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A semi-mechanistic model based on glutathione depletion to describe intra-individual reduction in busulfan clearance

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1 Abstract

1.1 Aim

To develop a semi-mechanistic model, based on glutathione depletion and predict a previously identified intra-individual reduction in busulfan clearance to aid in more precise dosing.

1.2 Methods

Busulfan concentration data, measured as part of regular care for allogeneic hematopoietic cell transplantation (HCT) patients, were used to develop a semi-mechanistic model and compare it to a previously developed empirical model. The latter included an empirically estimated time-effect, where the semi-mechanistic model included theoretical glutathione depletion. As older age has been related to lower glutathione levels, this was tested as a covariate in the semi-mechanistic model. Lastly, a therapeutic drug monitoring (TDM) simulation was performed comparing the two models in target attainment.

1.3 Results

In both models, a similar clearance decrease of 7% (range: -82% to 44%), with a proportionality to busulfan metabolism, was found. After 40 years of age, the time-effect increased with 4% per year of age (0.6-8%, $p = 0.009$), causing the effect to increase more than a 2-fold over the observed age-range (0-73 years). Compared to the empirical model, the final semi-mechanistic model increased target attainment from 74% to 76%, mainly through better predictions for adult patients.

1.4 Conclusion

These results suggest that the time-dependent decrease in busulfan clearance may be related to glutathione depletion. This effect increased with older age (>40 years) and was proportional

to busulfan metabolism. The newly constructed semi-mechanistic model could be used to further improve TDM-guided exposure target attainment of busulfan in patients undergoing HCT.

2 What is already known about this subject

- Busulfan has a narrow therapeutic window, necessitating precise exposure-guided dosing.
- Within-patient busulfan clearance varies over time, compromising the efficacy of pharmacokinetic guided dose adjustments.

3 What this study adds

- Intra-individual decrease of busulfan clearance is proportional to busulfan metabolism.
- Older age (> 40 years) is associated with a stronger time-dependent decrease.
- Therapeutic drug monitoring can be further improved by using a newly developed semi-mechanistic model implementing aforementioned effects.

4 Introduction

Allogeneic hematopoietic cell transplantation (HCT) is a high-risk, but potentially curative treatment for a variety of malignant and nonmalignant hematological disorders. Unfortunately, treatment related mortality is substantial (10-40%), implying an urgent need of further optimization of this procedure[1].

Prior to HCT, the bone marrow and immune system of the host are ablated by means of a preparative conditioning regimen. In these conditioning regimens, busulfan is the most frequently used drug[2]. Busulfan is usually administered over a 4-day period and has a narrow therapeutic window, where an exposure corresponding with an area under the plasma-concentration-time curve (AUC) from the first dose until infinity ($AUC_{t_0-\infty}$) of 80 – 100mg·h/L ($\approx 20000 - 25000\mu\text{Mol} \cdot \text{min}$) has been associated with optimal treatment outcomes in a myeloablative setting[3–5]. Lower exposures have been associated with more frequent relapse or graft failure, and higher exposures with more often severe toxicity and treatment related mortality.

Therapeutic drug monitoring (TDM)-guided dosing is recommended to better attain this narrow target exposure[6]. Indeed, the use of TDM has been proven to increase overall survival by 20% compared to fixed dosing in a randomized controlled trial setting[7]. However, the attainment of the desired busulfan target exposure is still challenging due to an intra-individually variable clearance reduction from day 1 to day 4 with associated variability of 11-15% as has previously been shown[8–10]. These effects respectively limit the accuracy and precision of TDM based on samples measured at the first day of conditioning. As the mechanism behind this time-dependent decrease is unknown, the effect has been implemented empirically, where a more mechanistic approach may better predict inter-individual differences in clearance-reduction.

The primary route of busulfan clearance is through extensive metabolism in the liver: only 2% of busulfan is excreted unchanged in urine. Initial inactivation occurs by conjugation to glutathione (GSH), both spontaneously and aided by the enzyme[11,12]. The busulfan-GSH conjugate is further metabolized via two parallel routes: β -elimination, catalyzed by cystathionine γ -lyase, forming tetrahydrothiophene, pyruvate and ammonium; or through conversion to an N-acetylated cysteine conjugate by N-acetyltransferase. Polymorphisms of the enzyme glutathione-S-transferase (GST) have been used to better predict busulfan clearance *a priori*[13–18], but such predictions are obviated by the use of TDM. Interestingly, it has also been shown that, in patients treated with high dose busulfan, levels of the substrate GSH decrease by approximately 75%[19]. In addition, higher baseline GSH concentrations were correlated with an up to 2-fold increased busulfan-clearance[19]. Therefore, we hypothesize that busulfan-mediated GSH-depletion causes the observed reduction in clearance. In lack of GSH concentration-time-data, a fully mechanistic approach to test this hypothesis was not possible. Nevertheless, patients with a high initial busulfan clearance may exhibit a higher decrease in clearance, following from more pronounced GSH-depletion. This hypothesis was explored in a semi-mechanistic population pharmacokinetic model, ultimately aiming to achieve more predictable busulfan exposure, and thus more predictable outcomes.

5 Methods

5.1 Patients

Included patients were those who received (non)myeloablative conditioning before HCT, between September 2005 and January 2017 at the UMC Utrecht and of whom plasma concentration data were available. Data consisted of plasma concentrations, measured as part of regular care for HCT patients. The dataset then contained all UMC patients included for the previously developed empirical model[8], which contained data up to 2008, plus all adult

patients and children transplanted after September 2009. Patients were included after written informed consent was acquired. Ethical approval by the institutional medical ethics committee of the UMC Utrecht was obtained under protocol number 11/063.

5.2 Procedures

The conditioning regimen consisted of intravenous busulfan combined with either fludarabine (+/- clofarabine) or cyclophosphamide. In selected patients transplanted before 2011, targeted busulfan was combined with cyclophosphamide at a cumulative dose of 120 or 200 mg/kg. Four days of busulfan were followed by 2 days (120 mg/m²) or 4 days (200 mg/m²) of cyclophosphamide, starting on day -7 and -9 respectively. Busulfan and fludarabine conditioning was administered on day -5 to -2 relative to HCT and consisted of a 1-hour-infusion of fludarabine-phosphate (40 mg/m²) directly followed by a 3-hour-infusion of busulfan (Busilvex, Pierre Fabre). A 1-hour infusion of clofarabine (30 mg/m²) preceded a reduced dose of fludarabine (10 mg/m²) in children with hematological malignancies. Rabbit anti-thymocyte globulin rabbit anti-thymocyte globulin (rATG) was added in the unrelated donor HCT setting: 4-hour infusions at 4 consecutive days from day -9 (10 mg/kg < 30kg, 7.5 mg/kg > 30kg) for children and 4 times a 12-hour infusion from day -12 (6 mg/kg) for adults. To patients receiving rATG, clemastine (0.03 mg/kg up to 2 mg), paracetamol (60 mg/kg up to 4g) and 2 mg/kg prednisolone with a maximum of 100 mg were given intravenously prior to rATG infusion. N-acetylcysteine was not routinely administered during conditioning.

