Appendix I

Body weight-dependent pharmacokinetics of busulfan in pediatric hematopoietic stem cell transplantation patients: towards individualized dosing

I.H. Bartelink[1,2], J.J. Boelens[3], R.G.M. Bredius[4], A.C.G. Egberts[1,5], C. Wang[2], M.B. Bierings[3], P.J. Shaw[6,7], C.E. Nath[6], G. Hempel[8], J. Zwaveling[9], M. Danhof[2], C.A.J. Knibbe[2,10]

(1) Department of Clinical Pharmacy, University Medical Center Utrecht, Utrecht, the Netherlands
(2) Department of Pharmacology, Leiden/Amsterdam Center for Drug Research, Leiden University, Leiden, the Netherlands
(3) Pediatric Blood and Marrow Transplantation Program, University Medical Center Utrecht, Utrecht, the Netherlands
(4) Department of Pediatrics, Leiden University Medical Center, Leiden, the Netherlands
(5) Department of Pharmaco-Epidemiology and Clinical Pharmacology, Utrecht University, Utrecht, the Netherlands
(6) Oncology Unit, Children's Hospital at Westmead, Sydney, NSW, Australia
(7) Discipline of Paediatrics and Child Health, Sydney Medical Program, Sydney, NSW, Australia
(8) Department of Pediatric Hematology and Immunology, University of Münster, Münster, Germany
(9) Department of Clinical Pharmacy, Leiden University Medical Center, Leiden, the Netherlands
(10) Clinical Pharmacy, St. Antonius Hospital, Nieuwegein, the Netherlands

Clinical Pharmacokinetics; 2012 51 (5): 331-345

Abstract

Background and Objectives: The wide variability in pharmacokinetics of busulfan in children is one factor influencing outcomes such as toxicity and event free survival. A meta-analysis was conducted to describe the pharmacokinetics of busulfan in patients from 0.1 to 26 years of age, to elucidate patient characteristics that explain the variability in exposure between patients and to optimize dosing accordingly.

Patients and Methods: Data were collected from 245 consecutive patients (from 3 to 100 kg) who underwent HSCT in four participating centers. The inter-patient, inter-occasion and residual variability in the pharmacokinetics of busulfan were estimated with a population analysis with the software program NONMEM VI (Globomaxx LLC, Hanover, MD, USA). Covariates were selected on the basis of their known or theoretical relationships with busulfan PK and were plotted independently against the individual PK-parameters and the weighted residuals of the model without covariates to visualize relations. Potential covariates were formally tested in the model.

Results: In a two-compartment model, body weight was the most predictive covariate for clearance, volume of distribution and inter-compartmental clearance and explained 65%, 75% and 40% of the observed variability, respectively. The relation between body weight and clearance was characterized best using an allometric equation with a scaling exponent that changed with body weight from 1.2 in neonates to 0.55 in young adults. This implies that an increase in body weight in neonates results in a larger increase in busulfan clearance than an increase in body weight in older children or adults. Clearance on the first day was 12% higher than that of subsequent days ($p < 0.001$). Inter-occasion variability on clearance was 15% between the four days. Based on the final PK-model, an individualized dosing nomogram was developed.

Conclusions: The model-based individual dosing nomogram is expected to result in predictive busulfan exposures in patients ranging between 3 and 65 kg and thereby to a safer and more effective conditioning regimen for hematopoietic stem cell transplantation in children.

I.1. Introduction

Children may differ from adults in drug pharmacokinetics (PK), in response to treatment (efficacy) and their susceptibility to side effects (safety). Variability in pharmacokinetics is one factor which alters drug exposure and this in turn might explain differences in the (un)wanted responses (Pharmacodynamics, PD) between children. Characterization of variability in PK between neonates, infants, children and adolescents is therefore important especially for drugs with a small therapeutic window such as busulfan. Busulfan is a chemotherapeutic drug which is standard of care in preparative chemotherapy in patients undergoing hematopoietic stem cell transplantation (HSCT) for a variety of malignant and non-malignant diseases [1]. Since the 1970s, total body irradiation (TBI) based conditioning regimens have been used [2]. Unfortunately, TBI is complicated by cataracts, endocrine disorders (including stunted growth), delayed intellectual development and secondary tumors. As an alternative to TBI, in the 1980s, chemotherapy-based conditioning (high-dose oral busulfan in combination with cyclophosphamide) was introduced [3, 4]. The therapeutic potential of oral busulfan was limited by the large PK-variability [5-8]. This PK-variability has major implications for outcomes of treatment; a low total exposure (expressed as the total area under the concentration curve using all administrations from day 0 to day 4, AUC_{day0-4}) to busulfan is associated with an increased risk of graft failure and relapse [9, 10],[9;10] whereas a high AUC_{day0-4} is associated with an increased risk of toxicity like veno-occlusive disease (VOD) and graft versus host disease [9, 10]. To reduce the variability in exposure and to improve safety of the regimen, intravenous (IV) busulfan was introduced in 2000 [11]. Although this excludes differences in absorption, wide inter-individual variability in busulfan pharmacokinetics is still observed with the IV formulation, particularly in children [12-17]. Therapeutic drug monitoring to guide IV busulfan dosing was therefore introduced [1, 14], which resulted in further improved of the event free survival in children [18]. It may be anticipated that patient-outcomes after HSCT may further benefit from an optimal starting dose for each individual, especially for centers where TDM is not available. Previous studies used body surface area (BSA) or body weight-based functions to characterize the variable PK of busulfan in children [17, 19, 20]. In addition some of the variability was suggested to be explained by the underlying disease of the patient [21-23]. However, most of these results

originated from relatively small studies. We describe a pharmacokinetic meta-analysis of 245 patients, in whom a large number of covariates related to the PK of busulfan, such as body weight, underlying diseaseand liver function tests, were measured. The meta-analysis was conducted with the objective of describing the pharmacokinetics of busulfan in patients from 0.1 to 26 years of age and to elucidate patient characteristics that explain the variability in exposure between patients. The resulting PK-model was used to derive an individualized dosing algorithm aiming for an optimal exposure of busulfan in each patient.

I.2. Methods

Setting and study population

In this prospective meta-analysis, patients were enrolled in the research protocol after the patient and/or their parents (the latter in patients under 12 years of age) provided written informed consent. The study was approved by the local ethics committees. Data were collected from all 245 consecutive patients who underwent HSCT between August 2000 to September 2009 in four centers and of whom concentration measurements after intravenous busulfan administration were available. The four centers were: the University Medical Center Utrecht and the Leiden University Medical Center in the Netherlands, the Universitätsklinikum at Münster, Germany, and the Children's Hospital at Westmead, Sydney, Australia. Part of the data (135 of the 245 patients) has been described in previous publications [17, 21, 24, 25]. The characteristics of the settings, busulfan administration and analysis and samples schemes are shown in table 1. Busulfan was administered during 3 to 4 consecutive days. In 98% of patients blood samples were collected on day 1 and in 68% of patients, blood samples were also collected on day 2-4.

Table 1: Participating centers.

		Leiden	Münster	Sydney	Utrecht
Busulfan preparation/formulation		Busilvex® Pierre Fabre Medicament, France	Busilvex® Pierre Fabre Medicament, France	Busulfex® Orphan, Australia	Busilvex® Pierre Fabre Medicament, France
Busulfan dosing algorithm	(day1)	until 2003: <4 years: 4mg / kg in 4 doses ≥4 years: 3.2 mg / kg in 4doses from 2003 <1 years: 80 mg / m2 in 1 dose ≥1years: 120 mg / m2 in 1 dose	3.2 mg / kg in 2 doses 120mg / m2 in 2 doses	120 mg / m2 in 1 dose 130 mg / m2 in 1 dose 3.2 mg / kg in 4 doses	until 2008: <1 years: 80 mg / m2 in 1 dose ≥1years: 120 mg / m2 in 1 dose from 2008: <0.5 years: 80 mg / m2 in 1 dose 0.5-1years: 120 mg / m2 in 1 dose ≥1years: 130 mg / m2 in 1 dose
Infusion duration day 1 (h)	median (range)	3 (2-3)	4	2(1-3.75)	3(2.75-3.25)
Dose adjustments based on TDM		yes	no	yes	yes
Sample scheme		predose, 1, 2, 4 hours after inf.	pre-dose, during infusion, 1, 1.5, 2, 3, 6 and 7.5 hours after inf.	4-8 samples following the dose between 0.08 to 7 hours after inf.	0.08, 1, 2 and 4 hours after inf.
Method of determination[a]		HPLC	LC-MS	GC	HPLC/LC-MS
LOQ		50 µg/L	5 µg/L	30 µg/L	50 µg/L
Precision within/between runs	(% variation)	3.5%,0.8%	<11%,<11%	<9%,<10%	2.3%, 0.2%

a. The methods Leiden, Utrecht (HPLC and LC-MS) and Münster were successfully cross validated.
HPLC = high pressure liquid chromatography, LCMS = liquid chromatography-mass spectrometry, GC = gas chromatography, CV = Coefficient of Variation (%), LOQ = limit of quantification, TDM = therapeutic drug monitoring

Transplantation details and other patient characteristics are shown in table 2. All patients received IV busulfan-based myeloablative conditioning according to the applicable (inter)national protocols. All patients were cared for in high-efficiency, particle-free, air-filtered, positive-pressure isolation rooms. Gut decontamination and infection prophylaxis was given according to the institutional protocol. Patients received anti-emetic drugs and prophylactic anticonvulsive therapy (clobazam, clonazepam, diazepam and in 10 cases phenytoin) during busulfan treatment.

