ORIGINAL ARTICLE

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

Running title: Population Pharmacokinetics of Busulfan in Children

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Statement of Translational Relevance

In this article we propose new individualized busulfan dosing regimens, in order 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 sinuosoidal obstruction syndrome of the liver and mucositis as a result 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 five 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 on. Two newly developed dosing regimens according to each individual’s body surface area or allometric body weight are expected to provide AUCs closer to the therapeutic target consequently increase efficacy and safety of therapy.
Abstract

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

Experimental Design: The development dataset consisted of plasma samples from 94 children, aged 0.4-18.8 years, receiving either oral or intravenous busulfan. The external model evaluation dataset included 24 children, aged 0.1-18.9 years, from 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] described the results sufficiently. Apart from interindividual variability on all pharmacokinetic parameters interoccasion variability was included for CL and V.

Results: CL values 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. Further, bioavailability was calculated to be 93-99% for the development dataset. Based on the final models we simulated two dosing schemes according to allometric BW and BSA showing that we estimated to get about 30% more patients into the proposed therapeutic area under the curve [AUC] range of 900-1500 µM*min and could further achieve a decrease in the AUC variability as when dosed according to the labeled EMA dosing recommendation.

Conclusion: We suggest a BSA or an allometric BW dosing regimen for individualizing busulfan therapy in children to reduce variability in busulfan exposure and in order to improve safety and efficacy of busulfan treatment.
Introduction

Busulfan, a DNA-alkylating agent, is used in high-dose conditioning regimens prior to bone marrow transplantation [BMT] in children and adults for hematological malignancies and non-malignancies as an alternative to total body irradiation [1]. Busulfan has a very narrow therapeutic index with side effects such as sinusoidal obstruction syndrome [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]. Based on these findings an AUC of 900-1500 µM*min after the 1st dose is documented to be a suitable target exposure range in adults [4,10]. Therefore, controlling patient’s AUC by therapeutic drug monitoring [TDM] became mandatory in many transplant centers especially within oral therapy of busulfan [11].

Busulfan is given in various combinations according to different regimens with one or two other drugs such as cyclophosphamide, thiotepa, melphalan or fludarabine according to tumor type or the underlying hematologic disorder of the patient. These combinations can result in increased toxicity which until now is not exactly defined in its appearance [12]. Oral busulfan is administered over 4 days every 6 h with a dose of 1.0 mg/kg. In most of the protocols, the intravenous [IV] formulation of busulfan is given in a similar regimen with 80% of the oral dose administered to achieve equivalent AUC values [13-15]. The European Medicines Agency [EMA] recommendation for IV busulfan [Busilvex®] dosing is a weight-based regimen with five dosing groups defined according to Nguyen as follows: 1.0 mg/kg for <9 kg; 1.2 mg/kg for 9 to <16 kg; 1.1 mg/kg for 16-23 kg; 0.95 mg/kg for >23-34 kg; 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 [IIV] and interoccasion variability [IOV] especially in children as well as 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 the glutathione S-transferase [GST] in the liver [29]. It is still controversially discussed if GST polymorphisms influence the busulfan PK [30-32]. Further it needs to be examined if 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, comediations like phenytoin, metronidazole and
azole antifungals which are given for seizure and infection prophylaxis [33] have shown to influence the busulfan clearance whereas they are not playing a major role when busulfan is administered IV. IV 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 IV 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 area under the curve [AUC] of a BW based dosing regimen as recommended in the labelling of Busilvex® according to Nguyen [13] with other dosing regimens such as a BSA based dosing regimen described earlier by Vassal et al. [16], Shaw et al. [18] and Yeager et al. [17]. Therefore, it remains unclear and needs further evaluation which dosing regimen for busulfan in children would be most feasible.

Materials and Methods

Patients

During this PopPK analysis three different datasets were used. Two datasets, one from oral and one from IV busulfan administrations, were used for model development and the third dataset from once-daily IV busulfan administrations served as external model evaluation dataset.