Busulfan, was targeted using a dosing algorithm⁸ and TDM to a myeloablative cumulative 4-day exposure of 90mg·h/L ≈ 22000 μMol·min (current target), 80mg·h/L ≈ 20000 μMol·min

(target before 2011), $60\text{mg}\cdot\text{h}/\text{L} \approx 15000\mu\text{Mol}\cdot\text{min}$ (reduced intensity) or $30\text{mg}\cdot\text{h}/\text{L} \approx 7300\mu\text{Mol}\cdot\text{min}$ for Fanconi anemia patients (expressed as area under the curve for all doses [$AUC_{T_0-\infty}$]). According to the busulfan TDM protocol, plasma samples were drawn on the first and/or second day of conditioning. In case of large dose adjustments ($> 50\%$), samples were also drawn on subsequent days for confirmatory reasons. Additional samples were taken for all patients at day 4 of conditioning to evaluate target exposure attainment. In general, plasma samples were taken at 5 minutes, 1, 2, and 3 hours, after the end of busulfan infusion. For a subset of patients, additional samples were collected from 4 to 20 hours post-infusion. Samples were analyzed with a validated LC-MS method according to Langman et al.[20]. In children treated before September 2008 a previously published HPLC-UV method was used[21,22]. For dose adjustment, the individual clearance was estimated with a Bayesian approach using the individual samples and a 1-compartmental pharmacokinetic analysis in the software package of MwPharm[23]. The individual clearance was estimated and the doses for the subsequent days were calculated using equation 1.

$$Dose_{adjusted} = (Target(AUC_{t_0-\infty}) - \frac{Dose_{administered}}{CL_{estimated}}) \cdot CL_{estimated} \quad (1)$$

5.3 Pharmacokinetic model design and evaluation

The previously published model by Bartelink et al. was used as the basis for a structural model[8]. In that model, weight was included as a covariate on clearance and volume of distribution of the central compartment (V_1). Clearance was included using an empirical weight-changing allometric exponent (equation 2) and V_1 was described by a constant empirically estimated exponent. A stepwise effect of time on clearance was estimated using

separate values for $day = 1$ and $day > 1$. No further covariates were included.

$$CL_i = CL_{43kg} \cdot \frac{BW_i^{l \cdot BW_i^p}}{43kg} \quad (2)$$

In addition to this model, henceforth referred to as the empirical model, allometric scaling to weight was added for volume of distribution of the peripheral compartment (V_2) and inter-compartmental clearance between V_1 and V_2 (inter-compartmental clearance between V_1 and V_2), using fixed exponents of 1 and 0.75 respectively. Next, the stochastic model was optimized with a higher number of subjects. Inter-individual variability (IIV) and correlations between IIV were tested for clearance, V_1 , V_2 , and Q . Inter-occasion variability (IOV) was tested only on V_1 and clearance to preserve parsimony. Both IIV and IOV were assumed to be log-normally distributed. The proportional residual variability from the original model was retained, but variances estimated for the different methods of quantification (HPLC-UV and LC-MS) differed.

For the semi-mechanistic model, the above mentioned expanded empirical model was used as a basis, but without the empirical time-effect. A compartment was then added representing the relative amount of GSH available at any time, where the initial amount was assumed to be 1. The model assumed a zero-order synthesis and first-order elimination of GSH. As the relative amount of GSH at baseline is set at 1, the zero-order synthesis rate constant equals the first-order elimination rate constant at equilibrium. Busulfan in the central compartment was assumed to be metabolized in a GSH-dependent way. The full model is depicted in figure 1 and described in equations 3, 4, and 5.

$$\frac{dA_{bu_1}}{dt} = -A_{bu_1} \cdot (k_{bu_{10}} \cdot A_{GSH} + k_{12}) + A_{bu_2} \cdot k_{bu_{21}} \quad (3)$$

$$\frac{dA_{bu_2}}{dt} = A_{bu_1} \cdot k_{bu_{12}} - A_{bu_2} \cdot k_{bu_{21}} \quad (4)$$

$$\frac{dA_{GSH}}{dt} = \frac{S_{GSH}}{V_1} \cdot A_{GSH} \cdot k_{10} \cdot A_{bu_1} - k_{GSH_{baseline}} \cdot A_{GSH} + k_{GSH_{synthesis}} \quad (5)$$

Compartments bu_1 and bu_2 represent the central and peripheral compartments of busulfan respectively, and GSH represents the theoretical GSH compartment. The elimination and distribution constants for busulfan are depicted by k_{bu} . The first-order elimination constant for GSH is depicted by $k_{GSH_{baseline}}$ and the zero-order synthesis constant by $k_{GSH_{synthesis}}$. S_{GSH} is a scaling factor between busulfan metabolism and relevant GSH depletion.

As no GSH concentrations were available in the current analysis, busulfan metabolism was used as a surrogate marker and full GSH dynamics could not be re-constructed. Therefore the following assumptions were made: (1) the last two terms of equation 5 represent the endogenous GSH turnover and were assumed sum up to 0 at baseline; (2) when A_{GSH} decreases as a result of busulfan metabolism, the sum of endogenous turnover terms exceeds zero, resulting in net GSH production; it was assumed that this net synthesis was negligible compared to busulfan dependent depletion, thus equation 5 was simplified to equation 6.

$$\frac{dA_{GSH}}{dt} = \frac{S_{GSH}}{V_1} \cdot A_{GSH} \cdot k_{10} \cdot A_{bu_1} \quad (6)$$

The factor S_{GSH} as such, scales busulfan metabolism to relevant depletion of GSH. Relevance is defined as depletion to an extent that it becomes a limiting factor in busulfan clearance (as this drives estimation of S_{GSH}). As GSH was set to an absolute amount equal for all individuals (1 at baseline), a scaling factor was necessary for consistent GSH amounts relative to busulfan amounts, to account for the highly variable body size and concurrent dosing in the current dataset. Therefore, S_{GSH} was scaled to individual values for V_1 , thus assuming the volume of distribution for GSH to be proportional to V_1 of busulfan.