Pharmacokinetic analysis

Model building and evaluation

The nonlinear mixed-effects modeling software NONMEM VI using ADVAN subroutines with first-order conditional estimation (FOCE) with η-ε interaction (Globomaxx LLC, Hanover, MD, USA) was used. Log-transformed busulfan concentrations were used for analysis. Twenty-nine of a total of 1775 busulfan concentrations were below the limit of quantification (LOQ). The values of these samples were set at ½ the LOQ [26]. Mixed effects models consist of a structural model (e.g. a one or two-compartment model) describing the relationship between dose and concentrations, and a stochastic model describing the random variability in the PK-parameters of the structural model. Random variability consisted of inter-individual in PK-parameters, inter-occasion variability (inter-occasion variability, IOV) in PK-parameters and intra-individual variability. Inter-individual and inter-occasion variability were modeled assuming a log-normal

distribution (eq. 1, appendix 1) [27]. Intra-individual variability was modeled using an additive error (eq. 2, appendix 1) which is equivalent to a proportional error model in the untransformed scale. Discrimination between different models was made by comparison of the objective function (-2 log likelihood). A value of $p < 0.005$, $df = 1$, representing a decrease in objective function of 7.8 on a χ^2 distribution, was considered statistically significant. In addition, goodness-of-fit plots (individual predicted *versus* observed concentrations, population predicted *versus* observed concentrations, conditional weighted residuals *versus* time and conditional weighted residuals *versus* population PK-parameters) were used for diagnostic purposes. Furthermore, the confidence interval of the parameter estimates, the correlation matrix and visual inspection of the distribution of the model parameters, were used to evaluate the model.

In order to determine whether the model was over-parameterized, the condition number of the final PK-model was calculated. The condition number should not exceed the critical value of 1000 [28]. [29] Finally, η- and ∈-shrinkage as defined by Karlsson *et al*. [29][30] was calculated for all model parameters for which inter-individual variability was estimated. A shrinkage-value below 20% was considered acceptable.

Covariate analysis

The covariates depicted in table 1 and 2 were selected on the basis of their known or theoretical relationships with busulfan PK. As described before, BSA and body weight are associated with busulfan PK [17, 19, 20]. Many drugs may interact with busulfan [30-32]. Therefore, all concomitant medications used by more than 10% of patients were included as a covariate. Underlying disease and clinical chemical parameters like liver function have been reported to influence busulfan clearance [13, 21-23, 33], while also blood counts may relate to variability in busulfan-PK.

All covariates were plotted independently against the individual PK-parameters (post hoc values) and the weighted residuals of the model without covariates to visualize relations. Potential covariates were formally tested in the model as follows. For continuous covariates such as body weight, age or BSA, the influence of the covariate on each PK-parameter was tested using a linear (eq. 4, appendix 1) or allometric function (eq. 5, appendix 1). In addition, other allometric functions were explored for the PK parameters of which the plot

of inter-individual variability of the PK-parameter *versus* the covariate showed that neither a linear nor an allometric function results in adequate description of the data. I.e., the plot of inter-individual variability of Cl *versus* body weight showed that an allometric function over-predicted patients < 10 kg and > 40 kg and under-predicted patients between 10 and 40 kg. In this respect, it has been reported in studies using small cohorts of children that the scaling exponent of the allometric function for clearance is larger in neonates and young children (i.e. a scaling exponent> 1) in comparison with older children (i.e. a scaling exponent < 1) [34-36]. Wang *et al*. have used an allometric function with a scaling exponent that varied with body weight between 1.35 in neonates to 0.57 in adults, when studying propofol clearance [35]. Therefore, beside a linear or standard allometric function, as a third approach, an allometric function was tested with a scaling exponent that varied with bodyweight, age or BSA (eq. 6, appendix 1). For categorical covariates, typical values of the PK-parameters were compared between categories (eq. 7, appendix 1).

Statistical evaluation of the incorporated covariate relationships was performed by forward inclusion and backward deletion [37]. A p-value < 0.005 was applied to evaluate the covariates in the forward inclusion (decrease of objective function of at least 7.8 points), while the backward deletion procedure used a stricter criterion (objective function > 10.83, $p < 0.001$). When two or more covariates were found to significantly improve the model, the covariate causing the largest reduction in objective function was left in the model. Additional covariates had to reduce this objective function further to be retained in the model. Moreover, to accept a covariate, a reduction in inter-individual variability of the PK parameter involved was required. In addition, individual and population PK-parameters were plotted against the most predictive covariate to evaluate whether the individual PK-parameters were equally distributed around the population parameters [38]. The choice of the model was further evaluated as discussed under model building and evaluation, whereby the results of the internal validation procedure were also considered.

Internal validation

The robustness of the population pharmacokinetic model was assessed by the bootstrap re-sampling method throughout the model-building process and on the final PK-model, using 1000 replicate datasets per bootstrap [39]. The mean

value, 95%-confidence intervals and covariance of all parameters obtained with the bootstrap replicates were compared with the estimates obtained from the original data set.

Derivation of model-based dosing regimen

The parameters of the final PK-model were used as a basis to determine an individualized dosing regimen in patients from 3-65 kg. The new dosing regimen aimed to reach a target exposure (defined as AUC_{day0-4}) of 90 mg*h/L (≈5400 μM*min/day) in myeloablative regimens and an AUC_{day0-3} of 60 mg*h/L for non-myeloablative conditioning regimens, based on the current literature of optimal exposures of busulfan in children [12, 15, 22, 24, 40, 41]. For each of the 245 patients, AUC_{day0-4} values were simulated in each patient upon four consecutive once daily IV doses, using ONLYSIM-option as implemented in NONMEM. All PK-parameters were fixed to the final PK-parameters and all covariate relations were included. The amounts were integrated into a dummy AUC-compartment. For each patient, an individual dose was derived for each of the two target-AUCs, which was plotted versus bodyweight resulting in two model-based dosing nomograms.

I.3. Results

Busulfan PK-data on 245 patients were obtained from the four participating centers. Patient characteristics such as body weight, underlying disease and concomitant medications were rather evenly distributed between centers and within the total dataset (table 2).

Table 2: patient characteristics.

		Leiden	Münster	Sydney	Utrecht	Total
Nr. of patients	n	102	18	55	70	245
Age (years)	median (range)	3.58 (0.2-17)	3.88 (0.8-17)	4.25 (0.2-18)	2.5 (0.1-26)	3.33 (0.1-26)
Body weight (kg)	median (range)	15.4 (4-73)	16.5 (9.5-74)	16.7 (3.1-109)	14.8 (3.7-107)	15.3 (3.1-109)
BSA (m2)	median (range)	0.65 (0.3-1.8)	0.70 (0.42-2.0)	0.69 (0.2-2.3)	0.61 (0.2 - 2.4)	0.65 (0.2-2.4)
Gender	male	66%	56%	56%	50%	58%
Number of samples	n (mean per patient)	472 (5)	205 (11)	466 (8)	632 (9)	1775 (7)
Frequency of Bu adm	n (4dd, 2dd, 1dd)	(39,-,63)	(-,18,-)	(4,-,51)	(-,-,70)	(43,18,184)
Underlying disease	malignancy / MDS	52	13	24	25	114
	bone marrow failure syndrome	7	1	7	6	21
	inborn errors (1)	13	4	7	20	44
	immunedef. + HLH	29	0	17	19	65
Chemotherapeutic	Bu + Cy	41	8	8	37	94
regimen (2)	Bu + Cy + Mel	44	8	0	8	60
	Bu + Flu	7	0	11	12	30
	Bu + Mel	1	1	6	0	8
	Bu + Cy + Eto	5	1	3	8	17
	Bu + Cy +Flu	4	0	19	4	27
	Bu+ Mel+Flu	0	0	8	1	9
Timing chemotherapy	Chemo before or during Bu	10	0	36	21	67
	Chemo after Bu	92	18	19	49	178
Serotherapy (3)	No serotherapy	39%	50%	60%	20%	40%
Nr of transplant	1	102	18	55	67	242
	>1				3	3
Type of transpants	CB,BM,PBSC (N)	(13,73,17)	(-,-,19)	(20,18,16)	(28,33,8)	(61,124,60)
GGT (u/L) (4)	median (range)	14 (6-870)		21 (4-275)	21 (7-1990)	18.5 (4-1990)
ALAT (u/L) (4)	median (range)	23 (6-253)	11 (4-41)	32 (11-840)	26 (11-550)	26 (4-840)
ASAT (u/L) (4)	median (range)	37 (9-145)	14 (6-366)	35 (16-166)	29 (12-350)	32.5 (6-366)
Hemoglobuline (mmol/L) (4)	median (range)	6.3 (4.4-8.5)	5.6 (3.7-8.6)	5.9 (3.9-8.6)	5.8 (4.1-9.6)	6.1 (3.7-9.6)
Leukocytes (*10E9/L) (4)	median (range)	5.4 (0.3-45)	3.3 (0.4-81)	3.1 (0.2-16.4)	2.9 (0.1-16.6)	3.9 (0.1-81)
Thrombocytes (*10E9/L) (4)	median (range)	121 (1-688)	141 (13-615)	192 (12-629)	109 (7-661)	127 (1-688)
Erythrocytes (*10E12/L) (4)	median (range)	3.5 (2.5-5.4)		3.5 (2.2-4.6)	3.1 (1.9-5.2)	3.5 (1.9-5.4)
Albumine (g/L) (4)	median (range)	40 (29-510)	39	37 (24-47)	31 (13-45)	37 (13-51)
Concomitant medications	median nr. (range)	2 (0-8)	8 (6-11)	5 (1-9)	6 (0-13)	5 (0-13)
Glucocorticoids (5)	yes	39%	78%	15%	36%	36%
Antibiotics (5)	yes	40%	100%	22%	99%	57%
Trimethoprim/cotrimoxazol (5)	yes	0%	89%	5%	0%	8%
Antivirals (5)	yes	2%	17%	11%	60%	22%
Omeprazol/pantoprazol (5)	yes	2%	28%	5%	24%	11%
Antimycotics (5)	yes	2%	19%	25%	32%	17%
Fluconazol (5)	yes	5%	39%	73%	89%	47%
Alizapride	yes	12%	0%	0%	4%	6%
Ondansetron (5)	yes	58%	72%	100%	97%	80%
Opiates (5)	yes	11%	0%	13%	0%	7%
Antihistaminics (5)	yes	10%	22%	11%	34%	18%
Diuretics (5)	yes	8%	61%	5%	19%	14%
Ursodeoxychol acid (5)	yes	0%	6%	75%	0%	17%
Benzodiazepines (5)	yes	21%	78%	73%	96%	58%
> 1 Drug cleared via renal clearance (6)	yes	54%	100%	80%	99%	76%
> 1 Drug cleared via P450 metabolism (7)	yes	33%	100%	76%	99%	67%
> 1 Drug cleared via phase 2 metabolism (8)	yes	79%	100%	100%	99%	91%