The development dataset consisted of 94 children [table 1A] receiving busulfan prior to bone marrow transplantation for either malignant or non-malignant conditions with a median age of 9.2 years [range 0.4 – 18.8 years]. 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 h. The PK of busulfan in these children were recently described by Schiltmeyer et al. [19].

The other 40 children received IV busulfan as an infusion. 19 children were recently described by Oechtering et al. [35] during a prospective, open, multicenter trial at the University Hospital Münster and additional transplant units. The other 21 children receiving IV busulfan were collected through routine drug monitoring at the University Hospital Münster. All 94 children received high-dose busulfan in combination with one or two other chemotherapeutic agents such as cyclophosphamide, melphalan, thiotepa, etoposide or fludarabine. To prevent seizures, children were treated with short-term infusions of clonazepam or phenobarbital starting the day before the first busulfan therapy.
The evaluation dataset consisted of 24 children [table 1B] with a median age of 2.6 years [range 0.1 – 18.9 years] who were treated with IV busulfan. These children were previously described by Bartelink et al. [36].

The patient distribution of the development and the evaluation dataset over the five different dosing groups in children is shown in table 1C.

**Drug administration and dosage form**

The patients treated with oral busulfan received it four times daily every 6 h over 4 consecutive days for a total of 16 doses. The standard oral busulfan dose was 1 mg/kg or 37.5 mg/m². 41 children were administered a total dose of 13-20 mg/kg busulfan and 7 children a dose of 600 mg/m². The formulations of busulfan were either prepared by the pharmacies on individual request or the licensed formulation Myleran®, a 2 mg tablet, was used. The patients receiving IV busulfan got 80% of the standard oral dose. This is calculated to a dose of 30 mg/m² or 0.8 mg/kg. In 18 patients out of the 40 patients receiving IV busulfan the first dose was given as a loading dose, giving a double dose of 1.4-2.0 mg/kg over 4 h. This dose was followed 12 h later by 15 doses of 0.7-1.0 mg/kg every 6 h. The other 22 patients received 16 doses of 0.7-1.0 mg/kg IV busulfan over 2 h infusions every 6 h. The whole regimen of IV busulfan treatment includes 4 consecutive days.

Patients from the evaluation dataset received busulfan as once-daily infusions over 3 h for 4 consecutive days. Patients > 1 year of age were started at a first dose of 120 mg/m² and patients < 1 year of age at a dose of 80 mg/m² [36]. The licensed product which was administered to all patients receiving IV busulfan was Busilvex®.

**Blood sampling and busulfan analysis**

Plasma samples of the development dataset were collected between December 1997 and April 2008. Samples from the oral busulfan patients were collected one to eight 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 HPLC-UV method based on a method reported by Blanz et al. [37] or by a validated LC-MS method with a limit of quantification of 5 µg/L and a linearity over the range of 5-2,000 µg/L [38]. The HPLC-UV assay had a quantification limit of 50 µg/L and linearity could be shown over the range of 50-10,000 µg/L.
Samples after administration of IV busulfan which were previously described in a study conducted by Oechtering et al. [35] were collected from the central venous line at 3, 4 (end of infusion), 4.5, 5, 6, 8 or 12 h after beginning the first infusion at day 1 of treatment. Optional sampling was designated prior to the 4th dose and 2, 3, 4 and 6 h 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 IV 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 IV administration. These samples were analyzed by the validated LC-MS method as described above [38].

Plasma samples of the evaluation dataset were collected during routine therapeutic drug monitoring [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 lab 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 ethic committee.

