Treosulfan pharmacokinetics and dynamics in pediatric allogeneic stem cell transplantation

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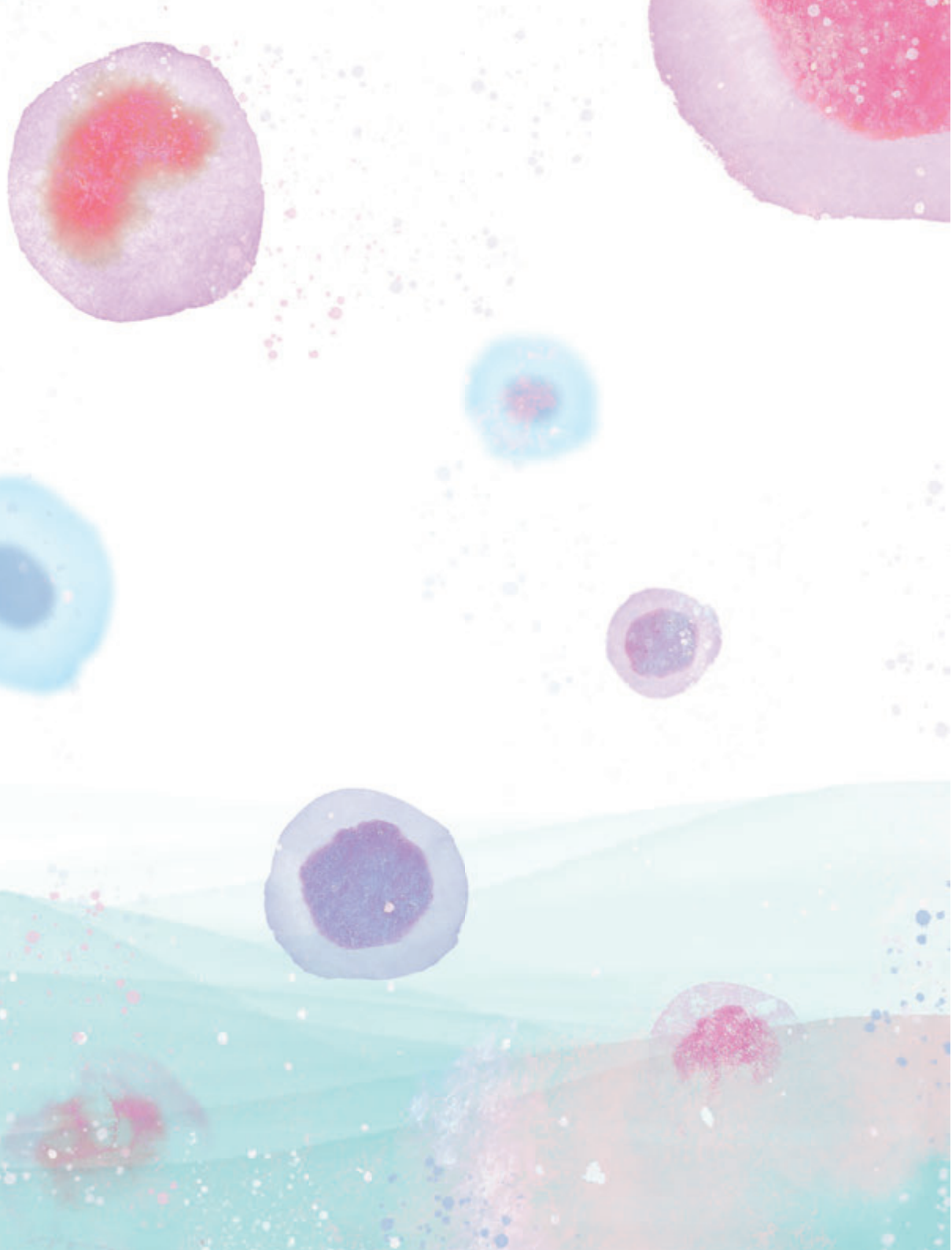

CHAPTER 01

GENERAL INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established curative treatment for malignant and nonmalignant diseases in both adult and pediatric patients. It involves eradicating the patient's hematopoietic stem cells (HSCs) and replacing them with HSCs from a healthy donor.

If a patient is deemed eligible for HSCT - based on indication, medical history and pre-transplantation tests - the search for a potential donor begins. The outcome of HSCT depends partly on the match between donor and patient for the human leukocyte antigens (HLA) in the major histocompatibility complex (MHC), which are encoded by a group of genes on chromosome 6, belonging to MHC class I (HLA-A, HLA-B, HLA-C) and MHC class II (HLA-DR, HLA-DQ and HLA-DP) [1]. A set of HLA genes, called haplotype, is inherited from each parent, which makes the probability for two siblings to inherit the same genes (haplotypes) 25%. These siblings are genotypically HLA identical and this is generally considered to be the optimal donor-recipient combination. That is why the search for a suitable stem cell donor usually begins within the family of the patient. If there is no eligible donor in the family, the search continues for an unrelated donor. This search for an unrelated donor is usually based on high-resolution DNA typing for 10 alleles (A,B,C, DR and DQ), while in some cases additional DP matching is included. In most cases, a 10/10 HLA identical unrelated donor is considered the second best choice in the absence of a geno-identical family donor. If there are no matched unrelated donors available, the search is expanded to mismatched (9/10 HLA identical) or mismatched family (haplo) donors. In case of a mismatched donor, T-cell depletion *ex vivo* (e.g. TCRα/β depletion) or *in vivo* with post-transplant cyclophosphamide is often deployed [2]. If there are multiple eligible donors, other factors besides HLA matching play a role, such as donor age and sex, CMV status and blood (blood group/ABO match) type. The stem cell sources for HSCT are bone marrow (BM), mobilized peripheral blood stem cells (PBSC) and cord blood. The preferred stem cell source depends on a variety of patient and donor-related factors, such as age of the donor and recipient, underlying disease, manipulation of the graft, but is also dependent on the experience with these approaches in the transplantation center. Once a suitable donor is selected, the transplantation course can be planned.

In order to eradicate the stem cells of the patient, high-intensity chemotherapy (with or without total body irradiation (TBI)) is given, the so-called conditioning regimen. Depending on the underlying disease, the conditioning regimen usually consists of agents that have myeloablative (MA) properties to create 'space' in the bone marrow of the patient and to eradicate the primary disease [3]. Immunoablative/-suppressive agents are applied to prevent rejection (host-versus-graft) as well as graft-versus-host disease (GvHD). After conditioning, the stem cell graft of the donor is infused and the cells migrate to the bone marrow niches to produce new blood cells, which is called 'engraftment'. After engraftment, immune and hematological recovery occurs; a gradual process that can take several months. **Figure 1** gives an overview of the HSCT procedure.

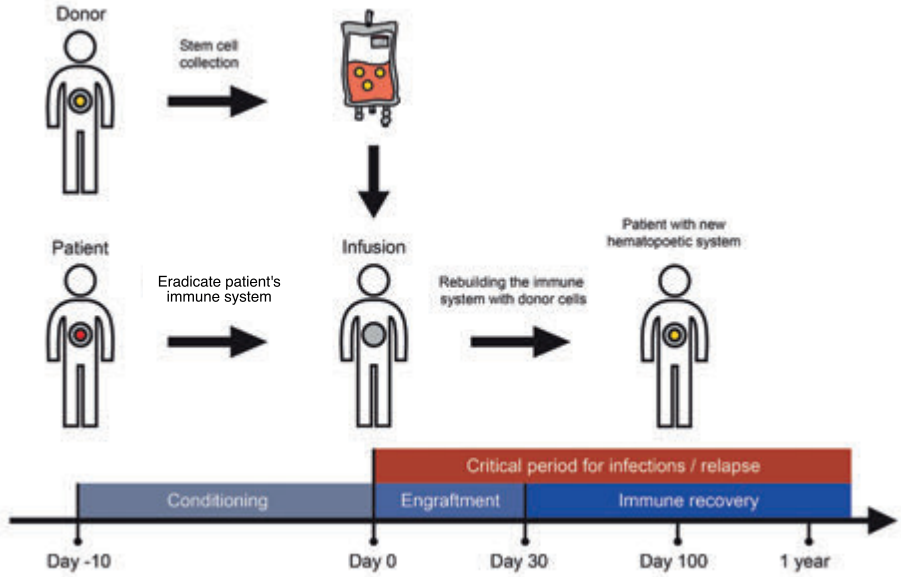

Figure 1: Overview of the allogeneic HSCT procedure.

INTENSITY OF CONDITIONING

Conditioning regimens are commonly defined as myeloablative, reduced intensity or minimal intensity. Although full consensus has not been reached, generally accepted definitions of these three types of regimens are as follows [2-4]:

Myeloablative conditioning (MAC): A combination of agents expected to produce profound aplasia and likely resulting in full donor chimerism, a situation where the newly developed hematopoietic system is of donor origin only [5]. Typical MAC protocols are based on high dose TBI, high dose busulfan or treosulfan (**Figure 2**).

Reduced intensity conditioning (RIC): Regimens containing reduced doses of myeloablative drugs (or radiotherapy), which are therefore less likely to achieve marrow ablation and more likely to produce mixed chimerism, a state where donor and recipient haematopoiesis coexist within the recipient. Examples are reduced busulfan with fludarabine or treosulfan with fludarabine, without thiotepa.

Minimal intensity conditioning (MIC): A regimen that causes minimal cytopenia and little early toxicity and can theoretically be given without stem cell support. In vulnerable patients MIC regimens are used to carefully eliminate their own bone marrow, followed by the infusion of donor HSCs. These regimens are mainly immunoablative. An example is fludarabine with low dose cyclophosphamide.

Figure 2 shows a classification of conditioning regimens, based on intensity and toxicity with some examples of common regimens.

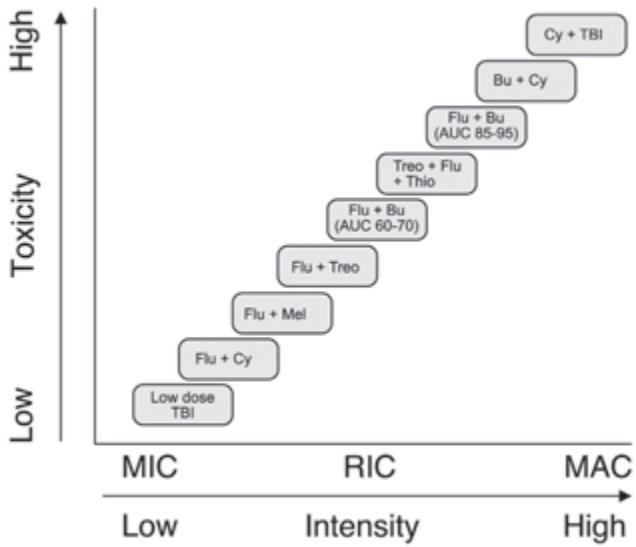

Figure 2: Classification of conditioning regimens, based on intensity (miminal intensity (MIC), reduced intensity (RIC) and myeloablative (MAC)) and toxicity. Bu: busulfan, Cy: cyclophosphamide, Flu: fludarabine, Mel: melphalan, TBI: total body irradiation, Thio: Thiotepa, Treo: treosulfan.

CHOICE OF CONDITIONING

The choice for the optimal conditioning regimen is dependent on different factors. To overcome rejection and ensure stable engraftment, the intensity of conditioning is traditionally higher in patients receiving transplants of unrelated and mismatched donors than when a transplant from a matched sibling is received. Serotherapy with either anti thymocyte globulin (ATG), anti-T lymphocyte globulin (ATLG) or alemtuzumab (Campath) is often added to the regimen to reduce the risk of GvHD and rejection. The underlying disease is also a factor that has to be taken into consideration. For nonmalignant diseases, less intense protocols can be sufficient to ensure engraftment, while reducing the risk of early (and possibly late) toxicity and GvHD. Other factors such as age, comorbidities and organ-specific toxicity risk

may determine the choice of the regimen. Especially in children, the influence of the conditioning regimen on growth and puberty (development) is an important aspect that has to be taken into account.

Historically, conditioning protocols were more myeloablative in nature. These regimens were associated with significant organ- and transplant-related toxicity and mortality [6]. Improvements have been made over the last decades to reduce transplant-related toxicity and mortality when using MAC. A number of pharmacological aspects, such as improvements in formulation and administration, more insight in the pharmacokinetic behavior of conditioning agents and increased availability of analytic tools have made an important contribution to these improvements. This is most explicitly illustrated with the alkylating agent busulfan.