Bu = busulfan, MDS = myelodysplastic syndrome, immunedef. = immune deficiencies, HLH = hemophagocytic lymphohistiocytosis, ALL = acute lymphocytic leukemia, AML = acute myeloid leukemia, JMML = juvenile myelomonocytic leukemia, CML = chronic myeloid leukemia, Cy = cyclophosphamide, Mel = melphalan, Flu = fludarabine, P450 = cytochrome P450 enzymes, BSA = body surface area.

1) Inborn errors were inborn errors of metabolism and hemoglobinopathies.
2) In general the dose of melphalan was 140 mg/m^2 once daily, etoposide 1200 mg/m^2 once daily, cyclophosphamide: 60 mg/kg once daily for 4 days, fludarabine 40 mg/m^2 for 4 days.
3) Patients with unrelated donors received serotherapy, either anti-thymoglobulin (ATG)-rabbit (Genzyme/Fresenius) or alemtuzumab (Genzyme).
4) These covariates were measured at day 1 or just before the day of the first busulfan administration. These covariates were also explored in relation to their age-related reference-values.
5) Covariate selection of concomitant medications used in these patients, based on the route of metabolism of the drugs (selections were made of medications which were used by more than 10% of patients).
6) Drugs cleared primarily via renal clearance were antibiotics, trimethoprim, antivirals, fluconazole, alizapride, metoclopromide, diuretics.
7) Drugs cleared primarily via P450 metabolism were phenytoin, antimycotics, proton-pump inhibitors, benzodiazepines, opiates.
8) Drugs cleared primarily via phase 2 metabolism were acetaminophen (if needed medication of acetaminophen was not included), metronidazole, co-trimoxazole, corticosteroids, metoclopramide, ondansetron, antihistaminics, diuretics, ursodeoxycholic acid.

Busulfan concentrations could adequately be described using a two-compartment model parameterized in terms of clearance (CL), inter-compartmental clearance (Q), volume of distribution of central compartment (V1) and peripheral compartment (V2, estimated as a factor times V1). The two-compartment model was superior over a one-compartment model for statistical reasons (decrease in objective function of 132 points ($p < 0.001$)) and improved goodness of fit plots. Inter-occasion variability on clearance in the structural model resulted in a large reduction of objective function of -309 ($p<0.001$). The addition of inter-occasion variability on V1 did not result in a significant improvement of the model (objective function -5.9). In the final PK-model, clearance at day 2-4 was estimated as a fraction of clearance at day 1 (eq 3, appendix 1) and was 12% lower in comparison with day 1 (objective function -45, $p<0.001$).

Figure 1a (left panel) shows that the two-compartment PK-model without covariates could adequately describe the observed concentrations. This model showed poor predictive performance, however, as shown by the population predicted *versus* observed concentrations (figure 1a, right panel). Particularly in the extremities of the body weight range (3-7 kg and >30 kg) concentrations were over- and under-predicted, respectively.

The covariate analysis identified body weight, BSA and age as most important covariates related to volume of distribution and clearance. The introduction of body weight as a covariate for V1 using a linear function (eq.4, appendix 1) resulted in a decrease in objective function of -442 ($p<0.001$). An allometric function for V1 (eq. 5, appendix 1) with a single scaling exponent of 0.89 was superior over a linear function (objective function -26 points, $p<0.001$) and resulted in an equal distribution of the individual V1 parameter estimates of all body weights around the population V1 parameter estimates. As a result, the inter-individual variability on V1 decreased from 76% to 20% in comparison with the PK-model without covariates. For clearance, inclusion of body weight using a linear equation (eq. 4, appendix 1) further improved the model (objective function -457, $p<0.001$). An allometric function for clearance (eq. 5, appendix 1) with a single scaling exponent of 0.91 was superior over a linear function (objective function -11 compared to a linear function, $p<0.001$), but the population predicted values of both models were biased compared to

the individual predicted PK-parameters. A linear or allometric function over-predicted patients < 10 kg and > 40 kg and under-predicted patients between 10 and 40 kg. An allometric equation with a scaling exponent, that changed with body weight from 1.2 in neonates to 0.55 in young adults (eq. 6, appendix 1) described the relation between bodyweight and clearance significantly better compared to the allometric equation with a single scaling exponent (decrease in objective function of 35, $p<0.001$). The population predicted values of this model described individual predicted PK-parameters without bias. This model resulted in an adequate distribution of the individual Cl parameter estimates over the entire body weight-range and resulted in a decrease in inter-individual variability on Cl from 99% to 27% in comparison with the PK-model without covariates. Body weight was linearly related to Q (objective function -26 , $p<0.001$) and resulted in a decrease in inter-individual variability from 148 to 88% in comparison with the PK-model without covariates.

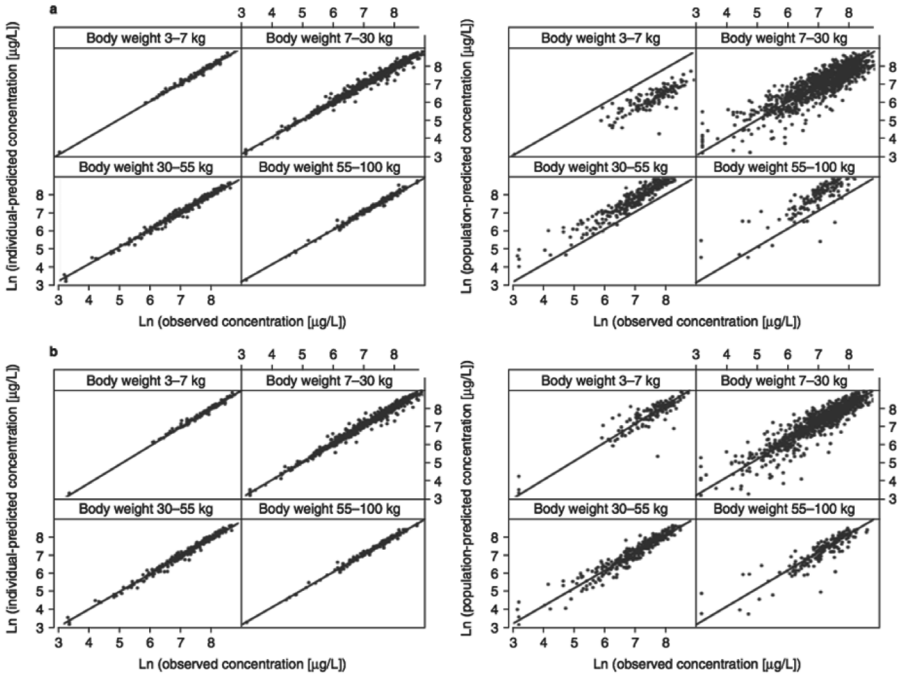


Figure1: Individual predicted concentrations vs observed concentrations (leftpanels) and population predicted concentrations vs observed concentrations (right panels) of the pharmacokinetic model without covariates (a) and the final pharmacokinetic-model (b). Data of four body weight categories (kg) are shown separately. Ln = lognormal.