**PopPK analysis**

The plasma concentration data of the model development dataset were analyzed using Nonlinear Mixed Effect Modelling with NONMEM [version VI, ICON Development Solutions, Ellicott City, MD, USA] [39]. Determination of the best PopPK model for our population was achieved by investigating one and two compartment structure models. Parameter estimation was performed with first-order conditional estimation method with interaction [FOCE-I]. Parameter variability in 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 bioavailability [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 based on 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 performed with prediction corrected Visual Predictive Checks [pcVPC] [43]. The pcVPCs were created with the Pearl-speaks-NONMEM [PsN] [44] toolkit and Xpose [Version 4.2.1] [45] for graphical analysis.

External model evaluation was carried out with the evaluation dataset [table 1 b] which was not used during the model development phase. The evaluation dataset was applied to the final development covariate model and PK parameters of the final development model were re-estimated on the basis of the evaluation dataset except of F, absorption rate constant [ka], IIV ka and IIV F as the evaluation dataset contained no oral busulfan concentrations. In addition, simulations by means of a pcVPC were performed with the evaluation dataset.

Comparison of dosing strategies
According to our two final models we simulated new dosing regimens according to BSA or allometric BW. The two new dosing formulas used for the simulations were

\[
\text{Dose in mg} = \text{AUC}_{\text{target}} \times \text{CL}_{\text{pop}} \times \text{BSA}_{\text{individual}} \quad [A]
\]

\[
\text{Dose in mg} = \text{AUC}_{\text{target}} \times \text{CL}_{\text{pop}} \times (\text{BW}_{\text{individual}})^{0.75} \quad [B]
\]

with AUC\text{target} as desired AUC, CL\text{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 [AUC\text{target}] to 1125 µM*min as proposed in the literature [13] and simulated individual steady state AUCs, CL values and busulfan concentrations for our simulation dataset consisting of 120 individuals from our development and evaluation dataset. The distribution of the BW and BSA were kept the same as in the original dataset.
The same was repeated for an AUC<sub>target</sub> of 1150 µM*min for testing if the number of individuals reaching the therapeutic AUC range would possibly increase. We further simulated a five weight strata dosing according to the EMA dosing recommendation and a dosing of 0.8 mg/kg for each individual as this dose was mainly administered to the patients in our dataset.

The median simulated AUCs and the percentage of patients within the AUC range of 900-1500 µM*min were compared and evaluated. Favored was the dosing regimen causing the lowest variability in AUC values and having a highest percentage of patients within the therapeutic AUC range of 900-1500 µM*min. Further, a TDM scenario based on a single plasma sample taken 0.5 h or 8 h after the end of the first infusion was investigated by simulation and re-estimation with the final model and the two suggested dosing algorithms.

**Results**

**Patients**

During routine drug monitoring we assessed the busulfan kinetics of 94 children included in the development dataset. Of these, 54 patients received oral busulfan and 40 patients IV 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 dataset consisting of 24 children receiving once-daily IV busulfan, were assessed during routine TDM performed 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 dataset. Apart from IIV on all PK parameters [CL, V, k<sub>a</sub> 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 IV 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 superior to a linear relationship between BW and CL. In the allometric model CL was modelled as a function of
BW raised to the power of $\frac{3}{4}$ [42,46]. CL and V values for the development dataset are: BSA model: CL 4.2 L h$^{-1}$ m$^{-2}$, V 18.4 L kg$^{-1}$; allometric BW model: CL 4.1 L h$^{-1}$ kg$^{-0.75}$, V 18.3 L kg$^{-1}$. 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 $\frac{3}{4}$ 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 increasing 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 93-99% and not around 80%, as reported by Léger et al. [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 when dosed according to the EMA dosing recommendation, per allometric BW or per BSA in different weight strata [figure 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 < 9 kg of BW and lower CL values [range 33-58%] for the children > 9 kg of BW [figure 1A]. Comparing the CL per allometric BW [figure 1C] or per BSA [figure 1D] for all five weight strata, revealed in no differences in the scaled CL between the five 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 [figure 2]. The CWRES are plotted versus TAD [supplementary figure S1 A,B]. 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 which is not observed for the 5th and 95th percentiles [supplementary figure S2 A]. By performing two separate pcVPCs, one for the oral data and one for the IV data, the underpredictions were only observed within the oral data [supplementary figure S2 B,C]. These findings could be confirmed by pcVPCs of the evaluation dataset which consisted of only IV data and did not show any underprediction of the median busulfan observations [supplementary figure S2 D]. As the underprediction was not very pronounced
and did not affect the predictions for the IV busulfan data, we decided both final models to be valid for predicting busulfan pharmacokinetics after IV administration.