In the early days, busulfan was only available as an oral formulation. Uniform dosing resulted in huge interindividual differences in exposure due to variation in absorption. Intravenous (i.v.) busulfan has widely replaced oral busulfan when this formulation became available, which reduced pharmacokinetic variability [7]. However, interpatient variability in clearance of i.v. busulfan is still reported to be up to 30% [8, 9]. Population pharmacokinetic studies were conducted to search for factors explaining this interpatient variability, which were age, body weight and GSTA1 genotype, among others [10]. Studies showed that a high area under the curve (AUC) of busulfan plasma concentration increased the risk of toxicity, while low busulfan concentrations may be associated with a higher risk of graft rejection and relapse [11]. Monitoring of busulfan levels and dose adjustments allowed for better control of the dose administered and reduction of the above mentioned risks; a clinical practice that is known as Therapeutic Drug Monitoring (TDM) [12]. With the introduction of intravenous busulfan, with more predictable pharmacokinetics (PK), tight control of plasma levels could be achieved and busulfan-mediated toxicity and mortality could be significantly reduced [13].

Unfortunately, not all (severe) toxicities can be avoided and long-term effects, such as infertility, alopecia and pulmonary diseases are still a problem in patients conditioned with busulfan [14-17]. Especially in children, these long-term effects can have a substantial

negative impact on quality of life. Reduced-intensity conditioning can be deployed to reduce transplant-related mortality (TRM), but the risk of relapse and graft failure or mixed chimerism may be increased [18]. Reducing toxicity without compromising HSCT efficacy could be of significant benefit. Replacing cyclophosphamide with fludarabine was an approach to reduce toxicity and demonstrated a significant reduction of TRM compared to busulfan with cyclophosphamide in patients with acute myeloid leukemia (AML) with no difference in relapse incidence [19]. Another strategy to reduce toxicity was replacing busulfan with treosulfan [20].

The research presented in this thesis focuses on the alkylating agent treosulfan.

TREOSULFAN

Treosulfan (L-threitol 1,4-bismethanesulphonate; dihydroxybusulfan) is a structural analogue of busulfan. It is a prodrug and a water-soluble alkylating agent. It is non-enzymatically and pH-dependently converted into a monoepoxide-(S,S-EBDM) and a diepoxide-derivative (S,S-DEB), in two consecutive reactions of intramolecular nucleophilic substitution (**Figure 3**). These epoxides are thought to be responsible for DNA alkylation, interstrand DNA crosslinking, chromosomal aberration and induction of apoptosis [21-23]. Treosulfan is originally used in oncology and approved for the treatment of ovarian carcinoma in most European countries [24]. Conventional doses are 5-8 g/m^2 every 3-4 weeks.

In preclinical studies, treosulfan has been shown to cause rapid and profound myelosuppression. In addition, it has more potent immunosuppressive characteristics than busulfan or cyclophosphamide [25]. In several clinical studies, a conditioning regimen containing Treo and Flu (with or without thiotepa) has been reported to result in rapid engraftment and complete donor chimerism. In addition, regimen related toxicity was low, as well as acute GvHD rates and transplant related mortality [26-32].

In contrast to busulfan, only a few studies were performed to investigate the pharmacokinetics of treosulfan in pediatric patients. These studies have shown great interpatient variability

in treosulfan exposure [33-36]. Factors that cause these great interpatient variability have not been investigated. Because clinical outcome of HSCT is associated with busulfan exposure, we hypothesize that treosulfan exposure might also be associated with clinical outcome. Also, treosulfan is relatively new in the field of HSCT and knowledge of acute and late side effects using treosulfan in the setting of HSCT (30-42 g/m^2) is limited.

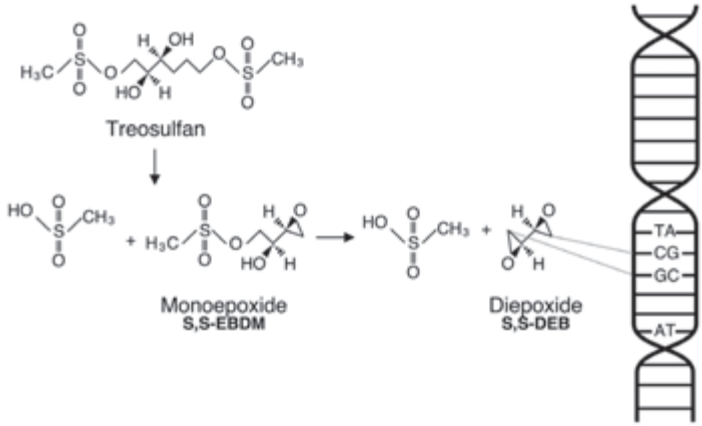

Figure 3. The conversion of treosulfan to the monoepoxide (S,S-EBDM) and the diepoxide (S,S-DEB), which causes interstrand DNA crosslinking.

AIMS AND OUTLINE OF THIS THESIS

This thesis focuses on treosulfan in the setting of pediatric stem cell transplantation. More specifically the aims of the thesis are:

- To investigate the pharmacokinetic behavior of treosulfan and develop a population PK model.
- To investigate the relationship between treosulfan exposure, early toxicity and clinical outcome.
- To acquire knowledge about the acute and late side effects.

Chapter 2 describes the development of a population PK model of treosulfan in a large cohort of pediatric patients undergoing HSCT and the exploration of covariates that are of possible influence on treosulfan pharmacokinetics. This population PK model is used to calculate treosulfan 'Area under the Concentration curve' (AUC) as a representation of treosulfan exposure.

Chapter 3 focuses on the relationship between treosulfan exposure and early toxicity in a multicenter pediatric cohort, while the focus of **Chapter 4** is more on the relationship between treosulfan exposure and clinical outcome in a cohort of patients transplanted for a nonmalignant disease.

In **Chapter 5**, the incidence and severity of myalgia - a side effect of treosulfan that was not mentioned in the original Summary of Product Characteristics (SmPC) - was identified using an electronic health record text mining tool and described in a cohort of patients that received treosulfan and compared to a cohort that received busulfan. **Chapter 6** describes the influence of busulfan and treosulfan exposure on long-term endocrine outcome, in particular gonadal function.

This thesis concludes with a review summarizing and discussing the evidence for TDM of the most commonly used conditioning agents in pediatric HSCT in **Chapter 7** and a discussion with future perspectives in **Chapter 8**.

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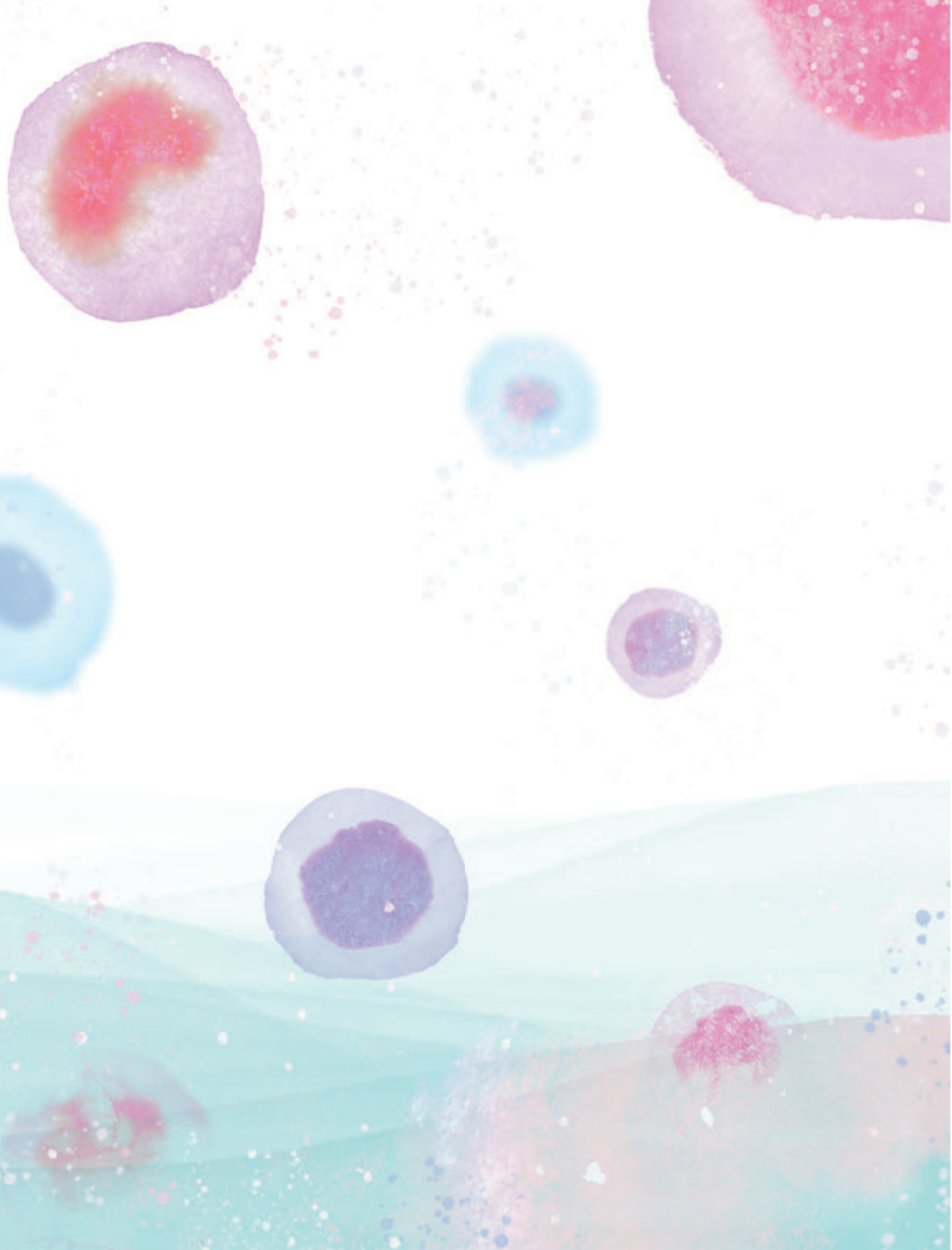

CHAPTER 02

POPULATION PHARMACOKINETICS OF TREOSULFAN IN PEDIATRIC PATIENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION

M.Y. Eileen C. van der Stoep, Juliette Zwaveling, Alice Bertaina, Franco Locatelli, Henk-Jan Guchelaar, Arjan C. Lankester, Dirk Jan A.R. Moes

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ABSTRACT

Aims:

Treosulfan is an alkylating agent increasingly used prior to hematopoietic stem cell transplantation (HSCT). The aim of this study was to develop a population pharmacokinetic model of treosulfan in pediatric HSCT recipients and to explore the effect of potential covariates on treosulfan pharmacokinetics (PK). Also, a limited sampling model (LSM) will be developed to accurately predict treosulfan exposure suitable for a therapeutic drug monitoring setting.

Methods:

In this multicentre study, 91 patients, receiving a total dose of 30, 36 or 42 g/m^2 treosulfan, administered over 3 consecutive days, were enrolled. A population pharmacokinetic model was developed and demographic factors, as well as laboratory parameters, were included as potential covariates. In addition, a LSM was developed using data from 28 patients.

Results:

A two-compartment model with first order elimination best described the data. Bodyweight with allometric scaling and maturation function were identified as significant predictors of treosulfan clearance. Treosulfan clearance reaches 90% of adult values at 4 postnatal years. A model-based dosing table is presented to target an exposure of 1650 mg*hr/L (population median) for different weight and age groups. Samples taken at 1.5, 4 and 7 hours after start of infusion resulted in the best limited sampling strategy.

Conclusions:

This study provides a treosulfan population PK model in children and captures the developmental changes in clearance. A 3-point LSM allows for accurate and precise estimation of treosulfan exposure.

INTRODUCTION

Treosulfan is an alkylating agent with both myeloablative and immunosuppressive properties [1]. In the last decade, treosulfan is increasingly being used in conditioning regimens prior to hematopoietic stem cell transplantation (HSCT), in children with both malignant and non-malignant disorders. It has been shown to be effective and has a relatively mild toxicity profile [2-7]. The most commonly reported toxicities are skin, mucosal, gastro-intestinal and hepatic toxicity [4, 6-8].

Treosulfan is an analogue of busulfan, from which it differs for two hydroxyl groups leading to a somewhat different mechanism of action [9]. Treosulfan is a prodrug and is non-enzymatically, pH-dependently converted into a monoepoxide and diepoxide derivative ((S,S)-EDBM and (S,S)-DEB, respectively) [10]. These metabolites are thought to be responsible for DNA alkylation, interstrand DNA crosslinking, chromosomal aberration and, finally, induction of apoptosis [11].