The inclusion of body weight on the PK-parameters resulted in a more significant reduction of the objective function in comparison with age on all parameters (objective function +108), while the model using BSA showed similar results in comparison with body weight-model given the criteria as defined under Methods.

The systematic covariate analysis did not identify any other covariates. No differences in the diagnostic-plots were observed between the participating centers (appendix 2), frequency of busulfan administration, concomitant medications or any of the other covariates.

For the final PK-model, the individual and population predicted *versus* observed concentrations per body weight-category are depicted in figure 1b. The right panel demonstrates that the predictive performance of the model is similar in all four bodyweight groups. The individual and population PK-parameters (Cl and V1) of the final PK-model *versus* body weight are shown in figure 2. The figure shows an equal distribution of the individual PK-parameter estimates of all body weights around the population PK-parameter estimates. All parameter estimates of the final PK-model and the results of the statistical evaluation (bootstrap validation, shrinkage and condition number) are presented in table 3. These statistical evaluation tools were within the limits, given the criteria as defined under Methods, except for shrinkage on Q and inter-occasion variability which were both >20%.

Based on the final PK-model, the model-based dosing nomogram was derived as depicted in figure 3. With this nomogram, a dose for each individual between 3-65 kg can be obtained, aiming for a myeloablative (AUC_{day0-4} of 90 mg*h/L) or non-myeloablative conditioning regimen (AUC_{day0-3} of 60 mg*h/L). Table 4 shows the model-based nomogram in mg/kg of patients between 3 and 65 kg and the deviations of their simulated AUC's in comparison with the target-AUC_{day0-4} of 90 mg*h/L. Deviations in the expected AUCs upon the currently approved dose in the summary of product characteristics by EMA [30] and a BSA based dosing regimen of 130 mg/m2are also shown in table 4. Both regimens are used in pediatric clinical practice by different institutes. The first dosing algorithm was based on a population-pharmacokinetic study of 24 children by Nguyen *et al*. in 2004 [20], aiming at a target AUC of 78mg*h/L (59-98mg*h/L in combination

with cyclophosphamide), which was prospectively validated in 55 children by Vassal *et al*. in 2008 [42]. The BSA-based dosing regimen was used in children by Nath *et al*. and Gordon *et al*. [22, 43] This dosing regimen was derived from a studies using oral busulfan using 130-150mg/m^2 per day in children [5, 44] and from a study of IV busuflan in adults [45].

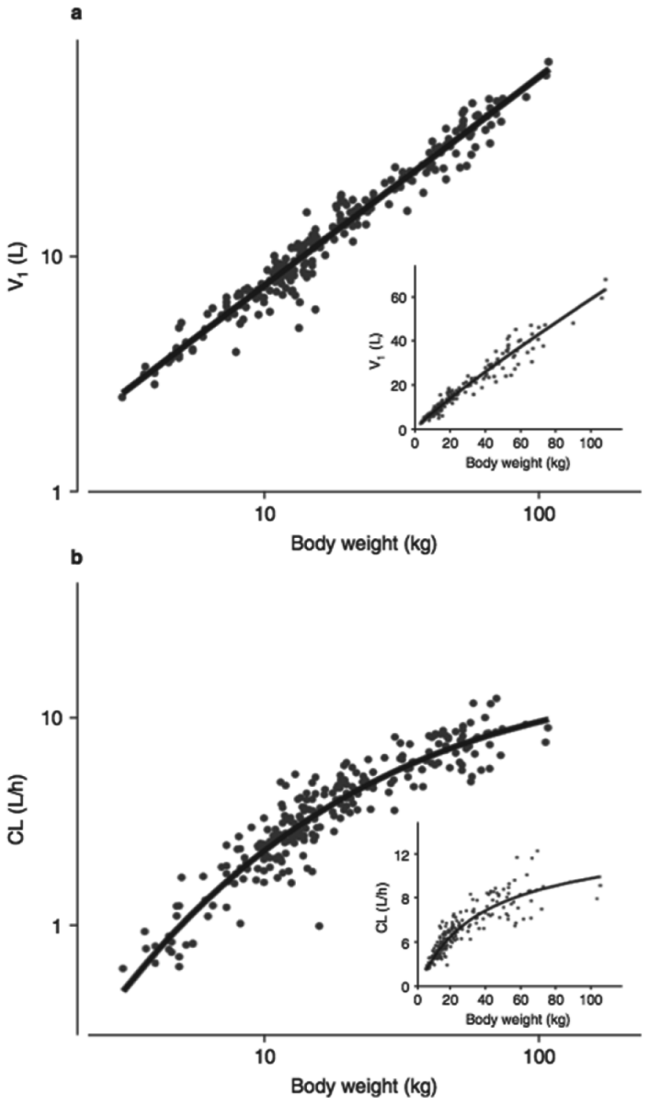


Figure 2: Individual predicted pharmacokinetic parameters (post hoc, pre- sented as dots) and population predicted pharmacokinetic parameters (black line) of volume of distribution of the central compartment (a) and clearance (b) vs body weight of the final pharmacokinetic model. The data are presented on log-scale and on normal scale (insert). CL = clearance; V1 = volume of dis- tribution of the central compartment.

Table 3: Population PK-parameter estimates, shrinkage and PK-parameter estimates obtained after bootstrap of the final PK-model.

	Dataset		Shrinkage	1000 Bootstrap replicates	
Parameter	Estimate	CV %		Median	5-95 percentile
Structural model					
$Cl_{15.3kg}$ (L/h)[a]	3.47	3.0		3.44	3.24-3.72
$V1_{15.3kg}$ (L)[b]	11.1	1.6		11.1	10.8-11.4
V2 expressed as factor (times V1)	6.92	6		6.89	1.56-13.5
$Q_{15.3kg}$ (L/h)[c]	0.495	12.2		0.509	0.29-0.67
L1 in: $Cl = Cl_{15.3kg} \times (BW/15.3)^{L1 \times BW^{M}}$	1.56	10		1.55	1.31-1.88
M in: $Cl = Cl_{15.3kg} \times (BW/15.3)^{L1 \times BW^{M}}$	-0.226	15		-0.224	-0.29--0.17
L2 in: $V1 = V1_{15.3kg} \times (BW/15.3)^{L2}$	0.890	2.0		0.889	0.86-0.92
Cl_{day2-4} expressed as reduction factor of day_1	0.12	14		0.12	0.08-0.15
Random variability					
Inter-individual variability on Cl (%)	27%	17	16%	27%	23%-31%
Inter-individual variability on V1 (%)	20%	21	19%	20%	17%-24%
Inter-individual variability on Q (%)	88%	24	50%	87%	64%-119%
Corr. IIV ClxV1	0.52	21		0.53	0.39-0.66
Inter-occasion variability Cl (%)	15%	21	42%	15%	12%-18%
Proportional residual error (%)	14%	12	18%	14%	12%-15%
Objective function	-3671			-3702	

$Cl_{15.3kg}$= clearance for a typical individual of 15.3kg $V1_{15.3kg}$ = volume of distribution of the central compartment for a typical individual of 15.3kg, $V2_{15.3kg}$ = volume of distribution of the peripheral compartment for a typical individual of 15.3kg, $Q_{15.3kg}$ = inter-compartmental clearance for a typical individual of 15.3kg, 15.3 kg was the mean value of the body weights in the dataset. In the allometric function, L1 represents the intercept and M is the exponent, which allows the scaling exponent to change with body weight. L2 represents the single scaling exponent. BW= body weight (kg) CV= coefficient of variation (%), inter-individual variability was calculated as the square root of the exponential variance -1).
a. Clearance was described, according to the following equations:

$$Cl = Cl_{15.3kg} \times \left(\frac{BW}{15.3}\right)^{L1 \times BW^{M}}$$

b. V1 was described as volume of distribution of the first compartment, according to the following equations:

$$V1 = V1_{15.3kg} \times \left(\frac{BW}{15.3}\right)^{L2}$$

c. Q was described as inter-compartmental clearance, according to the following equations:

$$Q = Q_{15.3kg} \times \left(\frac{BW}{15.3}\right)^{1}$$

Table 4: The model-based individualized dosing nomogram of busulfan, expressed in mg/kg for 20 patients with body weight ranging between 3 and 65 kg (aiming at a target-AUC_{day0-4} of 90 mg*h/L[a] in combination with fludarabine). The deviations of their corresponding AUC_{day0-4}-values in relation with this target-AUC_{day0-4} are shown (left column). For comparison, the dosing nomogram of the currently approved dose in the EMA-summary of product characteristics (SPC) (43) and deviations in their corresponding AUC_{day0-4} values of the target-AUC_{day0-4} of 90mg*h/L[a] are shown (middle column). Doses in mg/kg derived from a BSA based dosing regimen of 130mg/m^2 1dd and their concurrent deviations of the target-AUC_{day0-4} of 90mg*h/L[a], are also shown (right column).