Age[24,25] has a reported relation to human GSH abundance and turn-over and was tested as a continuous covariate on S_{GSH} .

A population approach based on non-linear mixed-effects modeling was applied[26], using the software package NONMEM (version 7.3.0, Icon, Hanover, MD, USA). Pirana (version 2.9.5)

and R (version 3.3.3) were used for workflow management and data handling and visualization, respectively[27,28]. The stochastic approximation and estimation maximization (SAEM) and Monte Carlo importance sampling estimation maximization assisted by mode a posteriori estimation (IMPMP) as implemented in NONMEM, were respectively used for estimation and objective function calculation.

The structural and covariate model with corresponding estimates had to be scientifically and biologically plausible. A visual inspection of model performance was done through standard goodness-of-fit plots. Examples of these plots are observed concentrations plotted versus individual and population predicted concentrations, and conditional weighted residuals (CWRES) versus time and observed concentrations[29]. Particular emphasis was given to goodness-of-fit plots stratified for different days (occasions) to assess the time-dependent performance. Hierarchical models were statistically compared after backward deletion of the term or covariate of interest. This comparison was done by the objective function value (OFV)(Δ OFV), which follows a chi-square distribution. A Δ OFV of -3.84 then corresponds to a p-value of 0.05 for addition of one parameter (i.e. 1 degree of freedom).

Several other evaluation techniques were performed, all in accordance with European Medicine Agency (EMA) and Food and Drug Administration (FDA) guidelines for population pharmacokinetic analyses[30,31]. A sampling importance resampling (SIR) evaluation (final step: 2000 samples, 1000 resamples) was performed to estimate parameter precision. To assess the simulation properties, prediction-corrected visual predictive checks (VPCs) were created to judge predictive performance of the final model as compared to the observed concentrations. The prediction-corrected VPC allows for variability in dosing[32]. In this analysis, the observed concentration data and its median and 95% confidence interval (CI) were compared to 95% CI of the predicted mean, 2.5th and 95th percentile, derived from 1000 model simulations.

5.4 TDM-guided target attainment evaluation

This analysis aimed to compare the Bayesian forecasting properties of both models in a TDM setting. For this, all patients targeted to an $AUC_{t_0-\infty}$ of 90 mg*h/L with samples available on at least day 1 and 4 were included. TDM was simulated by using both models to predict busulfan clearance throughout conditioning, using only the samples available on day 1. Subsequently, equation 1 was used to calculate the required dose for days 2, 3 and 4. The predicted $AUC_{t_0-\infty}$ was calculated by using the post-hoc estimates estimated using all available pharmacokinetic data and dosing as calculated from the day 1-TDM simulations. The target $AUC_{t_0-\infty}$ attainment rates were assessed for both models.

5.5 Nomenclature of Targets and Ligands

Nomenclature of Targets and Ligands Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY.

6 Results

6.1 Patient characteristics

A total of 385 patients were included with a median age of 14 years (range 0.16-73), from whom 3994 samples were collected. Of these patients, 292 received busulfan targeted to 90 mg*h/L and 94 received doses targeted to a lower exposure (as described in methods section 5.2). Most patients (n=259) received 160 mg/m² fludarabine next to busulfan. Alternative conditioning consisted mostly of either 120 mg/m² clofarabine with 40 mg/m² fludarabine (n=68) or 120-200 mg/kg cyclophosphamide (n=54) in addition to the targeted busulfan. Serotherapy (rATG) was given to 78% (n=303) of patients. Detailed patient characteristics are

shown in table 1.

6.2 Pharmacokinetic models

6.2.1 Empirical model and exposure

Parameter estimates and 95% CI of the adjusted empirical model (as described in section 5.3) can be found in table 2. IIV on clearance, V_1 and V_2 were estimated at 14%, 19%, and 28% respectively. A correlation of 71% between IIV of V_1 and clearance was found. IOV was implemented on V_1 (11%) and clearance (11%). A proportional residual error was separately estimated for samples measured with UV (9.0%) and MS (6.6%). The population mean clearance for day>1 was estimated to be 7% (95% CI: 5-8%) lower than day 1. Compared to random IOV, the predicted decrease was limited illustrated by an estimated difference in clearance between day 1 and 4 ranging from 87% (increase) down to -44% (decrease) as is depicted in figure 2A. In figure 2B the intra-individual change in clearance from day 1 to 4 is depicted, stratified for tertiles of individual clearance relative to the population predicted clearance (equation 2). The figure suggests that patients with a relatively higher clearance compared to the population value ($CL_{relative}$) have a relatively stronger reduction in clearance compared to patient with a low $CL_{relative}$, who regularly had an increased busulfan clearance over time. No relationship was found between busulfan exposure measures and outcomes (graft-versus-host disease, graft failure, relapse, non-relapse mortality, and survival). Occurrence of veno-occlusive disease (VOD) or other hepatotoxicity events was not reported.

6.2.2 *Semi-mechanistic model*

The semi-mechanistic model was developed and final estimates with corresponding SIR-derived 95% CI are shown in table 2. A similar overall reduction of clearance was estimated compared to the empirical model (figure 3). However, with the semi-mechanistic model a gradual reduction in clearance was assumed as GSH was presumed to decrease proportional to busulfan metabolism. The S_{GSH} was estimated at 0.0026 h/mg, implying a net relevant GSH reduction of 0.26% per hour for each mg of busulfan metabolism scaled to 1 liter V_1 .

Age appeared to have an effect on the time-dependent decline of clearance (figure 4A), but the effect was only relevant above an age of 40 years ($p = 0.009$). The effect was modelled as a proportional increase of the S_{GSH} of 4% for each year of age (equation 7). This resulted in a more than 2-fold increase of the effect from 40 to 73 years of age. (figure 4B).

$$S_{GSH,i} = S_{GSH,pop} \cdot (1 + (Age_i - 40) * Slope_{Age}) \quad (7)$$

Herein, $Slope_{Age}$ was assumed to be 0 below 40 years of age and was estimated for patients older than 40.

rATG was tested on the time-dependent decline of clearance as a surrogate covariate for paracetamol usage, but no improvement in the model was found.