To date, only a few papers have described the clinical pharmacokinetics of treosulfan in children, often based on small sample size datasets [12-18]. Three population pharmacokinetic models in children have been published, including one of our own group (see Supplemental Material 1). However, the sample size of two of the three studies was limited and besides bodyweight (BW), no significant covariates could be identified to explain interindividual variability in pharmacokinetics [16, 17, 19]. Also, the inclusion of infants (children <2 years) was limited; a population particularly of interest because variability and total exposure seems especially high in this subgroup [13, 18].

In order to perform PK-guided dosing and to accurately establish the exposure, intensive blood sampling is required. This may be laborious for both patients and staff employing PK-guided dosing in daily practice. In a pilot study, we reported that a limited sampling model (LSM) based on PK data from 20 pediatric patients based was capable of accurately predicting the area under the concentration-time curve from zero to infinity ($AUC_{0-\infty}$), with a model based approach, requiring only 2 blood samples [17].

The primary aim of the current study is to develop a population pharmacokinetic model of treosulfan in pediatric HSCT recipients with improved predictive performance compared to previously published models using a comprehensive multi-institutional dataset. The secondary aim is to identify patient-related factors that may explain pharmacokinetic variability by means of a covariate analysis. Finally, a limited sampling model will be developed to accurately estimate treosulfan systemic exposure suitable for a therapeutic drug monitoring (TDM) setting.

METHODS

Patient population

All pediatric patients who had participated in a prospective, observational, multicentre study and who had received treosulfan as part of conditioning prior to HSCT between June 2011 and March 2017 in the Leiden University Medical Center (LUMC), The Netherlands, and the Bambino Gesù Children's Hospital (OPBG) in Rome, Italy were included in this population pharmacokinetic analysis. Patients without permanent central venous access were excluded. The LUMC institutional Ethics Committee approved the study protocol (P12.267) which was subsequently approved in OPBG. Written informed consent to participate in the study was obtained from either parents or legal guardian, and patients older than 12 years were asked to give their assent, according to the Helsinki Declaration (last amended in 2013, Fortaleza, Brazil). In line with current dosing recommendations, patients older than 1 year received intravenous treosulfan in a total dose of 42 g/m^2, administered over 3 consecutive days (14 g/m^2 per day, 3-hour infusion). Patients under the age of 1 year received a total dose of 30 g/m^2 or 36 g/m^2 (10 g/m^2 or 12 g/m^2 per day, 3-hour infusion). Patients who underwent a second transplantation (n=7) in which treosulfan was also part of the conditioning regimen were included twice in the analysis. Samples were taken at first and second transplantation. Because the time between first and second transplantation was more than several months, these results were considered as distinct individuals.

Sampling and analysis

For treosulfan PK assessment, blood samples were collected in serum tubes (BD Vacutainer® Plus plastic serum tube) on day 1. In patients who gave additional consent, blood samples were also collected on day 3 to determine intra-patient variability. Samples were collected at 1.5, 3.5, 4, 5, 7 and 9 hours after start of infusion (extensive sampling) or at 4 and 7 hours after start of infusion (limited sampling). Samples were centrifuged as soon as possible (i.e. within 5 hours), and serum stored at -20˚C. A validated reversed-phase high-pressure liquid chromatography (RP-HPLC) using ultraviolet (UV) detection was used to determine treosulfan concentration in serum, as previously reported [17]. Briefly, treosulfan and the internal standard busulfan were made detectable through derivatization with sodium diethyldithiocarbamate (DDTC). Linearity was established up to 500 mg/L with a lower limit of quantification (LLOQ) of 6.8 mg/L. Accuracy of quality control (QC) samples was within the 90-110% limit. The intra-day imprecision, expressed as coefficient of variation (CV%), ranged from 2.0% to 3.3% and inter-day imprecision ranged from 2.1% to 2.8%.

Pharmacokinetic modelling

Nonlinear mixed effect modelling was used to estimate pharmacokinetic parameters as implemented in the NONMEM software package (version 7.3.0; Icon Development Solutions, Ellicott City, MD, USA), using PsN toolkit 4.7.0 and Piraña version 2.9.7 as modelling environment. Plotting of the results was performed using statistical software package R (v3.4.4) and R studio Version 1.0.456.

Base model

Initially, a base model was developed without covariates. Plots of observed concentration-time data of treosulfan were examined. One-, two- and three compartmental pharmacokinetic models with first-order elimination were compared to find the optimal fit for the concentration-time data. Interindividual variability (IIV) was assumed to follow a log-normal distribution and was implemented in the model as follows (Eq.1):

$$P_i = P_{POP} \times exp^{\eta_i} \quad (1)$$

where P_i is the pharmacokinetic parameter of i^{th} individual, P_{pop} is the population mean value of the parameters and η_i is a normally distributed random value with mean zero and variance ω^2. In 24 patients, interoccasion variability (IOV) could be evaluated and implemented similarly (Eq. 2) with each dose and subsequent sampling defined as a separate occasion.

$$P_i = P_{POP} \times exp\ (\eta_{1x1+}\eta_{2x2+\cdots\cdots}\eta_{ixi+}) \quad (2)$$

A proportional error model and a combined proportional and additive error model were examined to describe the residual error. Eventually, a proportional error model was implemented as follows (Eq. 3):

$$Y_{ij} = Y_{PREDij} \times (1 + \varepsilon exp_{proportional}) \quad (3)$$

where Y_{ij} is the j^{th} measured concentration in the i^{th} subject, Y_{PREDij} is the predicted concentration based on the model and $\varepsilon xp_{proportional}$ is the proportional error component.

Four of 410 (1%) serum concentration time points were below the lower limit of quantification. These measurements (actual values) were included in the dataset as proposed by Hecht *et al* [20].

Covariate analysis

The parameter values were standardised for a body weight of 70 kg and allometrically scaled (Eq. 4):

$$F_{size} = \left(\frac{BW}{70\ kg}\right)^{\alpha} \quad (4)$$

where F_{size} is the fractional difference in allometrically scaled size compared with a 70 kg individual. When scaling clearance (Cl) and intercompartmental clearance (Q) α is fixed to 0.75 and for volume of distribution of the central (V1) and peripheral compartment (V2) α is fixed to 1 [21].

Furthermore, a sigmoid E_{max} model was used to describe the maturation of treosulfan Cl on postmenstrual age (PMA) as follows (Eq. 5):

$$F_{mat} = \left(\frac{1}{1+\left(\frac{PMA}{TM_{50}}\right)^{-Hill}} \right) \quad (5)$$

where F_{mat} is the fraction of adult treosulfan clearance value, TM_{50} is the PMA at which maturation is 50% of the adult value, and the Hill coefficient is associated with the slope of the developmental profile [22]. PMA was estimated by adding a gestational age of 40 weeks to postnatal age.

Total clearance (Cl_{tot}) could then be described as follows (Eq. 6):

$$Cl_{tot} = Cl_{pop} \times F_{size} \times F_{mat} \quad (6)$$

where Cl_{pop} is the overall population value of parameter. A similar model was used for intercompartmental clearance (Q).

Potential other covariates were chosen based on biological or physiological plausibility and clinical relevance. Assessed covariates included: gender, underlying disease, conditioning regimen, hemoglobin, hematocrit, serum albumin and estimated glomerular filtration rate (eGFR) as a measure of renal function. This was calculated using the revised Schwartz formula (see Supplemental Material 2) and to avoid implausible high eGFR values, these were capped at 120 ml/min/1.73 m^2 [23]. There were no missing covariate values. All preselected covariate relationships were used for a systematic stepwise covariate modelling (SCM), with stepwise forward inclusion and backward deletion [24]. In the forward inclusion and backward deletion, the levels of statistical significance were set at $P<0.05$ and $P<0.01$, respectively, corresponding to differences in the NONMEM objective function value (OFV) of 3.84 and 6.64, respectively (1 degree of freedom). A covariate effect was only maintained in the model if the inclusion resulted in reduction of random variability of the PK parameter and improved model fit.

Final model evaluation

Model selection was based on physiological plausibility, visual inspection of goodness-of-fit plots (e.g. observed concentrations versus individual and population-predicted concentrations) and statistical significance. Throughout the model building process, an adjusted model was chosen over the original model if the drop in the objection function value (OFV) [-2 log likelihood] was >6.63 ($P<0.01$, with 1 degree of freedom, assuming chi-squared [χ^2] distribution). Shrinkage in interindividual variability and residual error were automatically calculated by NONMEM. Values below 30% were deemed acceptable [25]. Evaluation of the precision of the pharmacokinetic parameters was performed with 1000 bootstrap replicates. The stability and performance of the final model were assessed using a prediction-corrected visual predictive check (VPC), since different dosages were used. Prediction-corrected VPC was performed with 1000 replicates by simulating concentrations from the final model with the use of the original dataset. The median and the 10th and 90th percentiles of the simulated concentrations at each time point were calculated and plotted together with the median and the 10th and 90th percentiles of the observed concentrations. The distribution of the observed concentrations was visually compared to the simulated distribution. Differences and overlap of the simulated and original distributions indicated the adequacy of the identified model. In addition, the previously published models by Ten Brink *et al.* [17], Danielak *et al.* [19], and Mohanan *et al.* [16] were compared with the final model to show their ability to describe the current extensive treosulfan PK dataset. The difference in predictive performance was shown by means of comparing the prediction corrected VPCs of the different models.

Simulations to individualize dosing

Based on our final model, individual treosulfan doses were estimated to target an $AUC_{0-\infty}$ of 1650 mg*hr/L, the daily median of treosulfan $AUC_{0-\infty}$ in patients receiving the most common dose of 14 g/m^2. Bayesian pharmacokinetic parameter estimates were obtained by post hoc estimation in NONMEM. $AUC_{0-\infty}$ was then calculated as:

$$AUC_{0-\infty} = \frac{Dose * F}{Cl} \quad (7)$$

where F is equal to 1.

Clinical covariates were based on the 5th, 50th and 95th percentile estimates of weight per age for boys as provided by the CDC standard growth charts for infants and children [26].

Limited Sampling Model

Patients and data collection

Thirty-five "full" pharmacokinetic profiles from 28 different patients were used to find the optimal limited sampling model for treosulfan. These "full" pharmacokinetic profiles consisted of six blood samples collected over 9 hours (1.5, 3.5, 4, 5, 7 and 9 hours after start of a 3-hour infusion).

Pharmacokinetic and statistical analysis

"True" exposure ($AUC_{full0-\infty}$) was calculated from all measured concentration-time points using post hoc estimation in NONMEM with the final model ((DOSE *F1)/Cl). Limited sampling model (LSM) predicted AUC ($AUC_{pred0-\infty}$) was calculated by selecting several concentration-time points and combinations of time points. Bias and imprecision were calculated to assess the performance of the different LSMs according to the guidelines proposed by Sheiner and Beal [27]. Formulas can be found in Supplemental Material 2. A Pearson correlation coefficient test was performed to determine the correlation between $AUC_{full0-\infty}$ and $AUC_{pred0-\infty}$.

RESULTS

Patients

A total of 91 pediatric patients were included in this study; 58 were male and 33 female. Patient characteristics are summarized in Table 1. Median age was 4.3 years (range 0.1 - 18.2) and median body weight was 15.6 kg (range 3.8 - 75.0). Seven

patients underwent a second transplantation in which treosulfan was also part of the conditioning regimen. The median time between the first and second transplantation was 8.5 months. The dataset consisted of 410 samples. The concentration-time data were reviewed for completeness and consistency of sampling and dosing times. For distribution of samples, see Supplemental Material 3. Full concentration-time profiles of treosulfan are shown in Figure 1.