Body weight	Model-based individualized dosing nomogram Myeloablative dose 4 days, 1dd, mg/kg target AUC_{day0-4} 90mg*h/L[a]		Approved dose in SPC 4 days, 1dd, mg/kg		Dose based on 130 mg/m2[b] 4days, 1dd, mg/kg	
kg	Dose (mg/kg)	± % deviation of target AUC	Dose (mg/kg)	± % deviation of target AUC	Dose (mg/kg)	± % deviation of target AUC
3	3.8	0%	4.0	5%	8.7	128%
5	4.7	0%	4.0	-15%	7.0	49%
7	5.1	0%	4.0	-22%	6.5	28%
8	5.2	0%	4.0	-23%	6.2	19%
9	5.2	0%	4.8	-8%	6.2	19%
11	5.2	0%	4.8	-9%	7.0	33%
13	5.2	0%	4.8	-8%	5.5	6%
15	5.1	0%	4.8	-6%	5.2	2%
16	5.1	0%	4.4	-13%	5.4	7%
20	4.9	0%	4.4	-9%	5.2	7%
23	4.7	0%	3.8	-19%	4.9	4%
25	4.6	0%	3.8	-17%	4.9	8%
30	4.3	0%	3.8	-12%	4.3	0%
35	4.1	0%	3.2	-22%	3.9	-4%
40	3.9	0%	3.2	-18%	3.9	0%
45	3.7	0%	3.2	-14%	3.9	6%
50	3.5	0%	3.2	-10%	3.8	8%
55	3.4	0%	3.2	-6%	3.5	5%
60	3.3	0%	3.2	-2%	3.7	12%
65	3.1	0%	3.2	2%	3.4	8%

a. A total exposure of 90 mg*h/L to 21.6 mM*min total, or 5400 µM*min/day
b. Doses in mg/kg of the BSA based dosing regimen were extracted from a BSA-for-body weight plot of all patients.

I.4. Discussion

This international pharmacokinetic meta-analysis of 245 patients was conducted to characterize the pharmacokinetics of busulfan from 1month to 26 years of age. Body weight was the most predictive covariate for clearance (Cl), volume of distribution (V1) and inter-compartmental clearance (Q) and explained 65%, 75% and 40% of the observed inter-individual variability, respectively. The relation between body weight and clearance was clearly non-linear and was described using an allometric function with a scaling exponent

that varied between 1.2 in neonates and 0.55 in young adults. This precise relation could be identified as a result of the large range in body weights and extensive number of patients and resulted in an individualized dosing nomogram for patients between 3-65 kg.

The PK-model was built based on a large dataset obtained from a multi-center setting. The dataset contained multiple treatment regimens, a wide range of age, bodyweights, underlying diseases and a large number of other covariates. The model adequately described the data and only 13% residual variability remained in the final PK-model. Statistical evaluation tools (CV of parameter estimates, bootstrap) show that this is a robust model. No differences were seen between the participating centers, even though the centers had different settings. This indicates that the results of the model may be extrapolated to other pediatric HSCT centers elsewhere, if patient characteristics are comparable to the characteristics in this dataset (like similar concomitant medications). The quality of the structural model (figure 1b, left panel) indicates that this PK-model can reliably predict exposures in new patients, ranging from 3-65 kg.

Body weight, rather than age, was the most predictive covariate that explained the variability in exposure between patients from 3-100 kg. Because BSA is a composite parameter taking account both length and body weight, and the BSA model did not result in improvement of the description of the data in comparison with body weight as the only parameter, the final body weight model was preferred over the BSA model. The non-linear relation between body weight and clearance reflects that an increase in body weight in neonates results in a larger increase in busulfan clearance than an increase in bodyweight in older children or adults. The maturation of activity and expression of glutathione S-transferese have been studied in enterocytes and after oral administration of busulfan [13, 46, 47]. Assuming that the expression and activity of liver and enterocyte enzymes show similar developmental patterns, the non-linear relation of body weight and clearance could be related to changes in maturation rate of glutathione S-transferase as described in these articles. The effect might also relate to differences in liver volume, blood flow and biliary functions in young infants as compared to adults [48, 49]. This relation between body weight and clearance could not be estimated using an allometric function with a single scaling exponent, an approach that

is commonly applied - also in busulfan PK studies [17, 19, 20]. In this dataset, the allometric function with a single scaling exponent of 0.89 on body weight overestimated the clearance of busulfan up to 1.5 times in patients < 10 kg and > 40 kg, and underestimated the clearance up to 1.25 times in patients between 10-40 kg. In this meta-analyis we characterized the clearance of busulfan using an allometric function with a scaling exponent that varied with body weight from 1.2 in neonates to 0.55 in young adults. These values are very similar to a recent study of busulfan in children which showed a single scaling exponents 1.25 in children <9 kg and 0.76 >9kg [50]. In studies using other compounds varying (single) scaling exponents are published of 1.3-1.5 in neonates to 0.56 in adults [34-36], such despite the fact that different routes of metabolism are concerned. Literature data suggest that scaling of PK-parameters between children could be performed using an allometric scaling function with a fixed exponent of 0.75 or 1 for bodyweight and subsequently estimating a function that describes maturation processes as a function of age [51]. However, postnatal age and body weight are highly correlated. Therefore, in the current analysis, one single function based on bodyweight was identified, which would adequately describe the pharmacokinetics of busulfan from neonates to young adults. It would be of interest to test this function for other drugs when studying the effects of maturation in the whole pediatric age range including neonates.

After inclusion of body weight in the model, the disease-group (e.g. immune deficiencies) was not a significant covariate while also the inter-individual variability was similar between the four groups. In our cohort, the patients treated for malignancies or bone marrow failures were older and heavier in comparison with immune deficiencies, or inborn errors of metabolism. While in other studies, the disease group (i.e. diseases like immune deficiencies or lysosomal storage disease) has been reported a covariate for busulfan PK [13, 21-23, 33] we suggest that body weight may be an effect modifier in the relation between busulfan-PK [21-23]. As Glutathione S-transferases play an essential protective role against reactive oxygen species [52], many drugs could interact with busulfan. However, no significant interactions were identified in this large dataset, but drugs like metronidazole and phenytoin which have shown to influence busulfan PK in previous studies [30-32], could not be studied in full extent in this dataset due to use in <10% of patients. Moreover, neither clinical

chemistry data nor blood counts showed any biomarkers which could predict variability between patients. This analysis shows that the nature of the body weight-dependent pharmacokinetics of busulfan, including the smallest and heaviest patients, should be adequately characterized first. Only thereafter, other less influential covariate relationships like disease can be studied, to overcome confounding by maturation.

In this study, 15% day-to-day variability of busulfan clearance between the four days of administration was shown, which is only slightly higher than 5-10% shown in adults [19, 49]. In addition we showed that busulfan clearance was 12% lower at day 2-4 compared to day 1. This finding is consistent with results of a study in children with thalassemia in which a decrease of 11% in clearance between the first and subsequent doses was found [41]. Other studies did not report a significant decrease in busulfan AUC on following days [15, 16]. Yeh *et al*. reported that the concurrent use of fludarabine might decrease the busulfan clearance from day 1 to day 4 [53]. However, in the current study, the difference in clearance between consecutive days was not significantly altered in fludarabine as compared to non-fludarabine users. While the decrease in clearance deserves further study, it should be accounted for in the design of new dosing algorithms and when performing TDM.

Based on the final PK-model, a body weight dependent-dosing nomogram was defined (figure 3). The nomogram leads to a smaller range in predicted AUC's between patients of different body weights than the currently approved dose in the EMA-summary of product characteristics [30] or BSA-based dosing nomogram of 130mg/m^2 as shown in table 4. The currently approved dose leads to deviations in exposures, especially in children near the edges of each dosing category. A limitation of our meta-analysis is however, that only 12 patients >65 kg were included and therefore dose recommendations for patients >65 kg could not be provided based on these analyses.

Table 4 also demonstrates that a dose of 130 mg/m^2 [22, 43] results in overdosing up to 220% in patients < 0.5 m^2 and should therefore not be used. Even though the model-based nomogram takes into account variation from differences in bodyweight, the remaining unexplained inter-occasion variability in apparent clearance in the final PK-model is 15% and the unexplained inter-individual

variability 27%. Validation studies are needed to establish the predictive performance of the model and dosing regimen. Based on these considerations, we advocate that combined with the new dosing nomogram, TDM remains needed.