6.2.3 *Model evaluation*

Figure 5 depicts the goodness-of-fit plots for both models. In both models, no time-dependent trends could be observed. In the VPC stratified for days of conditioning no other

misspecifications were seen for either model (data not shown).

6.3 TDM-guided target attainment evaluation

258 patients were available for TDM simulation, of which the results are reported in figure 6. Overall, target attainment was slightly better when the semi-mechanistic model was used (top panel, 75%), compared to the empirical model (74%). The final model with age on the S_{GSH} further increased target attainment to 76%. Severe over-exposure (>25% above target) was similar for all models, while $AUC_{t_0-\infty}$ of > 25% below target was not simulated for any of the scenarios. Because of the apparent effect of age, a subset analysis was conducted in children and adults separately. Here it was found that in adults the semi-mechanistic model outperformed the empirical model: 80% vs 76% target attainment (figure 6: bottom panel).

7 Discussion

To our knowledge, this is the first pharmacokinetic model describing the decrease in busulfan clearance in a large cohort of both children and adult HCT recipients. We demonstrated an overall 7% decline in busulfan clearance over time, which was more pronounced in older adult patients (>40 years of age). The observed clearance reduction of busulfan is of clinical relevance due to a combination of the narrow therapeutic window and a large between-patient variation in clearance over time during the pre-HCT conditioning phase. Furthermore, patients with a high initial busulfan clearance showed a more pronounced decrease compared to patients with a lower initial clearance. For the same dose this would imply that patients with a lower exposure on day 1 (due to a higher clearance) may be overcorrected. Therefore, this metabolism-dependent clearance reduction should be taken into account for precise and

accurate targeting of busulfan using TDM. We hypothesized that this reduction in clearance is due to GSH depletion and constructed a semi-mechanistic model, which captured the metabolism-dependent clearance reduction well. Next to biology of GSH homeostasis and GST conjugation, the main arguments in support of the hypothesis are proportionality of the time-effect to busulfan metabolism and the increased effect in older age. In TDM simulations, adult patients showed the most improvement in target attainment using the semi-mechanistic model. Under-exposure occurred less in these patients, reducing the risk of relapse and graft failure. In the studied patient cohort, however, no direct relationship between busulfan exposure and outcome was found. TDM was applied for all included patients, causing the range of exposures to be more favorable than in studies where such exposure-outcome relationships were shown[3-5,7]. It's likely, however, that the still somewhat unfavorable exposures observed here would result in unfavorable outcome probability in a larger population, and/or increased non-lethal toxicity.

Besides the direct association between GSH levels and busulfan clearance[19], there is also indirect evidence from metabolomics. Glycine levels, an important substrate in GSH synthesis, were positively associated with busulfan clearance[33]. Also the age effect is supported by literature and can biologically be explained with older age (60-80 years) being associated with decreased GSH-synthesis and thereby absolute levels[24,25]. Here, we found a linear increase in theoretical GSH-depletion from the age of 40. Perhaps, the latter effect is caused by a relatively low initial GSH reservoir, which results in the same absolute busulfan dependent depletion of GSH to have a more relevant effect on clearance in patients aged 40 years and older. In addition, GST polymorphisms have been linked to busulfan clearance with variable results[13–18], which can be explained using the presented hypothesis. Though patients with increased GST activity would initially have a higher clearance, they would also have faster

GSH depletion. Thus, the average clearance over multiple doses may be similar to patients with less active GST subtypes. In the proposed setting, TDM accounts for the difference in initial clearance and the semi-mechanistic model predicts the concurrent extent of GSH depletion.

Furthermore, the GSH-dependent time-effect might have other implications, that were not quantified in this study. For example, other drugs that affect busulfan pharmacokinetics or GSH stores such as antifungal agents or paracetamol[34] could interact with the busulfan time-effect. In addition, treatment with N-acetylcysteine may be helpful in preventing severe side effects during treatment with busulfan. In previous research N-acetylcysteine was found to potentially serve as prophylactic agent against sinusoidal obstructive syndrome induced by busulfan[35,36]. Also, evidence was provided that N-acetylcysteine does not interfere with the myeloablative effect of busulfan. Thus N-acetylcysteine may be suitable to reduce the risk of hepatotoxic side effects of busulfan during conditioning regimens for hematopoietic stem cell transplantation. However, a randomized prospective study assessing N-acetylcysteine as a prophylactic agent for VOD did not show any relevant effect[37] as VOD occurrence was rare in both arms suggesting that this trial was underpowered.

A major strength of the current study is the large sample size with a good distribution of patient over different age groups and limited missing data. As these time-dependent effects are subtle and variable, information including sufficient data over a wide age range was essential for proper quantification of effects. Nevertheless, some weaknesses remain. As direct measurement of GSH was unavailable, busulfan clearance was used as a surrogate. Future studies should focus on measuring active GSH levels before and during the conditioning and implement these in the constructed semi-mechanistic model. The developed mechanistic model can then be expanded with re-synthesis of GSH. GSH levels can be measured also in the time-course after a busulfan dose has been cleared and before administration of the subsequent dose,

where most GSH re-synthesis is expected to take place. Preferably, this should be preceded by in vivo (animal) data to support a relationship between plasma and liver levels, as it is known that most GSH is stored in red blood and hepatic cells[38].

In summary, these data suggest the intra-individual decrease in busulfan clearance may be related to GSH depletion. This effect increases after an age of 40 years and is proportional to busulfan metabolism. Therefore, busulfan dosing guided by TDM, taking into account the decrease in clearance using the newly constructed semi-mechanistic model, can increase target attainment in patients undergoing conditioning prior to HCT.

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JL, JB, JJB, CvK, AE, AL, AH and EvM designed the study. JL, JB and AH analyzed the data.

JJB and JK included patients, provided medical insight, and critically appraised the manuscript.