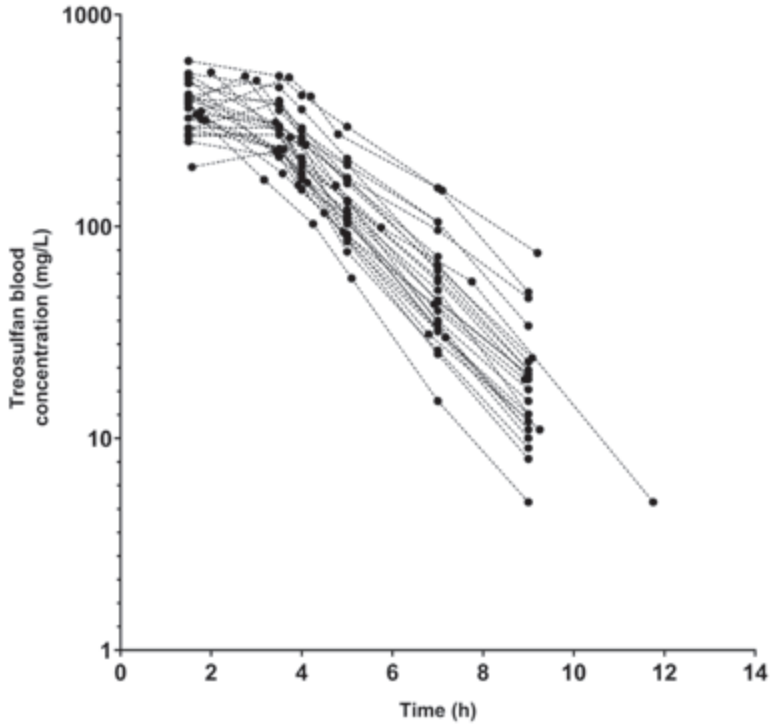

Figure 1. Full concentrations-time profiles of treosulfan in 27 pediatric patients undergoing HSCT, receiving 14 g/m^2.

Table 1. Patient characteristics (n=91)

Characteristic	
Age (years)	4.3 (0.1-18.2)
No. of infants (≤2 years old)	33 (36%)
Bodyweight (kg)	15.6 (3.8-75.0)
BSA (m^2)	0.7 (0.3-1.9)
Gender (% male)	63.7
Creatinine (μmol/L)	26 (8-166)
Albumin (g/L)	38 (20-52)
Hematocrit (L/L)	0.291 (0.199-0.384)
Hemoglobin (mmol/L)	6.6 (4.6-10.5)
eGFR (mL/min/1.73m^2)	111 (16-120)
Underlying disease (*n*)	
Hemoglobinopathy	35 (38.5%)
Hematological malignancy	17 (18.7%)
Primary immune deficiency	26 (28.6%)
Bone marrow failure	11 (12.1%)
Other	2 (2.2%)
No. of transplants (*n*)	
1	84 (92.3%)
>1	7 (7.7%)
Donor (*n*) [a]	
MSD	29 (31.9%)
MUD (≥ 9/10)	41 (45.1%)
MMFD (haplo)	20 (22.0%)
Stem cell source (*n*)[1]	
BM	56 (61.5%)
PBSC	23 (25.3%)
CB	10 (11.1%)
BM + CB	1 (1.1%)
Conditioning regimen (*n*)	
Treo+Flu+Thiotepa	59 (64.8%)
Treo+Flu	29 (31.9%)
Treo+Other (e.g. Mel)	3 (3.3%)
Treosulfan dose (*n*)	
10 g/m^2	16 (17.6%)
12 g/m^2	2 (2.2%)
14 g/m^2	73 (80.2%)
Transplant centre (Leiden/Rome)	63/28
Exposure	
Treosulfan $AUC_{0-\infty}$ (mg*hr/L)	1658 (643-3371)

Data are presented as median (range) unless stated otherwise. [a]: one patient died before transplantation, but after completing conditioning. BSA: body surface area, eGFR: estimated glomerular filtration rate, BM: bone marrow, PBSC: peripheral blood stem cells, CB: cord blood, MSD: matched sibling donor, MMFD: mismatched family donor, MUD: matched unrelated donor, Treo: treosulfan, Flu: fludarabine, Thio: thiotepa, Mel: melphalan, $AUC_{0-\infty}$: area under the curve from zero to infinity

Structural model development

Treosulfan PK was best described by a two-compartment model with first-order elimination from the central compartment. Adding the second compartment showed a significant improvement compared to the one-compartment model (ΔOFV = -127.78). The two-compartment model was parameterized in terms of volume of distribution of the central (V1) and peripheral (V2) compartment, and clearance from the central compartment (Cl) and intercompartmental clearance between V1 and V2 (Q). The base model showed the following PK parameters: average clearance (Cl) of 5.94 L/h (CV: 79.9%), average central distribution volume (V1) of 0.77 L (CV: 141.4%), average peripheral distribution volume (V2) of 8.73 L (CV: 90.5%) and average inter-compartmental clearance (Q) of 24.6 L/h (CV: 128.5%).

Covariate model

A bodyweight-based allometric model was added to all clearance and volume of distribution parameters and significantly improved the model (ΔOFV = -90.22). The addition of maturation of treosulfan Cl based on PMA on Cl and Q improved the model even further (ΔOFV = -39.63). The maturation of treosulfan clearance reaches 50% of adult values at 38 weeks PMA, that is 2 weeks prior to birth assuming a full-term gestational age of 40 weeks. Clearance reaches 90% of adult values at approximately 4 years old (Figure 2). In the stepwise covariate modelling process, eGFR was found to be a significant covariate on Cl (ΔOFV = -16.72), but the VPC worsened when eGFR was incorporated in the model and interindividual variability of the PK parameters increased. Therefore, we decided not to include eGFR to the model and only incorporate bodyweight and maturation of clearance in the final model.

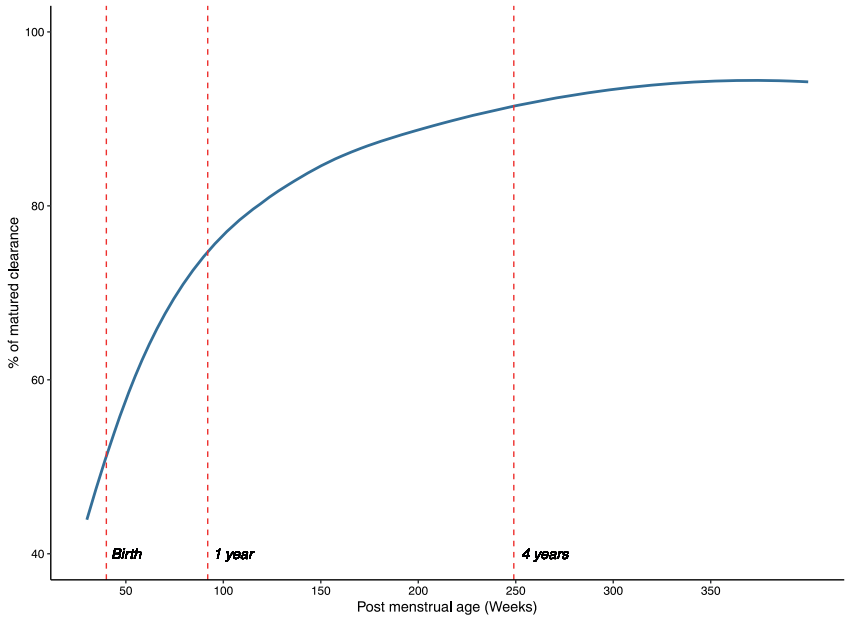

Figure 2. Maturation of treosulfan clearance as percentage of adult values.

Model evaluation

Parameter estimates of the base model, the model with only allometric scaling and the final model are presented in Table 2. Diagnostic plots of the final model are shown in Figure 3. The final model file code is provided in Supplemental Material 4. The relative standard error for the estimated V2 and Q_IIV was over 100%, 201% and 153% respectively. Interestingly, this was not seen when parameters were normalized to the median weight (15.6 kg) (RSE 53% and 46% for V2 and Q respectively, data not shown). However, evaluation with a bootstrap procedure with 1000 bootstrap replicates showed estimates that are in line with the estimates of the PK parameters and their random variability of the final model. The prediction-corrected VPC confirmed an acceptable agreement between the observed data and model-based simulated values (Figure 4A). The median PK parameter estimates and 95% confidence intervals (CI) from the bootstrap analysis are presented in Table 2.

Table 2. Summary of model parameter estimates

Parameter	Base model			Model with allometric scaling			Final model			1000 Bootstrap runs	
	Estimate	RSE (%)	Shr. (%)	Estimate	RSE (%)	Shr. (%)	Estimate	RSE (%)	Shr. (%)	Median value	95% CI
Cl (L/h)	5.94	6		15.9[a]	5		18.8[a]	7		19.4[a]	16.6 - 26.2
V1 (L/)	0.77	17		18.8[a]	17		20.2[a]	18		19.8[a]	5.1 - 29.6
Q_(L/h)	24.6	26		17.3[a]	28		21.3[a]	31		22.0[a]	9.7 - 68.9
V2 (L)	8.73	10		16.8[a]	14		16.8[a]	16		16.8[a]	10.9 - 29.6
Hill							1.2	34		1.1	0.3 - 3.2
TM_{50}							38	19		43	22.2 - 74.4
Inter-individual variability											
Cl (CV%)	79.9	11	1	36.9	11	11	31.8	13	15	31.4	22.8 – 40.2
V1 (CV%)	141.4	16	4	45.5	42	20	45.9	42	26	48.4	24.9 – 87.1
V2 (CV%)	90.5	14	2	15.7	208	19	17.3	201	19	20.7	9.1 – 46.0
Q_(CV%)	128.5	22	17	45.5	103	21	41.4	153	24	43.3	15.7 – 77.4
Interoccasion variability											
Cl (CV%)	13.1	21	32	13.3	22	38	13.9	23	27	13.0	9.8 – 17.1
Residual variability											
σ (proportional error)	11.8	7	30	12.4	7	27	12.3	4	27	12.0	9.4 – 14.6

Cl = clearance; CV = coefficient of variation; Hill = Hill coefficient for maturation, Q_= intercompartmental clearance; RSE = relative standard error; Shr.= shrinkage; TM50 = postmenstrual age at 50% maturation; V1 = volume of distribution of central compartment; V2 = volume of distribution of peripheral compartment; [a] normalised to a bodyweight of 70 kg

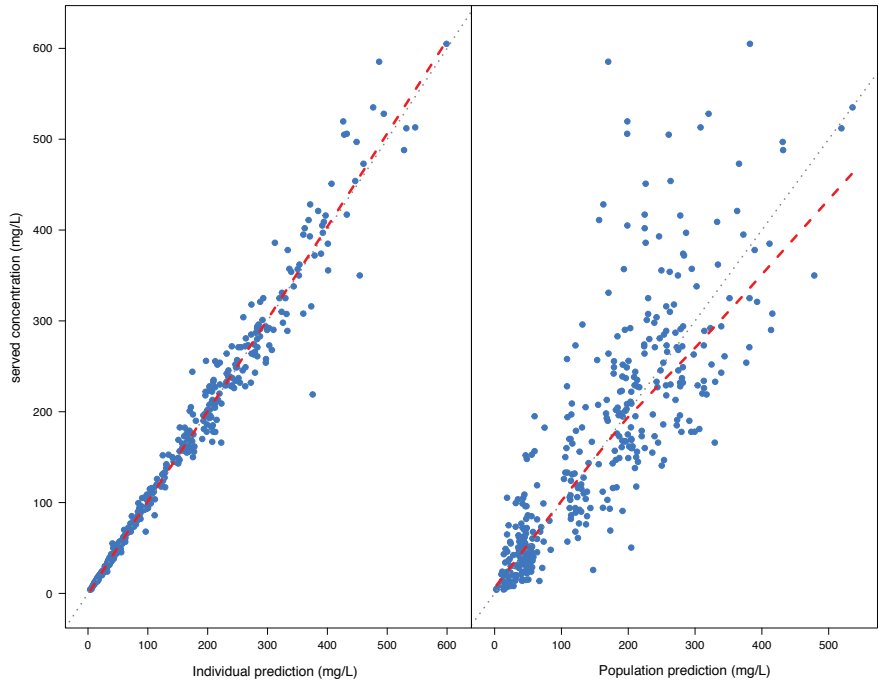

Figure 3. Goodness-of-fit plots for the final pharmacokinetic model. *Left:* individual-predicted concentrations versus observed concentrations. *Right*: population-predicted concentrations versus observed concentrations. Blue dots represent the observations and the red dashed line is a local regression fit of these values. Grey dashed line is the line of unity.

Comparison with previously published population pharmacokinetic models

Our model accounted for age and size differences over a big age range in children (1 month - 18 years). To evaluate the prediction accuracy in children, we performed prediction corrected VPCs with the previously published treosulfan pharmacokinetic models (Figure 4B, C and D) build on pediatric data [16, 17, 19]. The prediction corrected VPCs show that all three models show poor predictions and are not able to properly describe the current dataset.