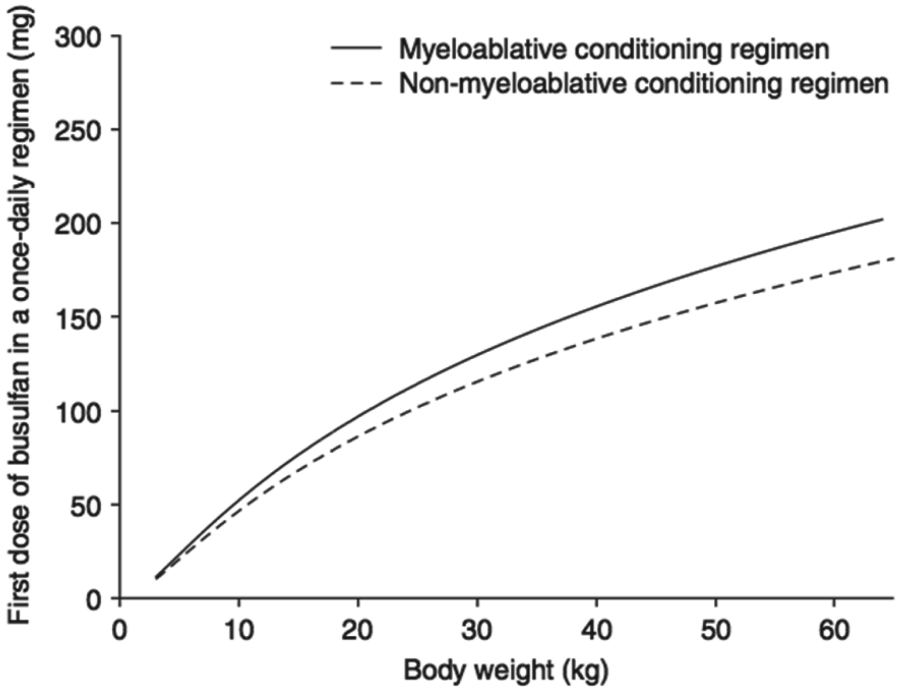


Figure 3: Model-based individualized dosing nomogram of busulfan related to the body weight of the patient, aiming for a myeloablative (AUC_{day0-4} of 90 mg*h/L[a] in combination with fludarabine) (*grey line*) and a non-myeloablative conditioning regimen (AUC_{day0-3} of 60 mg*h/L) (*dotted black line*).

The model-based dosing nomogram described in this article targets a narrow AUC_{day0-4} of 90 mg*h/L (≈5400 μM*min/day) in myeloablative and 60 mg*h/L in non-myeloablative conditioning regimens, in a platform with a (preferably non-alkylating) immunosuppressive agent (e.g. fludarabine) [45, 54, 55]. In the literature, different values for optimal AUC's have been proposed for patients with varying underlying diseases, disease severity and differences in concomitant medications given during the conditioning regimens [12, 15, 22, 24, 40, 41]. Comparing busulfan exposures between studies should be performed with care, as AUC's may have been calculated using different

sampling schedules and different methods. Most pediatric literature data are based on conditioning regimens containing busulfan and cyclophosphamide, resulting in a total AUC_{day0-4} of approximately 80 mg*h/L in children [24, 41]. We recently showed that busulfan with a target AUC_{day0-4} between 80 and 100 mg*h/L combined with fludarabine (n=40) was as effective, but less toxic in comparison with Busulfan-cyclophosphamide-melphalan (n=45) [56]. Also adult data shows that busulfan-fludarabine targeted to an AUC_{day0-4} < 100 mg*h/L results in optimal outcome [55, 57]. In children, a target AUC_{day0-4} of approximately 45-65 mg*h/L has been published using busulfan-fludarabine as a non-myeloablative conditioning regimen [40, 58]. Perhaps in the future, additional agents may be added to this busulfan-fludarabine combination to enhance the anti-leukemia effect (e.g. clofarabine, which was shown *in vitro* to have a synergistic anti-leukemic effect) [59, 60]. These developments and optimizations will lead to further individualization of the target exposures to busulfan. Using the structural parameters of this PK-model, simulations can be performed leading to a dosing nomogram that can target any desired AUC for busulfan.

In conclusion, in this population pharmacokinetic model for busulfan in patients ranging between 1 month and 26 years of age, body-weight was the most predictive covariate for all PK-parameters of busulfan and explained a major part of the observed inter-individual variability. The model-derived individualized dosing nomogram is expected to result in predictive busulfan exposures in patients ranging between 3 and 65 kg when combined with TDM, resulting in a safer and more effective HSCT in children.

I.5. Acknowledgements

We would like to thank T.J. van Steeg, C.van Kesteren, J.H. den Breeijen, J.Den Hartigh, M. Ansari, S. Davenport and H. van den Hoek for their valuable input.
This pharmacokinetic meta-analysis was performed within the framework of the Dutch Top Institute Pharma project number D2-104. The authors have no other conflicts of interest to disclose.

Appendix:

Appendix 1: Equations:

Model of random variability:

$$P_{ig} = P_{pop} \times e^{\eta i + \kappa ig} \quad \text{(eq. 1)}$$

Equation 1 describes the inter-individual variability and day-to day (inter-occasion) variability[27] of the structural parameters within the population, in which lognormal distribution was assumed. P_{ig} represents the individual PK-parameter for subject *i* on occasion *g*. P_{pop} is the typical value of the population PK-parameter. An occasion (g) was defined as all measurements performed in 1 day, κ_{ig} is the random effect between days. η and κ are random variables that follow the normal distribution with a mean value of 0 and variance ω^2 and π^2, respectively.

$$\log C_{ij} = \log C_{pred\,ij} + \varepsilon \quad \text{(eq. 2)}$$

Equation 2 describes the intra-individual variability; the differences between the observed and predicted concentrations. This residual error includes among other factors, model misspecification and measurement errors. The intra-individual variability was modeled using an additive error, equivalent to a proportional error model in the untransformed scale. C_{ij}, is the observed concentration for subject i at time j, and C_{predij} is the predicted concentration for individual *i* at time *j*. ε is a random variable that follows the normal distribution with a mean value of 0 and variance σ^2.

Other model-equations.

$$Cl_{day_1} = CL_{pop} \times (1 - fraction_{day_{2-4}}) \quad \text{(eq. 3)}$$

Equation 3 describes the clearance at $day_{2\text{-}4}$, estimated as a fraction of clearance at day_1.

Cl_{day1} is the typical value of clearance at day 1. Cl_{pop} is the typical value of clearance. Fraction day2-4 is the clearance at $day_{2\text{-}4}$ expressed as reduction factor of day_1.

Covariate functions:

The nature of the influence of continuous covariates on each PK parameter was tested using a linear (eq.4) and allometric (eq. 5) function**:**

$$P_i = P_{pop} \times \left(\frac{Cov_i}{Cov_{mean}} \right)^1 \quad \text{(eq. 4)}$$

In equation 4, P_i is the individual parameter for subject *i* with Cov_i. P_{pop} is the typical value of the population PK-parameter. Cov_i represents the covariate such as body weight, BSA or age for subject i and Cov_{mean} represents the mean value of the covariate.

$$P_i = P_{pop} \times \left(\frac{Cov_i}{Cov_{mean}} \right)^{L1} \quad \text{(eq. 5)}$$

In equation 5, P_i is the individual parameter for subject *i* with Cov_i. P_{pop} is the typical value of the population PK-parameter. Cov_i represents the covariate such as body weight, BSA or age for subject i and Cov_{mean} represents the mean value of the covariate. L1 represents the scaling exponent of the allometric function, which is one fixed estimated value in case of an allometric function with a single scaling exponent.

$$P_i = P_{pop} \times \left(\frac{Cov_i}{Cov_{mean}} \right)^{L2 \times Cov_i^M} \quad \text{(eq. 6)}$$

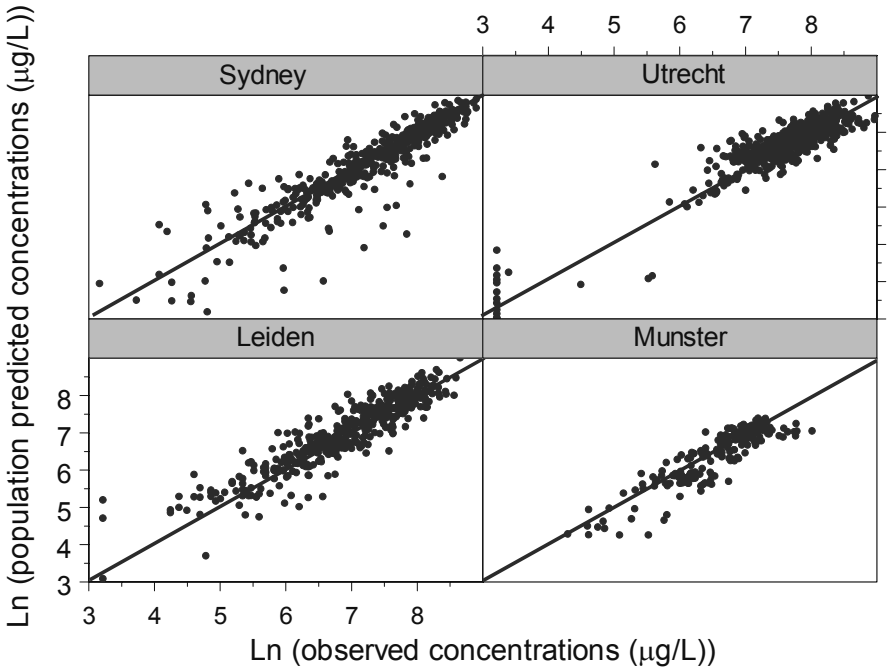
In equation 6, the allometric function with a scalingexponent that varies with body weight, BSA or age is shown, in which P_i is the individual parameter for subject *i* with Cov_i. P_{pop} is the typical value of the population PK-parameter. Cov_i represents the covariate such as body weight, BSA or age for subject i and Cov_{mean} represents the mean value of the covariate. In the scaling exponent, L2 represents the intercept and M is the exponent, which allows the scaling exponent to change with the covariate body weight, BSA or age.