The evaluation dataset was further used for external model evaluation by estimating the PK parameters for the evaluation dataset on the basis of the developed PopPK models. No distinct discrepancies are seen between the fixed effects of the two datasets [CL and V values for the evaluation dataset: BSA model: CL 5.0 L h\(^{-1}\) m\(^{-2}\), V 15.2 L kg\(^{-1}\); allometric BW model: CL 4.8 L h\(^{-1}\) kg\(^{-0.75}\), V 15.2 L kg\(^{-1}\)]. Assessing the GOF plots for the evaluation dataset [supplementary figure S3] a regular distribution around the line of identity without indicating a distinct bias and without showing discrepancies towards the developmental dataset could be observed. These results confirmed our previous findings through external model evaluation and simulation. In addition, we could confirm our findings regarding the busulfan CL versus weight relationship using the evaluation dataset [figure 1 B]. 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 two final models we simulated AUC and CL values according to BSA or allometric BW by implementing two new dosing formulas [equations A and B]. Comparing the median simulated AUCs and the percentage of patients within the AUC range of 900-1500 µM*min best results were achieved with the new allometric BW and the new BSA dosing regimen. With these two new dosing regimens we were able to have 70-71% of the simulated patients within the therapeutic AUC range of 900-1500 µM*min when the AUC\(_\text{target}\) was set to 1125 µM*min [table 2]. 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 2]. More patients were found to be below the therapeutic AUC range of 900-1500 µM*min than above [table 2]. Further simulations with an AUC\(_\text{target}\) of 1150 µM*min did not increase the percentage of patients being within the therapeutic AUC range of 900-1500 µM*min but resulted in an even distribution of patients being outside the therapeutic AUC range [table 2]. Therefore, we compared the median simulated AUCs of the simulations with the AUC\(_\text{target}\) set to 1150 µM*min with the simulated AUCs of the EMA regimen and the simulated AUCs with a 0.8 mg/kg dosing. Compared to the EMA dosing regimen, we achieved lower median AUCs and decreased variability in AUC values with both our new dosing regimens [figure 3 A]. Comparing the AUC values by weight
strata according to the EMA dosing regimen resulted in higher median AUC values than 1500 µM*min in children > 9 kg of BW [figure 3 B]. In contrast, a higher percentage of patients [69-73%] within the therapeutic AUC range is seen using our new dosing regimens [supplementary figure S4]. Therefore, we suggest two new dosing regimens for dosing busulfan according to the following formulas:

BSA dosing regimen:
Dose in mg = 4.72 mg h L\(^{-1}\) * 4.16 L h\(^{-1}\) m\(^{-2}\) * BSA m\(^{2}\) = 19.6 mg m\(^{-2}\) * BSA m\(^{2}\) [C]

Allometric BW dosing regimen:
Dose in mg = 4.72 mg h L\(^{-1}\) * 4.11 L h\(^{-1}\) kg\(^{-0.75}\) * (BW/27.2) kg\(^{0.75}\) = 19.4 mg kg\(^{-0.75}\) * (BW/27.2) kg\(^{0.75}\) [D]

In equations C and D, AUC\(_{target}\) = 1150 µmol min L\(^{-1}\) = 4.72 mg h L\(^{-1}\).