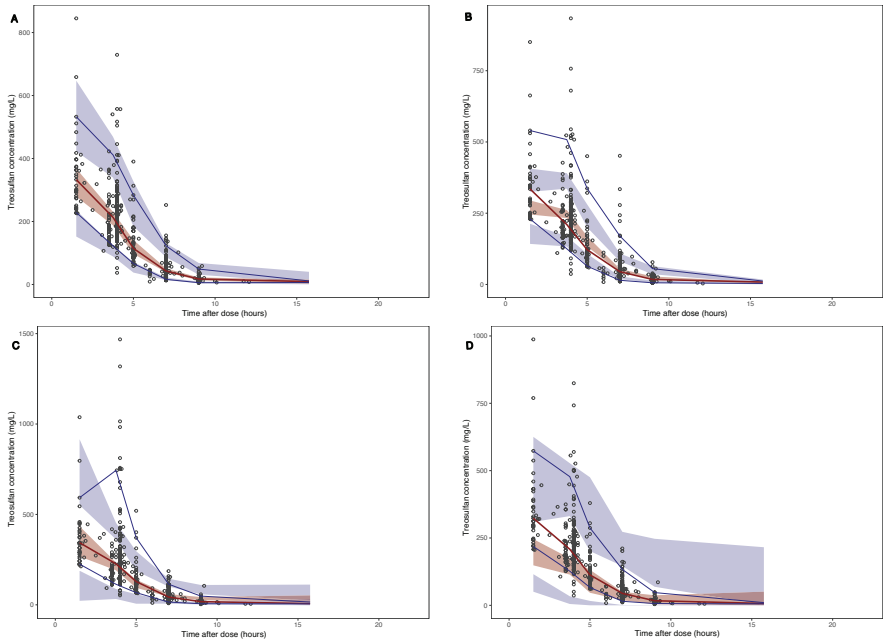

Figure 4. Prediction corrected visual predictive check with median, 10th and 90th observation percentile. The observed treosulfan serum concentrations are shown as open circles. The red and blue lines represent the observed median and 10th and 90th percentile. The shaded areas represent the 95% confidence interval around each of the prediction percentiles. A: present study, B: Ten Brink *et al.*, C: Danielak *et al.*, D: Mohanan *et al.*

Simulations to individualize dosing

The derived population PK parameters from our model were used to calculate the required treosulfan dose to reach an $AUC_{0-\infty}$ of 1650 mg*hr/L (median estimated $AUC_{0-\infty}$ in our cohort) for a set of virtual patients (normal weight and age range). In Table 3, the treosulfan dose per day required to target an $AUC_{0-\infty}$ of 1650 mg*hr/L can be found for each age category for the three different corresponding normal weight percentiles (5th, 50th, 95th). Figure 5 shows that the amount of treosulfan required varies per age, indicated as the grey ribbon between the dotted lines. The recommended treosulfan dose per kg is lower in early years of life and reaches a maximum at

approximately 4 years accounting for maturation of clearance and because dose per kg is higher in younger children based on allometric theory (Figure 5A). Figure 5B shows the absolute treosulfan dose, increasing with age and weight, but with a steeper slope in the beginning accounting for maturation.

Table 3. Recommended treosulfan dose for different age and weight categories (5th, 50th and 95th percentile)

Age	Weight (kg)	Treosulfan dose (mg) per day	Age	Weight (kg)	Treosulfan dose (mg) per day
	2.6	1350		20.7	11900
0 months	3.3	1600	8 years	25.8	14000
	4.2	1950		35.3	17700
	5.2	2650		22.7	12800
3 months	6.4	3100	9 years	28.7	15250
	7.7	3550		40.4	19700
	6.6	3500		24.9	13800
6 months	7.9	4000	10 years	32.1	16700
	9.5	4600		46.2	21900
	7.4	4100		27.5	14900
9 months	8.9	4700	11 years	36.1	18300
	10.6	5300		52.6	24200
	8.1	4600		30.6	16200
1 year	9.6	5200	12 years	40.7	20000
	11.5	6000		59.3	26600
	10.1	6100		34.2	17600
2 years	12.2	7000	13 years	45.8	22000
	14.7	8000		66.1	28900
	12.0	7300		38.5	19300
3 years	14.3	8300	14 years	51.2	24000
	17.3	9600		72.7	31100
	13.6	8250		43.0	21000
4 years	16.3	9450	15 years	56.5	25800
	20.3	11100		78.8	33100
	15.2	9100		47.3	22600
5 years	18.5	10500	16 years	61.1	27400
	23.5	12700		84.3	34900
	16.9	10000		50.8	23900
6 years	20.8	11700	17 years	64.7	28700
	27.0	14250		88.8	36300
	18.7	11000		53.2	24800
7 years	23.2	12800	18 years	67.3	29500
	30.9	16000		92.0	37400

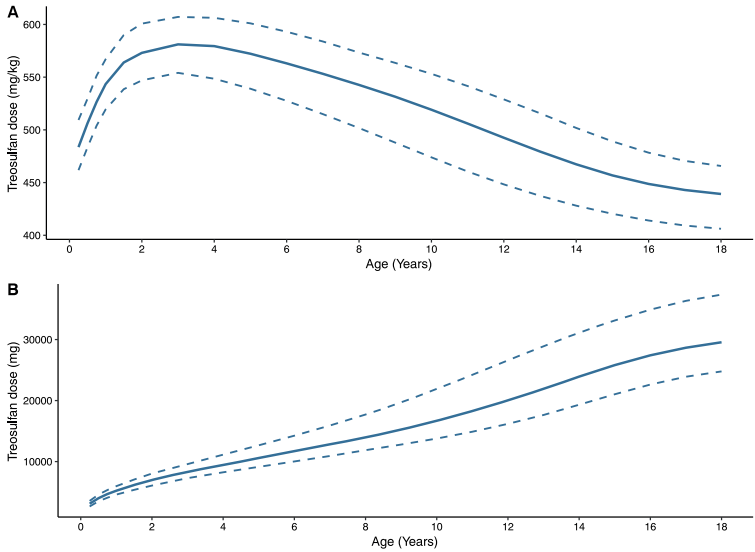

Figure 5. Required treosulfan daily dose in order to obtain a median $AUC_{0-\infty}$ of 1650 mg*hr/L against age **A**: in mg/kg and **B**: absolute dose (mg). The solid line represents the 50th weight percentile for that age, the upper dashed line represents the 5th weight percentile and the lower dashed line represents the 95th weight percentile.

Limited sampling model

The results of the LSM are shown in Table 4 and Figure 6. Predictive performance measurements used are: correlation, percentages of predicted AUCs within 10, 15 and 20% range of the 'true' $AUC_{0-\infty}$ and different ways of describing bias (mean prediction error, MPE; mean percentage prediction error, MPPE) and precision (root mean squared prediction error, RSME; mean absolute percentage predictive error, MAPE). Figure 6 shows results of four LSMs, including both regression lines with 95% confidence intervals as measurements of predictive performance. The best two-point markers were T=4 and 7 hours (R^2 = 0.97, MAPE = 5.06%, MPPE = 0.59%), with 97% of $AUC_{pred0-\infty}$ falling within 15% range of $AUC_{full0-\infty}$. The best three-point marker was T=1.5, 4 and 7 hours (R^2 = 0.99, MAPE = 2.84%, MPPE = -0.05%), with 100% of $AUC_{pred0-\infty}$ falling within 15% and even within 10% range of $AUC_{full0-\infty}$. With the tested single-point marker (T=1.5), prediction performance is far less compared to the two- and three-point markers. The percentage of $AUC_{pred0-\infty}$ that lies within 15% range of $AUC_{full0-\infty}$ is 69%. Population prediction without sampling has a very poor predictive performance and less than 35% of $AUC_{pred0-\infty}$ lies within the 15% range of $AUC_{full0-\infty}$.

Table 4. Limited sampling schemes based on one or multiple time points

Time points blood sampling	R^2 pearson	Percentage of AUC_{pred} within 10% range of AUC_{full}	Percentage of AUC_{pred} within 15% range of AUC_{full}	Percentage of AUC_{pred} within 20% range of AUC_{full}	MPE (mg*hr/L)	MPPE (%)	RMSE (mg*hr/L)	MAPE (%)
No sampling (population prediction)	0.01	22.86	31.43	40.00	-52.60	4.23	543.45	23.83
T=1.5	0.67	54.29	68.57	82.86	-29.17	0.41	291.71	11.65
T=4 / 7	0.97	91.43	97.14	100	11.01	0.59	101.79	5.06
T=1.5 / 3.5	0.93	77.14	94.29	97.14	-26.76	-0.66	138.88	5.62
T=5 / 7 / 9	0.96	82.86	94.29	100	-6.23	-0.49	112.20	5.86
T=3.5 / 4 / 7	0.96	82.86	97.14	100	-0.81	-0.06	103.74	5.07
T=1.5 / 3.5 / 4	0.97	91.43	97.14	100	-2.86	0.53	100.09	3.92
T=4 / 5 / 7	0.97	91.43	100	100	2.54	0.13	89.83	4.82
T=1.5 / 4 / 5	0.98	97.14	100	100	-9.00	0.01	77.84	3.28
T=1.5 / 4 / 7	0.99	100	100	100	0.61	-0.05	61.62	2.84
T=1.5 / 3.5 / 4 / 5	0.99	100	100	100	-7.91	0.01	61.93	2.41
T=1.5 / 4 / 5 / 7 / 9	0.99	100	100	100	-0.67	-0.07	45.98	1.91
T=1.5 / 3.5 / 4 / 7 / 9	1.00	100	100	100	1.43	0.06	18.72	0.82

AUC_{pred}: Predicted area under the curve; AUC_{full}: Full or 'true' area under the curve; MPE: Mean prediction error; MPPE: Mean percentage prediction error; RMSE: Root mean squared prediction error; MAPE: Mean absolute percentage predictive error

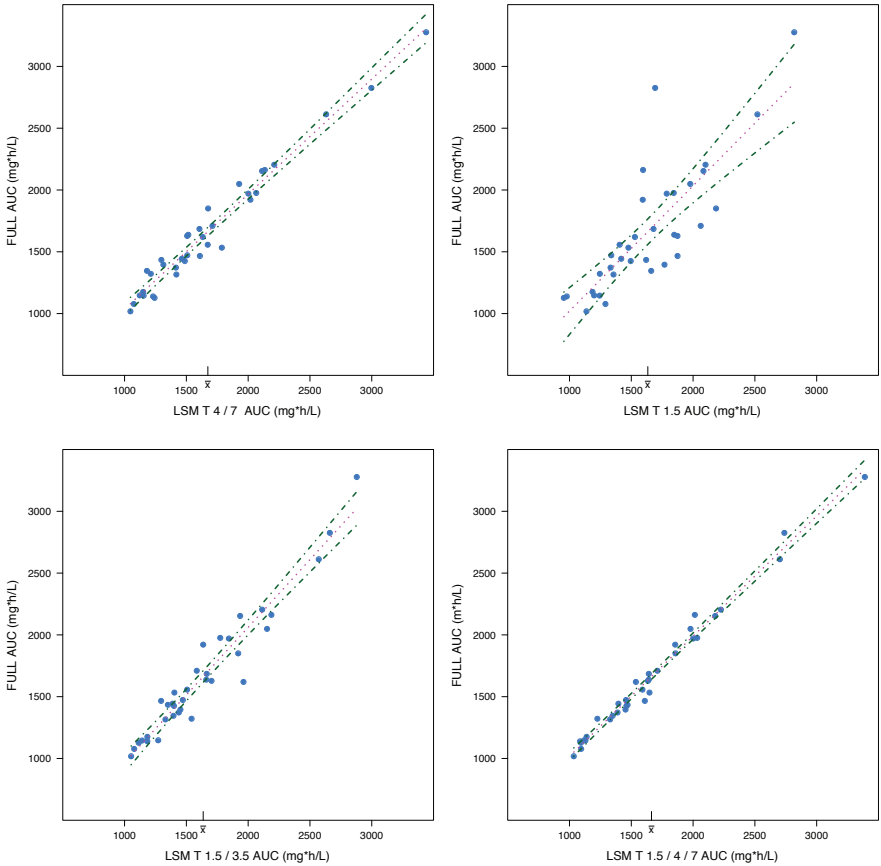

Figure 6. Regression line *(dotted lines)* plots of different limited sampling methods with 95% confidence intervals *(dot-dashed lines)*. *Upper left*: predictive performance of T=4 and 7 as limited sampling model; *upper right*: predictive performance of T=1.5 as limited sampling model; *lower left*: predictive performance of T=1.5 and 3.5 as limited sampling model; *lower right*: predictive performance of T=1.5, 4 and 7 as limited sampling model.