Potential categorical variables were modeled using:

$$P_i = P_{pop} \times P_c^{CCov} \quad \text{(eq. 7)}$$

In equation 7, CCov is the categorical covariate, P_i is the individual parameter for subject *I,* P_{pop} is the typical value of the population PK-parameter in absence of the covariate of interest (CCov=0) and P_c is the fractional change in the typical value of the PK-parameter caused by the covariate.

Appendix 2: Population predicted concentrations versus observed concentrations of the four participating centers.



References

1. Ciurea SO, Andersson BS. Busulfan in hematopoietic stem cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2009; 15: 523-36.
2. Thomas ED, Buckner CD, Banaji M, Clift RA, Fefer A, Flournoy N, Goodell BW, Hickman RO, Lerner KG, Neiman PE, Sale GE, Sanders JE, Singer J, Stevens M, Storb R, Weiden PL. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. Blood 1977; 49: 511-33.
3. Lu C, Braine HG, Kaizer H, Saral R, Tutschka PJ, Santos GW. Preliminary results of high-dose busulfan and cyclophosphamide with syngeneic or autologous bone marrow rescue. Cancer treatment reports 1984; 68: 711-7.
4. Santos GW, Tutschka PJ, Brookmeyer R, Saral R, Beschorner WE, Bias WB, Braine HG, Burns WH, Elfenbein GJ, Kaizer H, et al. Marrow transplantation for acute nonlymphocytic leukemia after treatment with busulfan and cyclophosphamide. The New England journal of medicine 1983; 309: 1347-53.
5. Shaw PJ, Nath C, Berry A, Earl JW. Busulphan given as four single daily doses of 150 mg/m2 is safe and effective in children of all ages. Bone marrow transplantation 2004; 34: 197-205.
6. Michel G, Gluckman E, Esperou-Bourdeau H, Reiffers J, Pico JL, Bordigoni P, Thuret I, Blaise D, Bernaudin F, Jouet JP, et al. Allogeneic bone marrow transplantation for children with acute myeloblastic leukemia in first complete remission: impact of conditioning regimen without total-body irradiation--a report from the Societe Francaise de Greffe de Moelle. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1994; 12: 1217-22.
7. Hassan M, Ljungman P, Bolme P, Ringden O, Syruckova Z, Bekassy A, Stary J, Wallin I, Kallberg N. Busulfan bioavailability. Blood 1994; 84: 2144-50.
8. Bleyzac N, Souillet G, Magron P, Janoly A, Martin P, Bertrand Y, Galambrun C, Dai Q, Maire P, Jelliffe RW, Aulagner G. Improved clinical outcome of paediatric bone marrow recipients using a test dose and Bayesian pharmacokinetic individualization of busulfan dosage regimens. Bone marrow transplantation 2001; 28: 743-51.
9. McCune JS, Gibbs JP, Slattery JT. Plasma concentration monitoring of busulfan: does it improve clinical outcome? Clin Pharmacokinet 2000; 39: 155-65.
10. Slattery JT, Clift RA, Buckner CD, Radich J, Storer B, Bensinger WI, Soll E, Anasetti C, Bowden R, Bryant E, Chauncey T, Deeg HJ, Doney KC, Flowers M, Gooley T, Hansen JA, Martin PJ, McDonald GB, Nash R, Petersdorf EW, Sanders JE, Schoch G, Stewart P, Storb R, Sullivan KM, Thomas ED, Witherspoon RP, Appelbaum FR. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. Blood 1997; 89: 3055-60.
11. Bhagwatwar HP, Phadungpojna S, Chow DS, Andersson BS. Formulation and stability of busulfan for intravenous administration in high-dose chemotherapy. Cancer chemotherapy and pharmacology 1996; 37: 401-8.
12. Kletzel M, Jacobsohn D, Duerst R. Pharmacokinetics of a test dose of intravenous busulfan guide dose modifications to achieve an optimal area under the curve of a single daily dose of intravenous busulfan in children undergoing a reduced-intensity conditioning regimen with hematopoietic stem cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2006; 12: 472-9.
13. Poonkuzhali B, Chandy M, Srivastava A, Dennison D, Krishnamoorthy R. Glutathione S-transferase activity influences busulfan pharmacokinetics in patients with beta thalassemia major undergoing bone marrow transplantation. Drug metabolism and disposition: the biological fate of chemicals 2001; 29: 264-7.
14. Russell JA, Kangarloo SB. Therapeutic drug monitoring of busulfan in transplantation. Current pharmaceutical design 2008; 14: 1936-49.
15. Tse WT, Duerst R, Schneiderman J, Chaudhury S, Jacobsohn D, Kletzel M. Age-dependent pharmacokinetic profile of single daily dose i.v. busulfan in children undergoing reduced-intensity conditioning stem cell transplant. Bone marrow transplantation 2009; 44: 145-56.

16. Wall DA, Chan KW, Nieder ML, Hayashi RJ, Yeager AM, Kadota R, Przepiorka D, Mezzi K, Kletzel M, Pediatric Blood MTC. Safety, efficacy, and pharmacokinetics of intravenous busulfan in children undergoing allogeneic hematopoietic stem cell transplantation. Pediatric blood & cancer 2010; 54: 291-8.
17. Zwaveling J, Press RR, Bredius RG, van Derstraaten TR, den Hartigh J, Bartelink IH, Boelens JJ, Guchelaar HJ. Glutathione S-transferase polymorphisms are not associated with population pharmacokinetic parameters of busulfan in pediatric patients. Ther Drug Monit 2008; 30: 504-10.
18. Bartelink IH, Bredius RG, Ververs TT, Raphael MF, van Kesteren C, Bierings M, Rademaker CM, den Hartigh J, Uiterwaal CS, Zwaveling J, Boelens JJ. Once-daily intravenous busulfan with therapeutic drug monitoring compared to conventional oral busulfan improves survival and engraftment in children undergoing allogeneic stem cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2008; 14: 88-98.
19. Trame MN, Bartelink IH, Boos J, Boelens JJ, Hempel G. External evaluation of a population pharmacokinetic model for dosing busulfan in children- body surface area better than body weight. In: Population Approach in Europe (PAGE) wwwpage-meetingorg/defaultasp?abstract=1909 2010, 2010.
20. Nguyen L, Fuller D, Lennon S, Leger F, Puozzo C. I.V. busulfan in pediatrics: a novel dosing to improve safety/efficacy for hematopoietic progenitor cell transplantation recipients. Bone marrow transplantation 2004; 33: 979-87.
21. Vassal G, Fischer A, Challine D, Boland I, Ledheist F, Lemerle S, Vilmer E, Rahimy C, Souillet G, Gluckman E, et al. Busulfan disposition below the age of three: alteration in children with lysosomal storage disease. Blood 1993; 82: 1030-4.
22. Nath CE, Earl JW, Pati N, Stephen K, Shaw PJ. Variability in the pharmacokinetics of intravenous busulphan given as a single daily dose to paediatric blood or marrow transplant recipients. Br J Clin Pharmacol 2008; 66: 50-9.
23. Bertholle-Bonnet V, Bleyzac N, Galambrun C, Mialou V, Bertrand Y, Souillet G, Aulagner G. Influence of underlying disease on busulfan disposition in pediatric bone marrow transplant recipients: a nonparametric population pharmacokinetic study. Ther Drug Monit 2007; 29: 177-84.
24. Bartelink IH, Bredius RG, Belitser SV, Suttorp MM, Bierings M, Knibbe CA, Egeler M, Lankester AC, Egberts AC, Zwaveling J, Boelens JJ. Association between busulfan exposure and outcome in children receiving intravenous busulfan before hematologic stem cell transplantation. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2009; 15: 231-41.
25. Oechtering D, Schiltmeyer B, Hempel G, Schwab M, Wurthwein G, Murdter T, Klingebiel T, Vormoor J, Gruhn B, Fleischack G, Boos J. Toxicity and pharmacokinetics of i.v. busulfan in children before stem cell transplantation. Anti-cancer drugs 2005; 16: 337-44.
26. Bergstrand M, Karlsson MO. Handling data below the limit of quantification in mixed effect models. The AAPS journal 2009; 11: 371-80.
27. Karlsson MO, Sheiner LB. The importance of modeling interoccasion variability in population pharmacokinetic analyses. Journal of pharmacokinetics and biopharmaceutics 1993; 21: 735-50.
28. Wilkins JJ. NONMEMory: a run management tool for NONMEM. Computer methods and programs in biomedicine 2005; 78: 259-67.
29. Karlsson MO, Savic RM. Diagnosing model diagnostics. Clin Pharmacol Ther 2007; 82: 17-20.
30. Pierre Fabre Médicament Production sAPI. Product information: Busilfex (R),busulfan. In, 2007.
31. Hassan M, Oberg G, Bjorkholm M, Wallin I, Lindgren M. Influence of prophylactic anticonvulsant therapy on high-dose busulphan kinetics. Cancer chemotherapy and pharmacology 1993; 33: 181-6.
32. Nilsson C, Aschan J, Hentschke P, Ringden O, Ljungman P, Hassan M. The effect of metronidazole on busulfan pharmacokinetics in patients undergoing hematopoietic stem cell transplantation. Bone marrow transplantation 2003; 31: 429-35.
33. Browning B, Thormann K, Donaldson A, Halverson T, Shinkle M, Kletzel M. Busulfan dosing in children with BMIs >/= 85% undergoing HSCT: a new optimal strategy. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2011; 17: 1383-8.