A TDM scenario was investigated by simulation and re-estimation with the final model and the two suggested dosing algorithms. Estimates of individual CL were made based on a single plasma sample taken 0.5 h or 8 h 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 higher percentage of subjects within the therapeutic AUC range of 900-1500 µM*min as seen in comparison with only the a priori dose [75-81% patients] [table 3]. Furthermore, to explore the maximum theoretical benefit with TDM another simulation was performed 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 within the desired AUC range.

Discussion
This busulfan PopPK analysis was conducted for comparison of different new dosing regimens with the recommended BW based dosing regimen according to the EMA. Earlier, a BSA dosing regimen was described by Vassal et al [16] proposing a total dose of 600 mg/m\(^{2}\), Shaw et al [18] reporting a single dose of 150 mg/m\(^{2}\)/d and Yeager et al [17] suggesting a dose of 4 x 38.9 mg/m\(^{2}\) to be more appropriate when dosing busulfan in children. Busulfan has a very narrow therapeutic range. Achieving higher AUC values than 1500 µM*min can cause more toxicities such as SOS or mucositis whereas lower AUC...
values than 900 µM*min can lead to increased incidence of graft failure [2-5,9,10]. Concerning these severe side effects of busulfan seen in adults a therapeutic AUC of 900-1500 µM*min is defined valid for dosing IV busulfan. In children the therapeutic AUC range is not yet defined. Some studies have shown graft rejections with an AUC < 900 µM*min in children but no studies have found a threshold limit for toxicities in children yet [6-8]. Therefore, until no further studies will define a different threshold limit in children an AUC range of 900-1500 µM*min based on the findings in adults is used in children [13].

In this PopPK analysis, data of 94 children who received either oral or IV busulfan were collected. Using a 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 two models. In both models IIV is exponentially scaled on CL, V, kᵣ 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, physiological functions and maturation effects on CL [42,46,47]. Estimating the allometric exponent for our dataset resulted to be 0.754 and thus confirmed the ¾ power function. As we could not see any difference between the allometric and the BSA model we decided both models to be valid for describing our development dataset. This close agreement between a BSA model and an allometric ¾ power model, when used for scaling of metabolic processes such as CL was already described by Anderson et al [47]. Therefore, allometric BW is recommended as the preferred choice along 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 [figure 1 A]. In addition, the higher CL in children < 9 kg of BW compared to CL values reported by Vassal et al. [14] might be due to the combination of oral and IV administrations in the dataset as two of the five 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 > 9 kg of BW is in line with the overexposure seen in AUC values by simulating the EMA dosing regimen [figure 3 B]. In addition, a recent study evaluating the EMA dosing recommendation for Busilvex® published by Michel et al. [48]
found a high PK variability in the studied children. Out of 67 children, 11 children developed over-exposure [AUC > 1500 µM*min] and 4 children under-exposure [AUC < 900 µM*min].

A second finding which differed from earlier studies was the high F value. In all our models F was calculated to be between 93-99%. The adult busulfan IV dose of 0.8 mg/kg was defined based on a F of 80% and should yield in the same AUC as achieved with the oral dose of 1.0 mg/kg busulfan [15]. Hassan et al. [20] observed a mean F of 80% for adults and 68% for children and reported a high variability in F of about sixfold in children and twofold in adults. In this investigation, only eight children and eight adults were included. The F value predicted by Hassan et al. 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 based on the data of several busulfan administrations of high-dose therapy in more 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 suggest not taking F into account for dosing busulfan.