DISCUSSION

In this study, the population PK of treosulfan in pediatric HSCT recipients was best described by a two-compartment model. Allometric scaling of all parameters using BW and the addition of a maturation function using PMA was found to best account for differences in size and age. Other covariates such as gender, underlying disease, conditioning regimen, hematocrit and serum albumin did not significantly influence treosulfan PK. Estimated glomerular filtration rate seems to influence treosulfan PK, because it is known from literature that up to 39% of treosulfan is excreted via the kidneys in unchanged form [28-30]. However, addition of this covariate led to an increased IIV and the prediction corrected VPC worsened compared to the model with bodyweight and maturation of clearance only. Therefore, we ultimately chose not to include this covariate in the final model. In our dataset, only a few patients had an eGFR below 60 ml/min/1.73m^2 (n=5). It is likely that this number might be insufficient to establish this potential relationship accurately.

Danielak *et al.* also studied covariates, but only weight and gender were examined with weight being a significant covariate [19]. Mohanan *et al.* considered more covariates such as age, body weight, BSA, sex, liver size, liver fibrosis and biochemical parameters [16]. Interestingly, none of these variables explained the wide IIV in their cohort. Our model was based on a larger PK dataset, accounting for a wide age range in children (1 month – 18 years) which allows us to incorporate a maturation component in the model and account for maturation of clearance in the first years of life. Treosulfan clearance reaches 90% of adult values at 4 postnatal years.

The parameter estimations obtained in this study are somewhat comparable to the other published models in terms of clearance, but differ in terms of intercompartmental clearance, and the volume of distribution parameters. However, comparison is rather difficult when the values are not reported in a standardized fashion. Standardizing to a bodyweight of 70 kg increased the RSE of IIV of V2 and Q in our model. However, evaluation with a bootstrap procedure with 1000 bootstrap replicates showed estimates that are in line with the estimates of the PK parameters and their random variability

of the final model. Standardizing to the median weight might be more appropriate, because standardizing to a weight outside the observed weight range can increase uncertainty of parameter estimates [31]. On the other hand, comparison with other models is more difficult when standardizing to the median weight, so in the final model the PK parameters were standardized to 70 kg. As we compared the prediction corrected VPCs of the current model versus the models of Ten Brink *et al.,* Danielak *et al.* Mohanan *et al.* it is clear that the current model has superior predictive performance both in the high and low concentration range.

The present study shows a model-based individualized dosing table of treosulfan, aiming for an $AUC_{0-\infty}$ of 1650 mg*hr/L, which was the median exposure of our population. The recommended treosulfan dose is dependent on age and weight. An increase in treosulfan daily dose per kg until the age of 4 years can be seen, reflecting the maturation of clearance and allometry. Recently we showed that there is a relationship between treosulfan exposure and early toxicity [18]. Patients with an exposure >1650 mg*hr/L have an increased risk of developing grade 2 or higher mucositis and skin toxicity. Our model could be used to establish the initial dose, prior to or during treosulfan administration to facilitate therapeutic drug monitoring and thereby prevent toxicity. Little is known about the relationship between treosulfan exposure and transplant outcome parameters yet; however, the study of Mohanan *et al.* reported an association between treosulfan clearance <7.97 L/h/m^2 and poor overall survival [HR 2.7; CI (1.09-6.76), p=0.032] and event-free survival [HR 2.4; CI (0.98-5.73), p=0.055] in 87 pediatric patients with thalassemia major undergoing HSCT [16]. More studies conducted in different disease settings are needed to establish how systemic exposure to treosulfan can influence patient outcome. Subsequently, the optimal target exposure can then be established.

We also studied a limited sampling strategy, which potentially minimizes the burden of sampling and is convenient for performing TDM in the future. Ten Brink *et al.* chose two time points at 4 and 7 hours after start of infusion, although MPE and MAPE values of the T=1.5 and 5 hours strategy were slightly better [17]. This was

done because of practical reasons to avoid sampling during infusion. In the current study, with the addition of new samples, a preference for sampling at T =1.5 hours besides a sample after infusion was shown. This results in 100% of predicted $AUC_{0-\infty}$ falling within 15% and even within 10% radius of full $AUC_{0-\infty}$. We recommend to add a sample at T = 1.5 hours to the two-sample strategy of 4 and 7 hours after infusion, not only to increase predictive performance, but also to make a TDM protocol more robust. For instance, if in clinical practice one of the samples needs to be discarded due to unforeseen sampling or storage errors one would still be able to accurately estimate the $AUC_{0-\infty}$.

Our study has some limitations. Our dataset consisted of rich (full curves) and sparse (2 point curves) data combined together, which is less useful for non-compartmental analysis. However, the current approach of population pharmacokinetics, using nonlinear mixed effects modelling, allows data from a variety of unbalanced sparse and rich data to be analysed. Moreover, drug levels of concomitantly given drugs (such as fludarabine and thiotepa), which might influence treosulfan pharmacokinetics, were not available. In addition, because treosulfan is a prodrug, the active metabolites could be of interest to incorporate in the population pharmacokinetic model. Danielak *et al.* found a weak correlation between exposure to treosulfan and the metabolite S,S-EBDM ($r = 0.1681$, $p < 0.0001$). Also, patients with treosulfan exposure above 1650 mg*hr/L were most likely to have a high S,S-EBDM exposure [32]. These issues should be addressed in future studies. We have capped the eGFR values at 120 ml/min/1.73m^2, which could introduce a bias. However, renal function was not a significant predictor for treosulfan clearance and therefore was not of influence in our analysis.

In conclusion, a two-compartment population PK model to describe the serum concentration-time profiles of intravenously administered treosulfan was developed. Bodyweight and age (as PMA) have been identified as significant and clinically relevant covariates influencing treosulfan PK. Treosulfan serum concentrations at 1.5, 4 and 7 hours after start of infusion can be used to accurately estimate treosulfan exposure, particularly in a TDM setting.

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Supplemental Material 1. Existing population pharmacokinetic models

Author	Patient population	Treosulfan dose and infusion length	Sampling & quantitation	Model	Pharmacokinetic parameters	Covariates
Present study	N = 91 (64% male) Age (y): 4.3 (0.1-18.2) Weight (kg): 15.6 (3.8-75.0) BSA (m2): 0.7 (0.3-1.9) Disease: Malignant 19%, non-malignant 81%	14 g/m^2 - 3h	1.5, 3.5, 4, 5, 7, 9 h or 4, 7 h after start infusion HPLC-UV	Population (two-compartment)	Cl: 18.8 L/h/70 kg Q: 21.3 L/h/70 kg V1: 20.2 L/70 kg V2: 16.8 L/70 kg	Weight, age
Ten Brink (17)	N = 20 (65% male) Age (y): 4.4 (1.1-16.7) Weight (kg): 15.6 (9.3-52.0) BSA (m2): 0.61 (0.01-1.49) Disease: Malignant 20%, non-malignant 80%	14 g/m^2 - 3h	1.5, 3.5, 4, 5, 7, 9 h after start infusion HPLC-UV	Population (one-compartment)	Cl: 6.85 L/h/20 kg V: 13.2 L/20 kg	Weight
Danielak (12)	N = 15 (80% male) Age (y): 7.8 (0.4-15) Weight (kg): 26.9 (7.7-52) BSA (m2): 0.95 (0.25-1.63) Disease: Malignant 67%, non-malignant 33%	10 g/m^2 - 1h, n = 1 12 g/m^2 - 2h, n = 4 14 g/m^2 - 2h, n = 4 12 g/m^2 - 1h, n = 6	7 patients: 0.5, 1, 3, 4, 6, 8 h after start infusion 8 patients: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12 h after start infusion HPLC-MS/MS	Population (two-compartment)	Cl: 14.7 L/h/70 kg Q: 2.25 L/h V1: 26.0 L/70 kg V2: 9.93 L/70 kg	Weight
Mohanan (16)	N = 87 (63% male) Age (y): 9.0 (1.5-25) Weight (kg): 23.2 (11.4-55.0) BSA (m2): 0.93 (0.48-1.56) Disease: Thalassemia Major only	14 g/m^2 - 5 g/h	0h, end of infusion, 1, 2, 3, 5, 7, 24 h after end of infusion UPLC-RID	Population (two-compartment)	Cl: 11.6 L/h/m^2 Q: 2.14 L/h/m^2 V1: 19.4 L/m^2 V2: 2.01 L/m^2	-

Supplemental Material 2. Formulas

Demographic covariate formulas:

Estimated Glomerular Filtration Rate (Schwartz Equation):

$$eGFR = \frac{0.413 \times Height\ (cm)}{Serum\ creatinine}$$

BSA (Mosteller (1987)):

$$BSA\ (m^2) = \sqrt{\frac{Height\ (cm) \times Weight\ (kg)}{3600}}$$

Limited sampling strategy statistical analysis formulas:

Bias

$$Mean\ prediction\ error\ (MPE) = mean\ (AUC_{pred} - AUC_{full})$$

$$Mean\ percentage\ prediction\ error\ (MPPE) = mean\ (\frac{AUC_{pred} - AUC_{full}}{AUC_{full}} \times 100\%)$$

Imprecision

$$Root\ mean\ squared\ prediction\ error\ (RMSE) = \sqrt{mean\ (AUC_{pred} - AUC_{full})^2}$$

$$Mean\ absolute\ percentage\ prediction\ error\ (MAPE) = mean\ (\frac{|AUC_{pred} - AUC_{full}|}{AUC_{full}} \times 100\%)$$

The percentage of AUC_{pred} within a *x*% radius of AUC_{full} is decreased by both greater bias and worse precision and is therefore a useful measure of overall predictive ability.

Supplemental Material 3. Distribution of collected samples

Time after start of infusion	Number of samples
1.5	39
3.5	45
4	115
5	47
6	10
7	105
8	4
9	41
10	2
12	2

Supplemental Material 4. Model file

```
;; Description: PK of treosulfan, 2 cmt model IV infusion
$PROBLEM  PK of treasulfan,2 cmt model IV infusion
$INPUT   ID TAD TIME DV AMT DOSE TEST AGE WT RATE ISM
     CLCR CREAT CMT EVID DAY BSA ULD ULD2 COND
     ALB HB HT PH PMA
$DATA   TREOSULFAN.csv IGNORE=#
$SUBROUTINE ADVAN6 TOL=3
$MODEL   COMP=(CENTRAL) COMP=(PERI)
$PK
DAY1=0
```

```
DAY2=0
DAY3=0
IF(DAY.EQ.1)DAY1=1
IF(DAY.EQ.2)DAY2=1
IF(DAY.EQ.3)DAY3=1
IOV=DAY1*ETA(5)+DAY2*ETA(6)+DAY3*ETA(7)
TVHILL=THETA(5)
HILL=TVHILL
TVTM50=THETA(6)
TM50=TVTM50
TVCL=THETA(1)
FSIZE=(WT/70)**0.75
FMAT=1/(1+(PMA/TM50)**(-HILL))
CL=TVCL*EXP(ETA(1))*EXP(IOV)*FSIZE*FMAT    ; clearance
TVV=THETA(2)
V=TVV*EXP(ETA(2))*(WT/70)          ; volume of distribution
Q=THETA(3)*EXP(ETA(4))*FSIZE*FMAT
V2=THETA(4)*EXP(ETA(3))*(WT/70)
;
```

```
K=CL/V

K12=Q/V

K21=Q/V2

;

S1=V

AUCCL=DOSE/CL

$DES

DADT(1) =  -K*A(1)-K12*A(1)+K21*A(2)

DADT(2) =  K12*A(1)-K21*A(2)

$ERROR

Y = F*(1+ERR(1))

IPRED=F

IRES=DV-IPRED

DEL=0

IF (IPRED.EQ.0) DEL=1

IWRES=(1-DEL)*IRES/(IPRED+DEL)

$THETA

(0, 18.8) ; TH_CL

(0, 20.2) ; TH_V
```

(0, 21.3) ; TH_Q

(0, 16.8) ; TH_V2

(0, 1.22) ; Hill

(0, 38) ; TM_{50}

$OMEGA BLOCK(4)

0.101 ; ET_CL

0.131 0.211 ; ET_Vc

0.0349 0.026 0.0299 ; ET_Vp

-0.0307 -0.0354 -0.05 0.171 ; ET_Q

$OMEGA BLOCK(1)

