults. In this investigation, only 8 children and 8 adults were included. The F value predicted by Hassan and colleagues is based on a single busulfan dose of just 2 mg busulfan given as a bolus injection. Plasma samples were only drawn until 10 hours after administration of oral busulfan. These issues could be reasons for an underestimation of F and could possibly explain the high variability seen in F. In contrast, F is calculated on the basis of the data of several busulfan administrations of high-dose therapy in a greater number of patients, with plasma samples drawn up to 18 hours after the last dose in our investigation. From our data, we assume F to be almost 100% and recommend disregarding of F for dosing busulfan.

These CL and F findings led to simulations of the 2 dosing regimens developed according to BSA and allometric BW based on our final models. If administered according to one of these dosing regimens, doses for children of less than 9 kg of BW will be in line with the EMA dose recommendation whereas children of more than 9 kg of BW would receive lower doses compared with the EMA dosing recommendation, resulting in lower AUC values (Supplementary Fig. S4). Decreasing doses in children of more than 9 kg of BW is of interest due to the high AUC values found with the EMA dosing simulations (Fig. 3B). Furthermore, as described above (Table 3), we estimated to include about 30% more patients into the therapeutic AUC range of 900 to 1,500 $\mu\text{M}\cdot\text{min}$ than with the EMA dosing regimen without using TDM for dose adjustments. However, our findings from the simulation experiments need to be confirmed by PK investigations in patients before generally applying the new dosing recommendations.

With the simulated TDM scenario based on 1 blood sample drawn 0.5 or 8 hours after the end of the first infusion and dose adjustment according to the new formulas, we were able to include more patients into the therapeutic AUC range of 900 to 1,500 $\mu\text{M}\cdot\text{min}$. As shown in Table 4, TDM dosing according to a later plasma sample after the end of infusion, such as 8 hours compared with 0.5 hours, resulted in a greater number of patients (8 hours: 81% patients vs. 0.5 hour: 75% patients) within the therapeutic AUC range of 900 to 1,500 $\mu\text{M}\cdot\text{min}$. By simulating a TDM scenario as if we were able to assess the true CL for each individual during the first treatment cycle and adjusting the

dose according to these individual CL values, we estimated to include 90% of the patients within the desired AUC range. This maximum theoretical benefit with TDM is limited by the magnitude of IOV in CL (49). By reduction of the variability in AUC, our results indicate that TDM based on a PopPK model could improve treatment outcome with busulfan in pediatric patients, but that a single plasma sample soon after end of infusion does not supply sufficient information. Bleyzac suggested using PopPK models and Bayesian individualization of dose regimens for busulfan based on 2 plasma samples when busulfan is given intravenously and based on 3 plasma samples for oral busulfan (50). The newly suggested dosing regimens for *a priori* and TDM dose adjustments based on allometric body weight or BSA appear to be superior to the regimen suggested by the EMA for dosing busulfan in pediatric oncology.

The CL estimated from our models are significantly lower than what has previously been published (13, 14, 19, 35); therefore, the simulation-based dosing regimens would result in lower doses than those recommended by the EMA. However, according to the simulations, the proposed dosing algorithm as shown in Tables 3 and 4 would have significantly improved the treatment outcome for the population studied. Before these dosing recommendations are generally applied, attention must be paid to understanding the differences in CL between our and previous studies. Furthermore, it must be pointed out that no adults and no patients heavier than 80 kg were included. Thus, we cannot extrapolate these results to patient groups weighing more than 80 kg.

In conclusion and according to our calculations, the labeling of Busilvex[®] (EMA) might be redeveloped for dosing intravenous busulfan in children in order to optimize the dose intensity. We would recommend using either the presented BSA-based dosing regimen or the allometric BW dosing regimen for individualized treatment of busulfan in children. As BSA dosing is most often established by clinicians and pharmacists for dose adjustment in pediatric oncology, the presented BSA-based dosing regimen may be of more clinical appropriateness than the allometric BW dosing regimen. However, before recommending specific treatment algorithms according to either of the 2 presented dosing regimens, these findings need to be confirmed in further studies in children and adults.

Disclosure of Potential Conflicts of Interest

Along with the principal study investigators, for example, M.N. Trame, J. Boos, and C. Hempel who act as the guarantors, all other investigators, namely, M. Bergstrand and M.O. Karlsson had full access to the data and took part in the design, execution and data analysis, and in writing the report. None of the authors listed have financial or personal relationships with other people or organizations that inappropriately influence their actions. No potential conflicts of interest were disclosed by other authors.

Acknowledgments

The authors thank the help of Drs. T. Linden, C. Chen, and the other medical colleagues involved in collecting plasma samples in Münster. The authors would also like to thank Drs. I. Bartelink and Jaap J. Boelens from the Departments of Hematology, Immunology, and Clinical Pharmacy at the

University Medical Center Utrecht, The Netherlands, for providing the external data set. In addition, the authors would like to thank the Uppsala Pharmacometrics Research Group for their helpful suggestions.

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Received January 12, 2011; revised August 11, 2011; September 6, 2011; published online November 1, 2011.

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Population Pharmacokinetics of Busulfan in Children: Increased Evidence for Body Surface Area and Allometric Body Weight Dosing of Busulfan in Children

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Clin Cancer Res 2011;17:6867-6877. Published OnlineFirst September 14, 2011.

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