9 Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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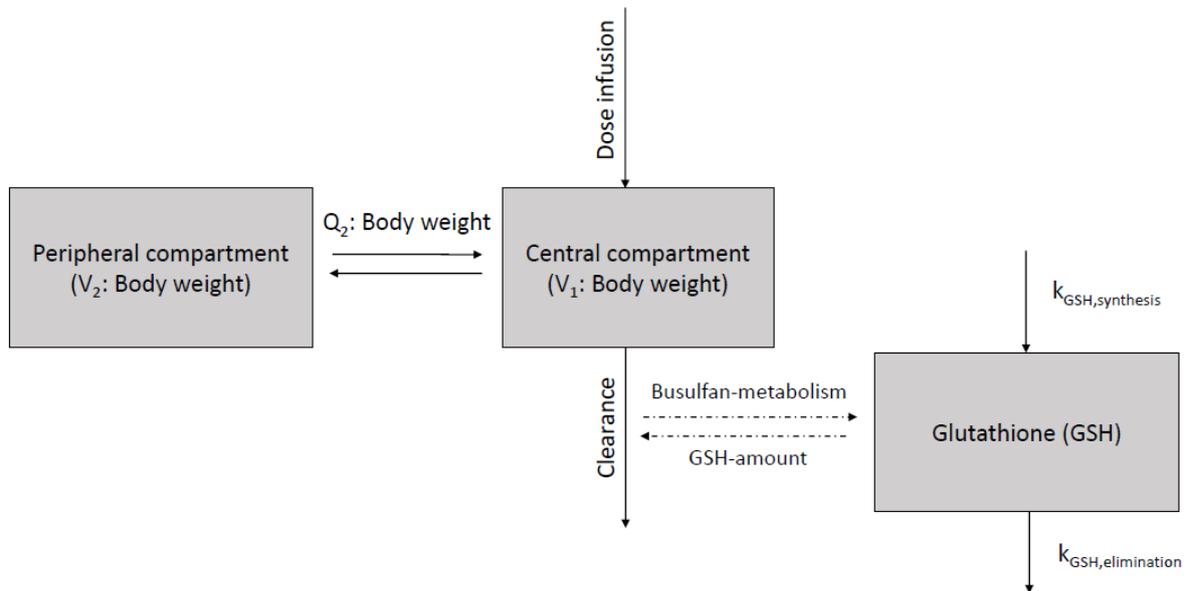


Figure 1: **Semi-mechanistic busulfan model structure:** busulfan is infused to and eliminated from (Clearance) the central compartment (V_1); busulfan distributes reversibly to the peripheral compartment (V_2) with a rate determined by the inter compartmental clearance: Q_2 . Glutathione is synthesized and eliminated according to $k_{GSH,synthesis}$ and $A_{GSH} * k_{GSH,elimination}$ respectively. These terms are assumed to be the same at steady state. The dashed lines indicate the influence busulfan-metabolism and glutathione amount have on each other, where the solid lines depict transport.

Accepted

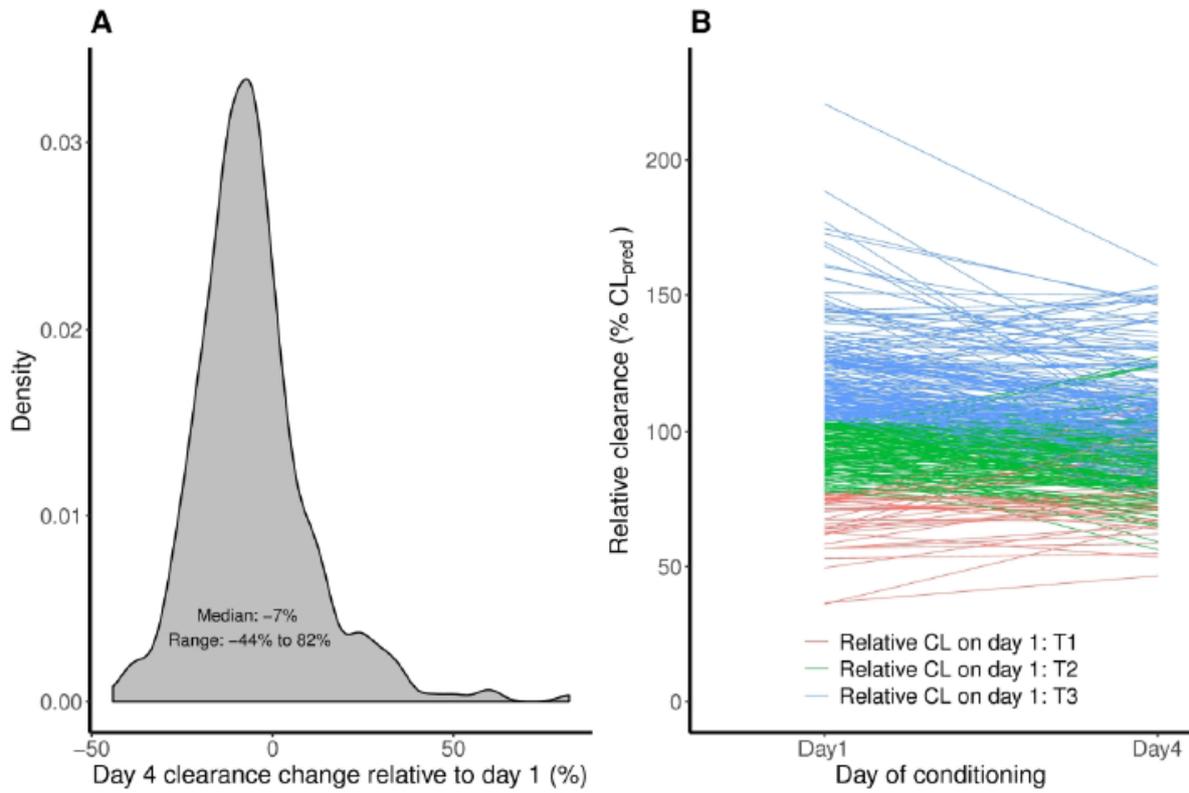


Figure 2: **Observed variability in busulfan clearance change.** Panel A depicts a density plot of the relative change of clearance from day 1 to day 4 (%). In panel B, this change is displayed per individual and stratified in tertiles for relative clearance day 1: defined as the individually estimated clearance divided by the weight-predicted clearance for that individual (%).