These CL and F findings led to simulations of the two developed dosing regimens according to BSA and allometric BW based on our final models. If administered according to one of these dosing regimens, doses for children < 9 kg of BW will be in line with the EMA dose recommendation whereas children > 9 kg of BW would receive lower doses compared to the EMA dosing recommendation resulting in lower AUC values [supplementary figure S4]. Decreasing doses in children > 9 kg of BW is of interest due to the high AUC values found with the EMA dosing simulations [figure 3 B]. Further, as described above [table 2] we estimated to get about 30% more patients into the therapeutic AUC range of 900-1500 µM*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 one blood sample drawn 0.5 h or 8 h after the end of the first infusion and dose adjustment according to the new formulas we were able to get even more patients into the therapeutic AUC range of 900-1500 µM*min. As shown in table 3, TDM dosing according to a later plasma sample after the end of infusion such as 8 h compared to 0.5 h resulted in a greater number of patients [8 h: 81% patients vs 0.5 h: 75% patients] within the therapeutic AUC range of 900-1500 µM*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 get 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 two plasma samples when busulfan is given IV and based on three 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 clearances estimated from our models are significantly lower than what has previously been published [13,14,19,35] hence the simulation based dosing regimens would result in lower doses than as recommended by the EMA. However, according to the simulations, the proposed dosing algorithm as shown in table 2 and 3 would have significantly improved the treatment outcome for the population studied. Before these dosing recommendations are generally applied, attention must be put into understanding the differences in clearance between our and previous studies. Also, it must be pointed out that no adults and no patients heavier than 80 kg were included. Thus, we cannot extrapolate to these patient groups.

In conclusion and according to our calculations the labeling of Busilvex® [EMA] might be redeveloped for dosing IV 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 one of the two presented dosing regimens, these findings need to be confirmed in further studies in children and adults.

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**Disclosure of Conflicts of Interest**

Along with the principal study investigators, e.g. Mirjam N. Trame, Joachim Boos and Georg Hempel who act as the guarantors, all other investigators, namely Martin Bergstrand and Mats 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.
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Tables:

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<th>Demographics</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>9.2</td>
<td>0.4</td>
<td>18.8</td>
</tr>
<tr>
<td>BW [kg]</td>
<td>27.2</td>
<td>4.2</td>
<td>80</td>
</tr>
<tr>
<td>Height [cm]</td>
<td>130</td>
<td>56</td>
<td>189</td>
</tr>
<tr>
<td>BSA [m²]</td>
<td>1.015</td>
<td>0.26</td>
<td>2</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>16.9</td>
<td>11.6</td>
<td>31.7</td>
</tr>
<tr>
<td>BW / BSA [kg/m²]</td>
<td>26.8</td>
<td>16.2</td>
<td>40</td>
</tr>
</tbody>
</table>

B

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>2.6</td>
<td>0.1</td>
<td>18.9</td>
</tr>
<tr>
<td>BW [kg]</td>
<td>15.1</td>
<td>4</td>
<td>66.6</td>
</tr>
<tr>
<td>Height [cm]</td>
<td>93</td>
<td>51</td>
<td>165</td>
</tr>
<tr>
<td>BSA [m²]</td>
<td>0.62</td>
<td>0.24</td>
<td>1.73</td>
</tr>
<tr>
<td>BMI [kg/m²]</td>
<td>17.6</td>
<td>13.2</td>
<td>25.7</td>
</tr>
<tr>
<td>BW / BSA [kg/m²]</td>
<td>24</td>
<td>16.7</td>
<td>38.5</td>
</tr>
</tbody>
</table>

C

<table>
<thead>
<tr>
<th>Patient body weight [kg]</th>
<th>n in development dataset</th>
<th>n in evaluation dataset</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 9</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>9 to &lt; 16</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>16 to &lt; 23</td>
<td>13</td>
<td>1</td>
</tr>
<tr>
<td>23 to 34</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>&gt; 34</td>
<td>36</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 1: [A] Patient characteristics of the development dataset; [B] Patient characteristics of the evaluation dataset; [C] Patient distribution over the five different weight strata

[Abbreviations: BW body weight, BMI body mass index, BSA body surface area, n number of patients]
## Table 2: Comparison of different dosing regimens