0.0194

$OMEGA BLOCK(1) SAME

$OMEGA BLOCK(1) SAME

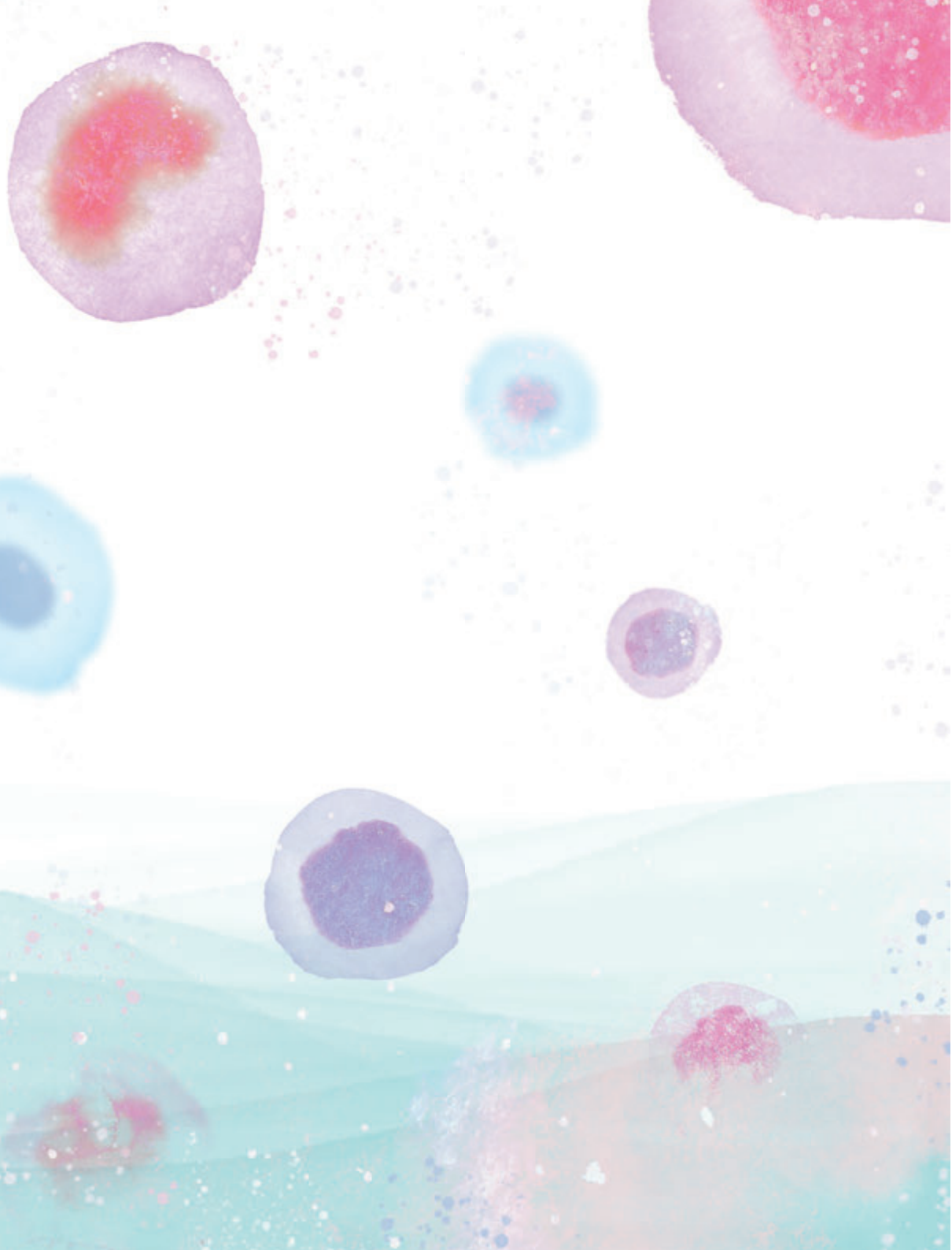
$SIGMA 0.0152 ; ER_Prop

$ESTIMATION METHOD=1 INTERACTION MAXEVAL=99999 PRINT=5 SIG=2 NOABORT POSTHOC MSFO=RFINAL8.nmv

$COVARIANCE unconditional matrix=s

$TABLE ID TIME DOSE IPRED IRES IWRES CL V ETA1 ETA2 IOV WT AGE CREAT

BSA ULD ULD2 COND TAD ISM CLCR AUCCL DAY ALB HB HT PH PMA EVID NOPRINT ONEHEADER FILE=RFINAL8.tab



CHAPTER 03

HIGH INTERPATIENT VARIABILITY OF TREOSULFAN EXPOSURE IS ASSOCIATED WITH EARLY TOXICITY IN PAEDIATRIC HSCT: A PROSPECTIVE MULTICENTRE STUDY

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ABSTRACT

Treosulfan-based conditioning is increasingly employed in paediatric hematopoietic stem cell transplantation (HSCT). Data on treosulfan pharmacokinetics in children are scarce, and the relationship between treosulfan exposure, toxicity and clinical outcome is unresolved. In this multicentre prospective observational study, we studied treosulfan pharmacokinetics and the relation with regimen-related toxicity and early clinical outcome in 77 paediatric patients. Treosulfan dose was 30 g/m^2, administered over 3 consecutive days in infants <1 year old (n=12), and 42 g/m^2, in children ≥1 year old (n=65), respectively. Mean day 1 treosulfan exposure was 1,744±795 mg*hr/L (10 g/m^2) and 1,561±511 mg*hr/L (14 g/m^2), with an inter-individual variability of 56 and 33%, respectively. High treosulfan exposure (>1,650 mg*hr/L) was associated with an increased risk of mucosal (OR 4.40; 95%CI 1.19-16.28, *P*=.026) and skin toxicity (OR 4.51; 95%CI 1.07-18.93, *P*=.040). No correlation was found between treosulfan exposure and the early clinical outcome parameters engraftment, acute graft-versus-host disease, and donor chimerism. Our study provides the first evidence in a large cohort of paediatric patients for high variability in treosulfan pharmacokinetics and an association between treosulfan exposure and early toxicity. Ongoing studies will reveal whether treosulfan exposure is related to long-term disease-specific outcome and late treatment-related toxicity.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment for a variety of malignant and non-malignant inherited or acquired diseases. The conditioning regimen given prior to HSCT has two goals: suppression of the immune system of the host to prevent graft rejection and allow donor engraftment, and ablation of dysfunctional or malignant host hematopoietic cells. Based on differences in underlying diseases, patient characteristics including age and co-morbidity, and HSCT strategy, various conditioning regimens, which differ in immuno- and myeloablative potential, have been developed. Myeloablative regimens based on total body irradiation (TBI) or high exposure busulfan are effective in facilitating engraftment and disease control, but share the disadvantage of being associated with significant early and late toxicity [1-3]. One of the major challenges to improve HSCT is reducing toxicity caused by the conditioning regimen given while maintaining efficacy. Treosulfan is an alkylating agent that is increasingly employed as part of conditioning regimens in HSCT for both malignant and non-malignant diseases due to its favourable toxicity profile in comparison to busulfan and TBI.

Treosulfan (L-threitol 1,4-bismethanesulphonate, Ovastat®) is a prodrug and a water-soluble alkylating agent. It is non-enzymatically, pH-dependently converted into a monoepoxide- and a diepoxide-derivative, which are thought to be responsible for DNA alkylation, interstrand DNA crosslinking, chromosomal aberration and induction of apoptosis [4, 5]. Treosulfan has potent myeloablative potential that is comparable with that of busulfan [6]. Furthermore, the immunosuppressive profile of treosulfan has been demonstrated to be stronger in comparison to that of busulfan and more durable than that of cyclophosphamide [6]. An *in vitro* study has shown that treosulfan has a stronger cytotoxic effect against paediatric leukemic cells compared to busulfan [7]. Recently, several studies have reported efficacy and tolerability of treosulfan-based conditioning regimens in paediatric HSCT for both non-malignant and malignant diseases [8-15].

Studies have shown that busulfan has highly variable pharmacokinetics, and clinical outcome of HSCT using busulfan-based conditioning is dependent on busulfan

exposure. Therefore, therapeutic drug monitoring (TDM) is required to achieve optimal drug exposure in individual patients [16, 17]. Similarly, we assume that clinical outcome with respect to both toxicity and efficacy after HSCT after a treosulfan-based regimen might also be dependent on actual drug exposure. To date, only a few studies, including small numbers of paediatric patients, investigated the pharmacokinetic profile of treosulfan. These studies focused on pharmacokinetics (PK) and reported substantial interpatient variability up to 70%. However, the association between treosulfan exposure and clinical outcome parameters was not addressed in these studies [18-21].

In this report of a multicentre prospective study, we describe the pharmacokinetics of treosulfan in paediatric HSCT recipients and the relationship between treosulfan exposure and early toxicity and clinical outcome.

PATIENTS AND METHODS

Characteristics of the study cohort

A total of 77 paediatric patients transplanted between June 2011 and July 2016 who received conditioning with treosulfan and fludarabine (TF) or treosulfan, fludarabine and thiotepa (TFT) prior to HSCT in the Leiden University Medical Center in The Netherlands and the Children's Hospital Bambino Gesù in Rome, Italy were included in this study. The institutional Ethics Committee approved the treosulfan PK study protocol (P12.267). Written informed consent for participation in the study was obtained from the parents or legal guardians, as well as consent from patients when they were older than 12 years according to the Helsinski Declaration (last amended in 2013, Fortaleza Brasil).

Patient and donor characteristics are summarized in Table 1. Forty-six (59.7%) patients were males and 31 (40.3%) females. The median age in our cohort at transplantation was 4.8 (IQR 1.6-11.4) years, 12 patients were <1 years old. Patients received HSCT for various malignant and non-malignant indications in line with the

EBMT–Working Party Inborn Errors and Paediatric Diseases recommendations and based on previous reports on treosulfan-based conditioning [9, 10, 13]. Most patients (84.4%) were transplanted for a non-malignant disease, including 31 (40.3%) patients with hemoglobinopathy, 22 (28.5%) patients with primary immune deficiency, 11 (14.3%) with an inherited bone marrow failure syndrome and 1 (1.3%) patient with a metabolic disease. Patients with malignant disease received treosulfan as part of a reduced toxicity regimen because of pre-existent co-morbidity. Seventy-three (94.8%) patients received treosulfan-based conditioning preceding a first HSCT, whereas in four patients the drug was used in preparation to a second transplantation. Sixty-five patients (84.4%) above the age of 1 year received treosulfan in a total dose of 42 g/m^2, administered over 3 consecutive days (14 g/m^2 per day). Twelve patients (15.6%) under the age of 1 year old received a total dose of 30 g/m^2 (10 g/m^2 per day). Treosulfan was combined with thiotepa (8-10 mg/kg) and fludarabine in 52 patients (67.5%) (total dose of 150-160 mg/m^2), whereas in 25 patients (32.5%) treosulfan was combined with fludarabine. Thiotepa was administered at day -8, treosulfan at day -7 to day -5 and fludarabine at day -7 to day -3. Serotherapy consisted of anti T lymphocyte globulin (ATLG), anti thymocyte globulin (ATG) or alemtuzumab. Twenty-seven patients (35.0%) received a transplant from a HLA identical sibling, 36 patients (46.8%) from a matched unrelated donor (≥9/10 allelic matching) and 14 patients from a HLA-haploidentical relative (18.2%). In patients with a mismatched related donor, peripheral blood stem cell grafts were processed by either CD34-positive selection or selective elimination of αβ+ T and CD19+ B cells [22]. Graft-versus-Host Disease (GvHD) prophylaxis was given according to institutional guidelines. Granulocyte colony-stimulating factor (G-CSF) was routinely given in cord blood transplants from day +8 onwards. All patients were cared for in high-efficiency, particle-free air (HEPA)-filtered positive-pressure isolation rooms and received intestinal decontamination using non-absorbable antimicrobials, as well as supportive care according to institutional guidelines.