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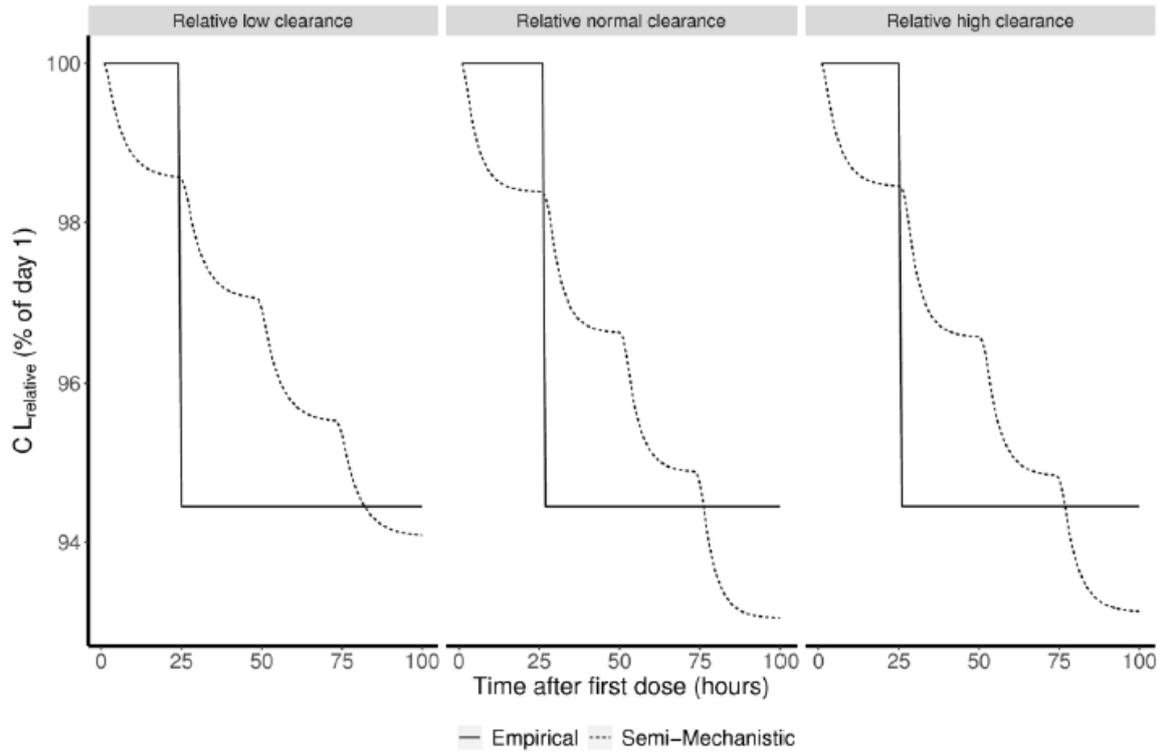


Figure 3: **Time-effect.** A display of the time-effect for both models. 3 individuals were randomly drawn from the each tertile of relative clearance (as defined in figure 2). The clearance over time is depicted, as predicted per model, based on the day 1 clearance. Values are relative to the day 1 clearance (%).

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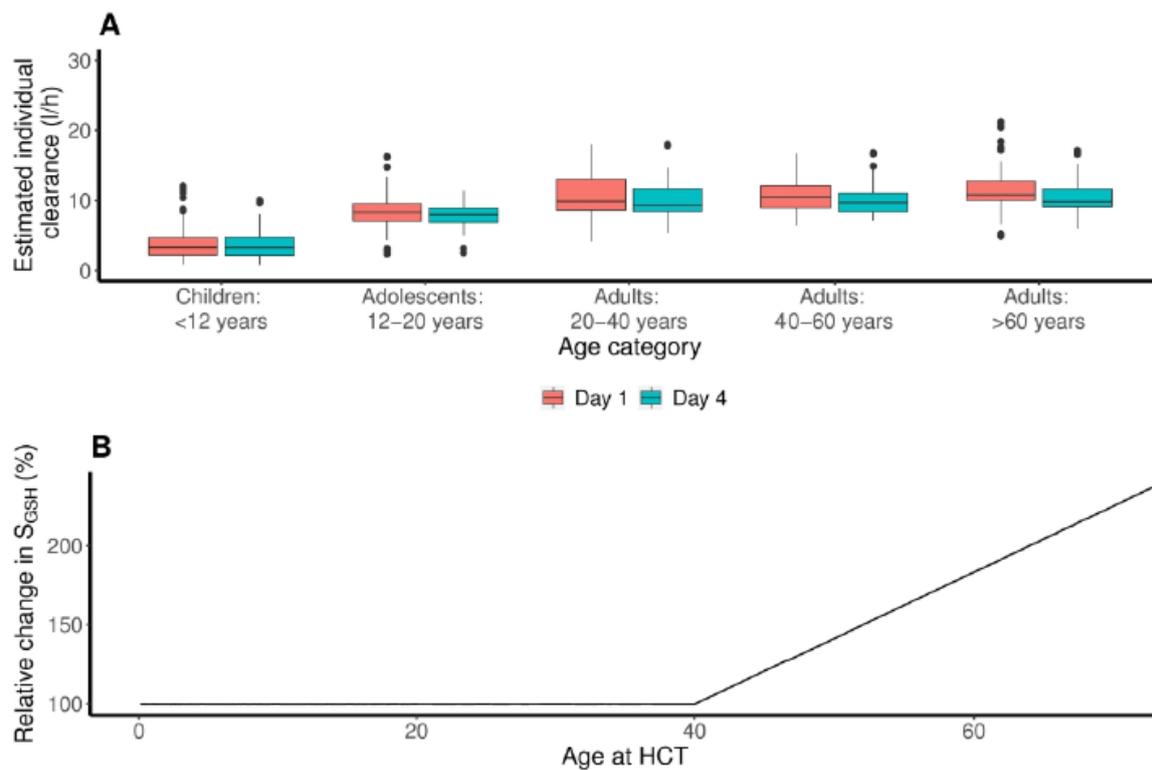


Figure 4: **Covariate effects.** Panel A depicts the observed clearance decrements from day 1 to 4 stratified for age at transplantation. Panel B depicts the model predicted decrease of S_{GSH} as implemented in the semi-mechanistic model.