<table>
<thead>
<tr>
<th>Dosing regimen</th>
<th>Target AUC [µM*min]</th>
<th>Median simulated AUC [µM*min]</th>
<th>RMSE</th>
<th>% patients within AUC range 900-1500 µM*min</th>
<th>% patients with an AUC &lt; 900 µM*min</th>
<th>% patients with an AUC &gt; 1500 µM*min</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.8 mg/kg</td>
<td>1125</td>
<td>1248</td>
<td>428</td>
<td>59</td>
<td>15</td>
<td>26</td>
</tr>
<tr>
<td>5 dosing regimen</td>
<td>1125</td>
<td>1533</td>
<td>610</td>
<td>44</td>
<td>3</td>
<td>53</td>
</tr>
<tr>
<td>TAUC<em>4.11</em>BW^0.75</td>
<td>1125</td>
<td>1127</td>
<td>283</td>
<td>71</td>
<td>17</td>
<td>12</td>
</tr>
<tr>
<td>TAUC<em>4.16</em>BSA</td>
<td>1125</td>
<td>1110</td>
<td>283</td>
<td>70</td>
<td>20</td>
<td>10</td>
</tr>
<tr>
<td>TAUC<em>4.11</em>BW^0.75</td>
<td>1150</td>
<td>1152</td>
<td>289</td>
<td>72</td>
<td>15</td>
<td>13</td>
</tr>
<tr>
<td>BSA Dosing</td>
<td>1150</td>
<td>1134</td>
<td>289</td>
<td>70</td>
<td>17</td>
<td>17</td>
</tr>
</tbody>
</table>

Abbreviations: BW body weight, BSA body surface area, TAUC target area under the curve, RMSE root mean square error: calculated the deviation in comparison to the target AUC, EMA European Medicine Agency.
### Table 3: TDM dosing

<table>
<thead>
<tr>
<th>Time of TDM samples after end of first infusion [infusion time 4 h]</th>
<th>Allometric BW Dosing</th>
<th>BSA Dosing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.5 h</td>
<td>8 h</td>
</tr>
<tr>
<td>target AUC [μM*min]</td>
<td>1150</td>
<td>1150</td>
</tr>
<tr>
<td>median simulated AUC [μM*min]</td>
<td>1204</td>
<td>1164</td>
</tr>
<tr>
<td>RMSE</td>
<td>287</td>
<td>245</td>
</tr>
<tr>
<td>% patients within AUC range 900-1500 μM*min</td>
<td>75</td>
<td>81</td>
</tr>
<tr>
<td>% patients with an AUC &lt; 900 μM*min</td>
<td>8</td>
<td>9</td>
</tr>
<tr>
<td>% patients with an AUC &gt; 1500 μM*min</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>Dosing regimen</td>
<td>TAUC<em>4.11</em>BW^0.75</td>
<td>TAUC<em>4.16</em>BSA</td>
</tr>
</tbody>
</table>

**Table 3:** TDM dosing

[Abbreviations: BW body weight, BSA body surface area, TAUC target area under the curve, TDM therapeutic drug monitoring, RMSE root mean square error: calculated the deviation in comparison to the target AUC]**
Figure Legends:

Figure 1: Individual clearance estimates in different weight strata; [A] per body weight for the development dataset; dashed black lines: mean clearance values, black solid curve: mean clearance values from a previous investigation [14]; [B] per body weight for the evaluation dataset; [C] per allometric body weight; [D] per BSA; in figure C and D data from the development and the evaluation dataset 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,B] development dataset with allometric body weight as covariate on CL; [C,D] development dataset 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.

Figure 3: [A] AUC simulations for the different dosing regimens with an AUC_target of 1150µM*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 [Abbreviations: BW^0.75 allometric body weight, BSA body surface area, EMA European Medicine Agency]
Population Pharmacokinetics of Busulfan in Children: Increased Evidence for Body Surface Area and Allometric Body Weight Dosing of Busulfan in Children

Mirjam N Trame, Martin Bergstrand, Mats O. Karlsson, et al.

Clin Cancer Res Published OnlineFirst September 14, 2011.

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