34. van den Broek MP, Huitema AD, van Hasselt JG, Groenendaal F, Toet MC, Egberts TC, de Vries LS, Rademaker CM. Lidocaine (lignocaine) dosing regimen based upon a population pharmacokinetic model for preterm and term neonates with seizures. Clin Pharmacokinet 2011; 50: 461-9.
35. Wang C, Peeters MY, Allegaert K, Blusse van Oud-Alblas HJ, Krekels EH, Tibboel D, Danhof M, Knibbe CA. A Bodyweight-Dependent Allometric Exponent for Scaling Clearance Across the Human Life-Span. Pharm Res 2012.
36. Knibbe CA, Krekels EH, van den Anker JN, DeJongh J, Santen GW, van Dijk M, Simons SH, van Lingen RA, Jacqz-Aigrain EM, Danhof M, Tibboel D. Morphine glucuronidation in preterm neonates, infants and children younger than 3 years. Clin Pharmacokinet 2009; 48: 371-85.
37. Wahlby U, Jonsson EN, Karlsson MO. Comparison of stepwise covariate model building strategies in population pharmacokinetic-pharmacodynamic analysis. AAPS pharmSci 2002; 4: E27.
38. Krekels EH, van Hasselt JG, Tibboel D, Danhof M, Knibbe CA. Systematic evaluation of the descriptive and predictive performance of paediatric morphine population models. Pharm Res 2011; 28: 797-811.
39. Lindbom L, Pihlgren P, Jonsson EN. PsN-Toolkit--a collection of computer intensive statistical methods for non-linear mixed effect modeling using NONMEM. Computer methods and programs in biomedicine 2005; 79: 241-57.
40. Pulsipher MA, Boucher KM, Wall D, Frangoul H, Duval M, Goyal RK, Shaw PJ, Haight AE, Grimley M, Grupp SA, Kletzel M, Kadota R. Reduced-intensity allogeneic transplantation in pediatric patients ineligible for myeloablative therapy: results of the Pediatric Blood and Marrow Transplant Consortium Study ONC0313. Blood 2009; 114: 1429-36.
41. Gaziev J, Nguyen L, Puozzo C, Mozzi AF, Casella M, Perrone Donnorso M, Gravina P, Sodani P, Marziali M, Isgro A, Simone MD, Andreani M, Formosa A, Testi M, Federici G, Bernardini S, Lucarelli G. Novel pharmacokinetic behavior of intravenous busulfan in children with thalassemia undergoing hematopoietic stem cell transplantation: a prospective evaluation of pharmacokinetic and pharmacodynamic profile with therapeutic drug monitoring. Blood 2010; 115: 4597-604.
42. Vassal G, Michel G, Esperou H, Gentet JC, Valteau-Couanet D, Doz F, Mechinaud F, Galambrun C, Neven B, Zouabi H, Nguyen L, Puozzo C. Prospective validation of a novel IV busulfan fixed dosing for paediatric patients to improve therapeutic AUC targeting without drug monitoring. Cancer chemotherapy and pharmacology 2008; 61: 113-23.
43. Gordon N, Mullen CA, Tran H, Worth L, Gomez Almaguer D, Chan KW. Fludarabine and once-daily intravenous busulfan for allogeneic bone marrow transplantation for Chediak-Higashi syndrome. Journal of pediatric hematology/oncology 2003; 25: 824-6.
44. Vassal G, Deroussent A, Challine D, Hartmann O, Koscielny S, Valteau-Couanet D, Lemerle J, Gouyette A. Is 600 mg/m2 the appropriate dosage of busulfan in children undergoing bone marrow transplantation? Blood 1992; 79: 2475-9.
45. de Lima M, Couriel D, Thall PF, Wang X, Madden T, Jones R, Shpall EJ, Shahjahan M, Pierre B, Giralt S, Korbling M, Russell JA, Champlin RE, Andersson BS. Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. Blood 2004; 104: 857-64.
46. Gibbs JP, Murray G, Risler L, Chien JY, Dev R, Slattery JT. Age-dependent tetrahydrothiophenium ion formation in young children and adults receiving high-dose busulfan. Cancer research 1997; 57: 5509-16.
47. Gibbs JP, Liacouras CA, Baldassano RN, Slattery JT. Up-regulation of glutathione S-transferase activity in enterocytes of young children. Drug metabolism and disposition: the biological fate of chemicals 1999; 27: 1466-9.
48. Kanamori M, Takahashi H, Echizen H. Developmental changes in the liver weight- and body weight-normalized clearance of theophylline, phenytoin and cyclosporine in children. Int J Clin Pharmacol Ther 2002; 40: 485-92.
49. Ginsberg G, Hattis D, Sonawane B, Russ A, Banati P, Kozlak M, Smolenski S, Goble R. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. Toxicological sciences : an official journal of the Society of Toxicology 2002; 66: 185-200.
50. Paci A, Vassal G, Moshous D, Dalle JH, Bleyzac N, Neven B, Galambrun C, Kemmel V, Abdi ZD, Broutin S, Petain A, Nguyen L. Pharmacokinetic behavior and appraisal of intravenous busulfan dosing in infants and older children: the results of a population pharmacokinetic study from a large pediatric cohort undergoing hematopoietic stem-cell transplantation. Ther Drug Monit 2012; 34: 198-208.

51. Anderson BJ, Holford NH. Mechanism-based concepts of size and maturity in pharmacokinetics. Annu Rev Pharmacol Toxicol 2008; 48: 303-32.

52. Orrenius S, MoldÈus P. The multiple roles of glutathione in drug metabolism. Trends in pharmacological sciences 1984; 5: 432-35.

53. Yeh RF, Pawlikowski MA, Blough DK, McDonald GB, O'Donnell PV, Rezvani A, Deeg HJ, McCune JS. Accurate targeting of daily intravenous busulfan with 8-hour blood sampling in hospitalized adult hematopoietic cell transplant recipients. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2012; 18: 265-72.

54. Andersson BS, de Lima M, Thall PF, Wang X, Couriel D, Korbling M, Roberson S, Giralt S, Pierre B, Russell JA, Shpall EJ, Jones RB, Champlin RE. Once daily i.v. busulfan and fludarabine (i.v. Bu-Flu) compares favorably with i.v. busulfan and cyclophosphamide (i.v. BuCy2) as pretransplant conditioning therapy in AML/MDS. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2008; 14: 672-84.

55. Russell JA, Tran HT, Quinlan D, Chaudhry A, Duggan P, Brown C, Stewart D, Ruether JD, Morris D, Glick S, Gyonyor E, Andersson BS. Once-daily intravenous busulfan given with fludarabine as conditioning for allogeneic stem cell transplantation: study of pharmacokinetics and early clinical outcomes. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2002; 8: 468-76.

56. Flinsenberg TWH, Bartelink IH, Ververs TFTT, Bierings M, Boelens JJ. Busulfan+Fludarabine, An Effective And Low Toxic Regimen In Children With Malignant And Non-Malignant Diseases. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2010; 16: S244.

57. Geddes M, Kangarloo SB, Naveed F, Quinlan D, Chaudhry MA, Stewart D, Savoie ML, Bahlis NJ, Brown C, Storek J, Andersson BS, Russell JA. High busulfan exposure is associated with worse outcomes in a daily i.v. busulfan and fludarabine allogeneic transplant regimen. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2008; 14: 220-8.

58. Güngör T, Schanz U, Seger R, Albert M, Hassan M. Successful Half-Dose Busulfan/Full-Dose Fludarabine Based Reduced Intensity Conditioning In High-Risk Pediatric And Adult Chronic Granulomatous Disease (CGD) Patients. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2010; 16: S181-S82.

59. Worth LL, Andersson BS, Kazerooni R, Petropoulos D, Kelly SS, Lee DA, Du M, Madden TL, deLima MJ, Champlin RE, Cooper LJN. Thiotepa (TT), Busulfan (Bu), And Clofarabine (Clo) As A Conditioning Therapy For Allogeneic Hematopoetic Stem Cell Transplant For Patients With High Risk Malignancies: Early Response And Engraftment Data. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2010; 16: S292-S93.

60. Andersson BS, de Lima M, Valdez BC, Thall PF, Worth LL, Popat U, Jones RB, Shpall EJ, Madden T, McAdams PL, Alousi AM, Rondon G, Kebriaei P, Champlin RE. Clofarabine±Fludarabine With IV Busulfan And Allogeneic Stem Cell Transplantation For Relapsed, Refractory Myeloid Leukemia (ML) And MDS. Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation 2010; 16: S271-S72.