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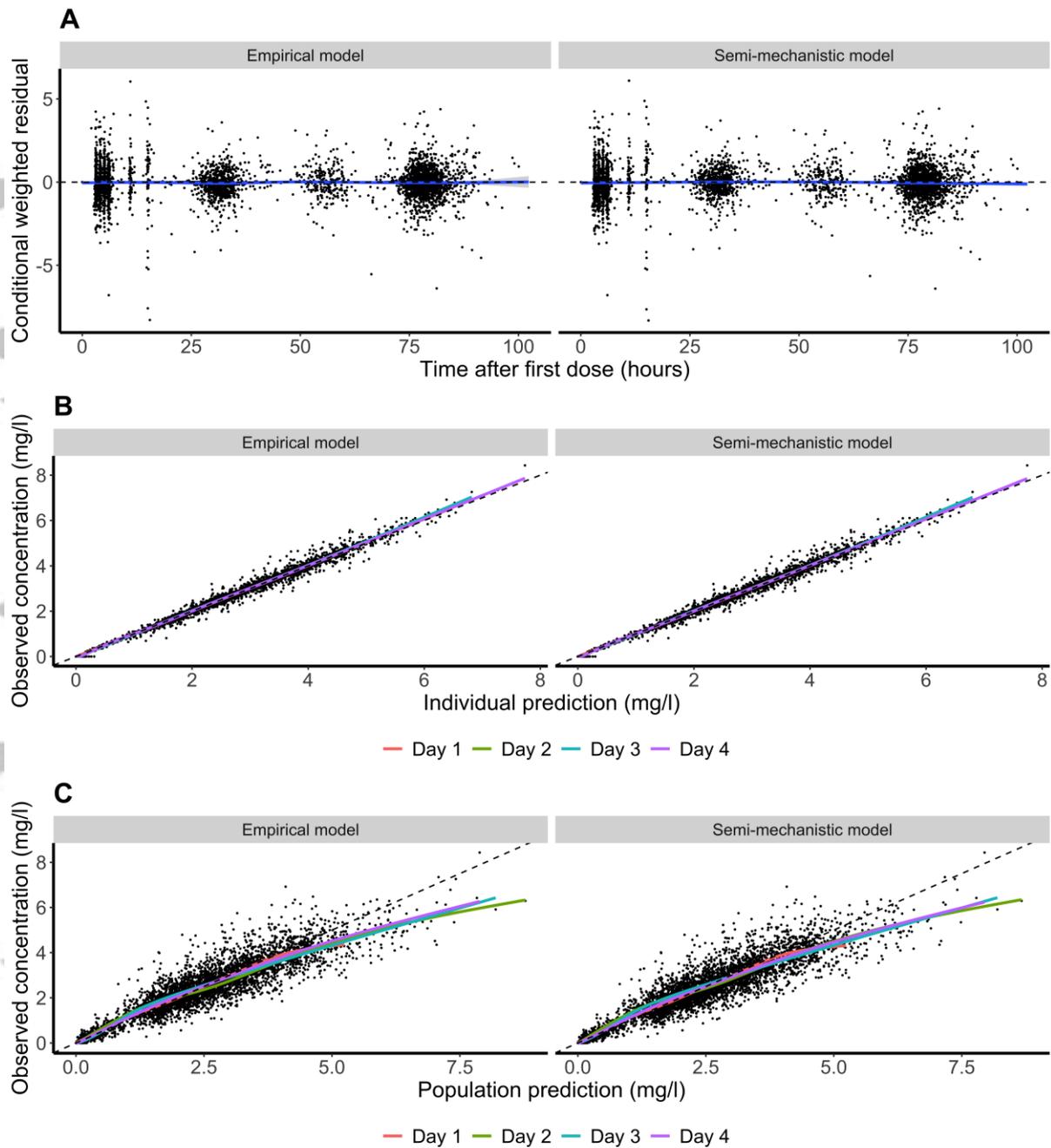


Figure 5: **Goodness of fit plots.** A display of the time-effect for both models. 3 individuals were randomly drawn from the each tertile of relative clearance (as defined in figure 2). The clearance over time is depicted, as predicted per model, based on the day 1 clearance. Values are relative to the day 1 clearance (%)

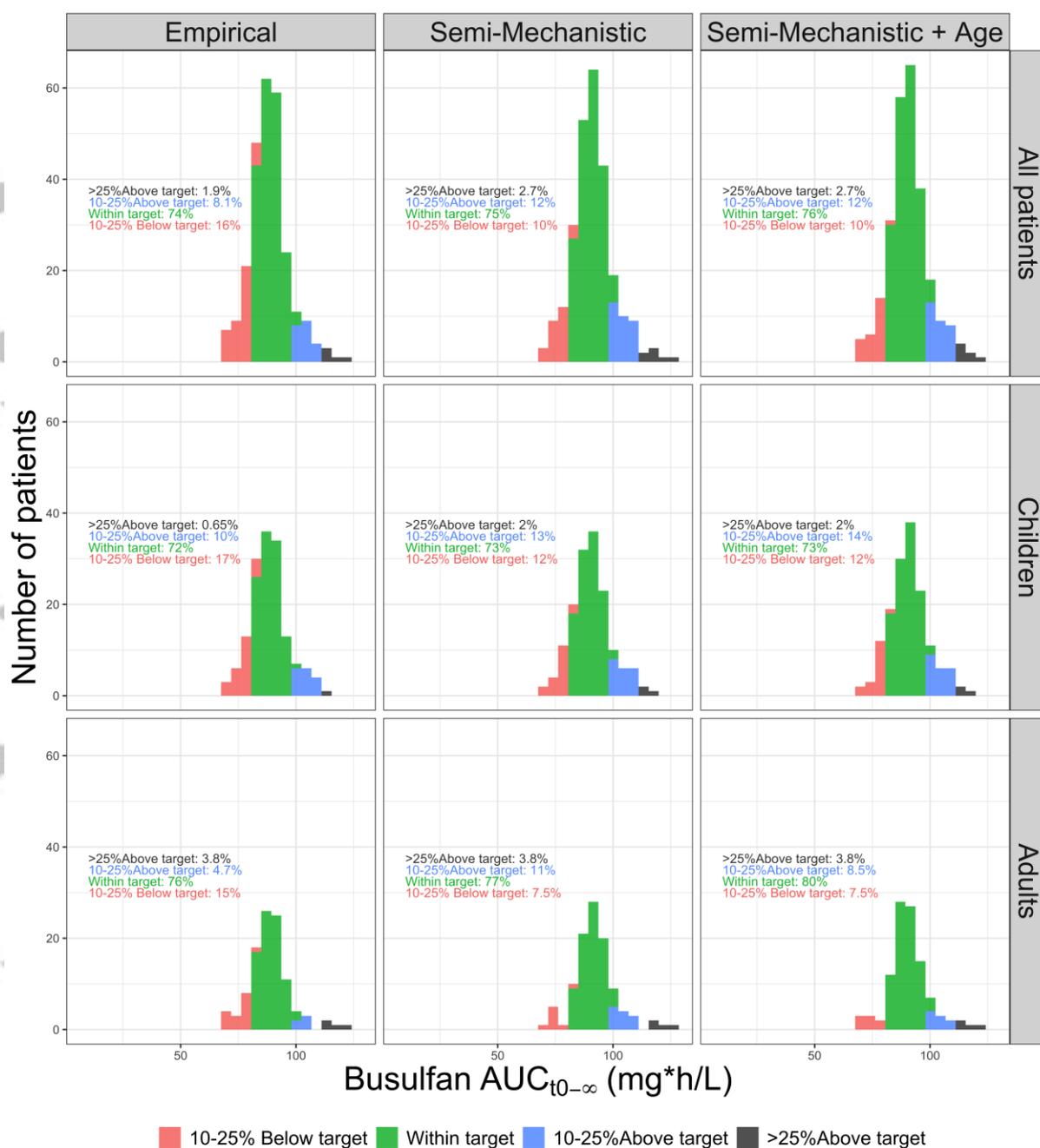


Figure 6: **Target attainment for different models.** Histograms of the simulated busulfan exposure using the empirical model or the semi-mechanistic model with or without age. Results are shown for the full population as well as for children (< 20 years) and adults (≥ 20 years) The target range is defined as $90 \text{ mg}^* \text{h/L} \pm 10\%$ (81-99 $\text{mg}^* \text{h/L}$)

Table 1: Patient characteristics^a

| | |
|-----------------------------------|--------------|
| Weight at HCT (kg) | 50 (3.7-130) |
| Age at HCT (years) | 14 (0.16-73) |
| Age category at HCT | |
| Children: 0-12 years | 159 (41%) |
| Adolescents: 12-20 years | 91 (24%) |
| Adults: 20-40 years | 35 (9%) |
| Adults: 40-60 years | 59 (15%) |
| Adults: 60+ years | 43 (11%) |
| Samples (No. per patient) | 15 (5-24) |
| Sex | |
| Male | 233 (60%) |
| Female | 154 (40%) |
| Cell source | |
| Cord blood | 179 (46%) |
| Peripheral blood stem cells | 120 (31%) |
| Bone marrow | 76 (20%) |
| Autologous | 7 (1.8%) |
| Haplo-Cord | 5 (1.3%) |
| Conditioning regimen | |
| Bu90/Flu | 215 (56%) |
| Bu90/Clo/Flu | 68 (18%) |
| Bu<90/Cy | 46 (12%) |
| Bu<90/Flu | 44 (11%) |
| Other | 14 (3.6%) |
| Diagnosis | |
| Leukemia | 179 (46%) |
| Benign ^a | 132 (34%) |
| MDS ^c | 33 (8.5%) |
| Plasma cell disorder ^c | 24 (6.2%) |
| Lymphoma ^c | 19 (4.9%) |
| Serotherapy | |
| Serotherapy | 303 (78%) |
| No Serotherapy | 84 (22%) |

^aCharacteristics are displayed per patient-transplantation combination (one patient was transplanted twice)

^bPatients transplanted for benign disorders were mostly pediatric (0-12 years: n=92/159; 12-20 years: n=33/91; 40-60 years: n=4/59; 60+ years: n=2/43)

^cPatients transplanted for MDS, plasma cell disorders and lymphoma were all adults

HCT: hematopoietic cell transplantation; BuXX: busulfan targeted to XX mg*h/L; Flu: fludarabine; Clo: clofarabine; MDS: myelodysplastic syndrome

Table 2: Final model parameter estimates

| Fixed effects: Empirical model | | | |
|---|----------|-----------------|-----------|
| Parameter | Estimate | 95% CI | |
| V_1 (L/43kg) | 23.4 | 22 - 24 | |
| Exponent V_1 | 0.869 | 0.84 - 0.88 | |
| Clearance at Day 1 (L/h/43kg) | 7.58 | 7.5 - 7.9 | |
| Exponent1 CL: l | 1.03 | 0.95 - 1.1 | |
| Exponent1 CL: p | -0.138 | -0.17 - -0.11 | |
| V_2 (L/43kg) | 4.83 | 4 - 5.8 | |
| Q (L/h/43kg) | 5.6 | 4 - 8.1 | |
| CL _{decrement} after Day1 (%) | 0.0676 | 0.05 - 0.082 | |
| Random effects: Empirical model | | | |
| Parameter | Estimate | Correlation | 95% CI |
| IIV V_1 | 13.8% | 74.9% (V_1) | 12 - 15 |
| IIV CL | 19.4% | | 18 - 21 |
| IIV V_2 | 27.4% | | 20 - 36 |
| IOV CL | 12.4% | | 11 - 13 |
| IOV V_1 | 12% | | 10 - 13 |
| Proportional error (HPLC) | 9% | | 7.8 - 11 |
| Proportional error (LCMS) | 6.6% | | 6.4 - 6.8 |
| Fixed effects: Semi-Mechanistic model | | | |
| Parameter | Estimate | 95% CI | |
| V_1 (L/43kg) | 23.3 | 22 - 24 | |
| Exponent V_1 | 0.863 | 0.84 - 0.88 | |
| CL at T=0 (L/h/43kg) | 7.61 | 7.4 - 7.8 | |
| Exponent1 CL: l | 1.04 | 0.95 - 1.1 | |
| Exponent1 CL: p | -0.14 | -0.17 - -0.1 | |
| V_2 (L/43kg) | 4.73 | 3.9 - 5.7 | |
| Q (L/h/43kg) | 5.9 | 4.1 - 8.6 | |
| S_{GSH} (h/mg, age \leq 40 years) | 0.00259 | 0.0017 - 0.0032 | |
| Age effect (proportional/year, age $>$ 40 years) | 0.0419 | 0.0062 - 0.081 | |
| Random effects: Semi-Mechanistic model | | | |
| Parameter | Estimate | Correlation | 95% CI |
| IIV V_1 | 13.8% | 68.4% (V_1) | 12 - 15 |
| IIV CL | 19.5% | | 18 - 21 |
| IIV V_2 | 28.6% | | 20 - 35 |
| IOV CL | 12.1% | | 11 - 13 |
| IOV V_1 | 11.5% | | 10 - 13 |
| Proportional error (HPLC) | 9.1% | | 7.7 - 10 |
| Proportional error (LCMS) | 6.6% | | 6.4 - 6.8 |