Table 1. Patient characteristics

	Total (n=77)
Characteristic	
Age (years, IQR (median))	1.6-11.4 (4.8)
Weight (kg, IQR (median))	10.8-34.2 (17.0)
Sex (n: M/F)	46/31
Diagnosis for HSCT	
Hemoglobinopathies (%)	31 (40.3)
Hematologic malignancy (%)	12 (15.6)
Primary immune deficiency (%)	22 (28.5)
Bone marrow failure (%)	11 (14.3)
Other (%)	1 (1.3)
Number of transplantation	
First (%)	73 (94.8)
Second (%)	4 (5.2)
Donor	
MSD (%)	27 (35.0)
MUD (≥ 9/10) (%)	36 (46.8)
MMFD (haplo) (%)	14 (18.2)
Stem cell source	
BM (%)	50 (64.9)
PBSC (%)	20 (26.0)
T cell replete (%)	6 (7.8)
TCR αβ/CD19 depletion (%)	11 (14.3)
CD34 enrichment (%)	3 (3.9)
CB (%)	6 (7.8)
BM + CB (%)	1 (1.3)
Conditioning	
Treo-Flu-Thiotepa (%)	52 (67.5)
Treo-Flu (%)	25 (32.5)
Treosulfan dose	
14 g/m^2 (%)	65 (84.4)
10 g/m^2 (%)	12 (15.6)
Serotherapy	
Yes (%)	69 (89.6)
ATG (%)	38 (49.3)
ATLG (%)	22 (28.6)
Alemtuzumab (%)	9 (11.7)
No (%)	8 (10.4)
GvHD prophylaxis	
CsA (%)	4 (5.2)
CsA / MTX (%)	50 (64.9)
CsA / Pred (%)	3 (3.9)
Other (%)	6 (7.8)
None (%)	14 (18.2)

BM: bone marrow, PBSC: peripheral blood stem cells, CB: cord blood, MSD: matched sibling donor, MMFD: mismatched family donor, MUD: matched unrelated donor, Treo: treosulfan, Flu: fludarabine, Thio: thiotepa, ATG: Anti thymocyte globulin, ATLG: Anti T lymphocyte globulin, GvHD: Graft-versus-Host Disease, CsA: Cyclosporine A, MTX: methotrexate, Pred: prednisolone.

Treosulfan assay

Blood samples were collected in serum tubes without gel. Samples were centrifuged as soon as possible (within 5 hours), and stored at -20 degrees Celsius. A validated reversed-phase high-pressure liquid chromatography (RP-HPLC) using ultraviolet (UV) detection was used to determine treosulfan concentration in serum, as previously reported [21]. Briefly, treosulfan and the internal standard busulfan were made detectable through derivatization with sodium diethyldithiocarbamate (DDTC). Linearity was established up to 500 mg/L with a lower limit of quantification of 6.8 mg/L. Accuracies of quality control (QC) samples were within the 90-110% limit. The intraday imprecision, expressed as coefficient of variation (CV%), ranged from 2.0% to 3.3% and interday imprecision ranged from 2.1% to 2.8%.

Population pharmacokinetics of treosulfan

The individual pharmacokinetic parameters of each patient were determined using a validated two-compartment population pharmacokinetic (PK) model, using non-linear mixed-effects modelling as implemented in the NONMEM software package (version 7 level 3; Icon Development Solutions, Ellicott City, Maryland, USA). This is an extended model based on the PK model published by Ten Brink et al [21]. A total of 384 samples were used in a range of 1.5, 3.5, 4, 5, 7 and 9 hours after the start of a 3-hour infusion to develop and validate the model. Clearance and volume of distribution were allometrically scaled using body weight. The structural PK model of treosulfan indicated the following PK parameters for a child with 20 kg body weight: average clearance (CL) of 6.98 L/h (CV: 37.9%), average central distribution volume (Vc) of 9.59 L (CV: 54.2%), average peripheral distribution volume (Vp) of 2.34 L (CV: 82.6%) and average inter-compartmental clearance of 2.74 L/h (CV: 69.1%). The allometric scaling exponent for clearance was fixed at 0.75 and for volume of distribution at 1.0. AUCs were calculated using post-hoc estimation using the final model based on all available samples. In 29 patients rich curves were obtained and used. Using the limited sampling strategy established by Ten Brink et al. and

reconfirmed with the extended model as described above, 2 samples were taken in the following 48 patients at 4 and 7 hours after start infusion to calculate AUC [21]. For determination of the intra-patient variability blood samples obtained on day 3 in a subgroup of patients (n = 19) were also used in the analysis.

Evaluation of clinical data

Primary endpoint of this study was early toxicity and secondary endpoints were early clinical outcome parameters (i.e. engraftment, acute GvHD and chimerism at day +30 and +100). Toxicity endpoints were evaluated until 28 days after HSCT and included mucosal, skin, hepatic and neurological toxicity measured according to CTCAE criteria and Bearman et al [23]. Engraftment was defined as the first of three days with a neutrophil count of $\geq 0.5 \times 10^9$/L. Platelet engraftment was defined as platelet count > 50×10^9/L, without platelet support for 3 consecutive days. Chimerism was determined in peripheral blood granulocytes and mononuclear cells by VNTR polymorphism at day 30 and 100 after transplantation. Acute GvHD was diagnosed and graded as defined by Przepiorka et al [24].

Statistical analysis

Normally distributed parameters are shown as mean ± standard deviation and all log-normally distributed parameters as median (interquartile range IQR). Inter-patient variability was calculated by the coefficient of variation (CV%) of the treosulfan exposure between individuals and intra-patient variability by calculating the mean difference between the AUC on day 1 and day 3 of each individual. The predictive value of systemic treosulfan exposure for the occurrence of toxicity within 28 days is evaluated using a logistic regression analysis for mucosal, skin, hepatic and neurological toxicity events. Cumulative toxicity was scored as the sum of these different toxicities, with a maximum score of 4 and tested as two groups (≥2 toxicities, yes/no). This is tested with AUC as a discrete variable, considering 3 exposure groups low [<1350 mg*hr/L (1^{st} tertile)], medium [1350-1650 mg*hr/L (2^{nd} tertile)] and high [>1650

mg*hr/L (3th tertile)]. The cumulative incidence of neutrophil engraftment and acute GvHD was estimated using the method of Fine and Gray for censored data subject to competing risks, taking into account graft failure, death without engraftment and subsequent HSCT as competing risk for neutrophil engraftment and death before day +100 as competing risk for acute GvHD [25]. The association between treosulfan exposure and acute GvHD and engraftment is tested with the Gray test using two AUC groups with the median as cut-off value (1500 mg*hr/L). The relationship between treosulfan exposure and chimerism at day +30, +100 and 1 year after HSCT was determined with ordinal logistic regression, using 3 groups (donor chimerism <50%, 50-90% and >90%) and AUC as a continuous variable. All *P*-values were 2-tailed and considered significant when $P < .05$. Statistical analyses were performed with SPSS statistics, version 23.0 (IBM Corp., Armonk, NY, USA). The competing risks analysis and Gray test was performed using R version 3.4.0.

RESULTS

Treosulfan pharmacokinetics

A total of 96 AUCs were determined in 77 patients. The results are shown in Figure 1 and Table 2. In the first 19 patients treosulfan AUC was determined on day 1 and day 3 to assess intra-patient variability. The mean intra-patient variability was 13.9% (Figure 2). Based on these results we decided to determine treosulfan exposure only on day 1 as a good representation of total exposure.

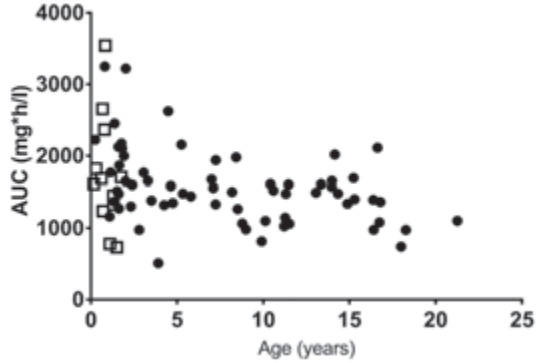

Figure 1. The relation between treosulfan exposure and age. The vertical axis represents the Area under the Curve (AUC) values in mg*hr/L. Symbols represent the different dosing schemes: 14 g/m^2 (●), 10 g/m^2 (□).

Table 2. Treosulfan pharmacokinetic parameters

Parameter	10 g/m^2 mean ± SD	14 g/m^2 mean ± SD
No. of patients	12	65
Age (yrs)	0.9 ±0.48	7.96 ± 5.75
Weight (kg)	7.4 ±1.9	27.59 ± 18.15
BSA (m^2)	0.37 ± 0.07	0.94 ± 0.41
AUC (mg*hr/L)	1744 ± 795	1561 ± 511
Cl (ml/min/kg)	2.17 ± 1.41	8.08 ± 5.04
Clp (ml/min/kg)	1.61 ± 0.46	3.00 ± 2.44
Vc (L/kg)	0.14 ± 0.09	0.74 ± 0.63
Vp (L/kg)	0.07 ± 0.04	0.17 ± 0.15

BSA: body surface area, AUC: area under the curve, Cl: clearance, Clp: inter-compartmental clearance, Vc: central volume of distribution, Vp: peripheral volume of distribution

The mean day 1 exposure was 1,561 mg*hr/L (range 511-3,250 mg*hr/L) and 1,744 mg*hr/L (range 732-3,544 mg*hr/L) for patients that had received 14 g/m^2 and 10 g/m^2, respectively (P = .263). The corresponding inter-patient variability (CV%) was 33% and 56% within the groups that had received 14 g/m^2 and 10 g/m^2, respectively, showing large inter-patient variability of treosulfan exposure, especially in young children. Because of dose adjustment to 10 g/m^2 in young children, mean exposure

did not significantly differ, however mean clearance was significantly lower in children receiving 10 g/m^2 compared to 14 g/m^2 (2.17 vs. 8.08 ml/min/kg, $P < .001$) and mean central volume of distribution was also lower (0.14 vs 0.74 L/kg, $P < .001$).

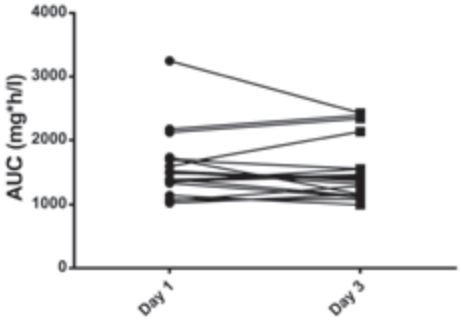

Figure 2. Intra-patient variability of treosulfan. AUC was measured at day 1 and day 3 in 19 patients (13.9%).

Early regimen-related toxicity

Early regimen-related toxicity was evaluated until 28 days after HSCT. The most common toxicities observed were mucosal-, hepatic-, skin- and neurological toxicity.

Mucositis. Thirty-six (46.8%) patients experienced mucositis, with 25 patients developing grade 2 (19.5%) or 3 (13.0%). Grade 4 mucositis was not seen in any of the patients. The occurrence of mucositis in the different exposure groups is shown in Figure 3. The odds of developing grade 2 or greater mucositis was significantly higher when AUC exceeded 1,650 mg*hr/L (OR 4.40; 95% CI 1.19-16.28, $P = .026$) compared to AUC under 1,350 mg*hr/L. Given the fact that there were two different conditioning regimens (TF and TFT), we corrected for this covariate together with age. The adjusted OR was 7.03 (95% CI 1.60-30.86, $P = .010$). A higher risk to develop grade 2-3 mucositis is also seen in the medium AUC group (1350-1650) compared to AUC under 1350 mg*hr/L, however this did not reach statistical significance (adjusted OR 3.66; 95% CI 0.93-14.52, $P = .065$). Accordingly, higher treosulfan AUC is associated with the risk of higher grade mucositis ($P = .006$) (Figure 4).

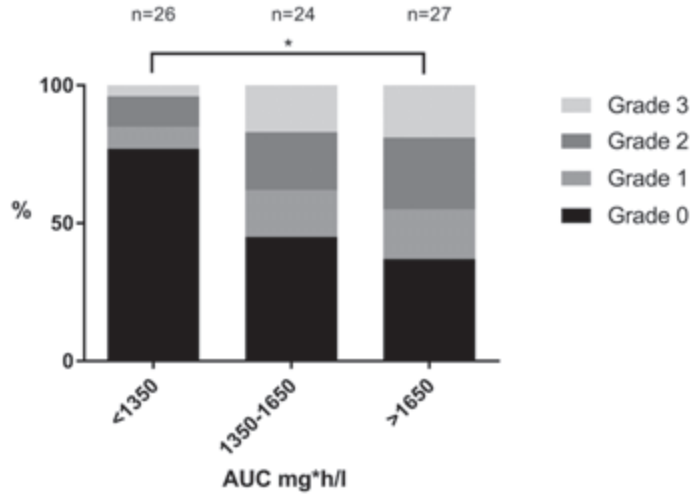

Figure 3. Incidence of mucositis in different treosulfan exposure groups. The incidence of mucositis is shown according to grade, with black being grade 0, progressing to light gray being grade 3. (*Grade 0/1 vs grade 2/3, *P* = .026).

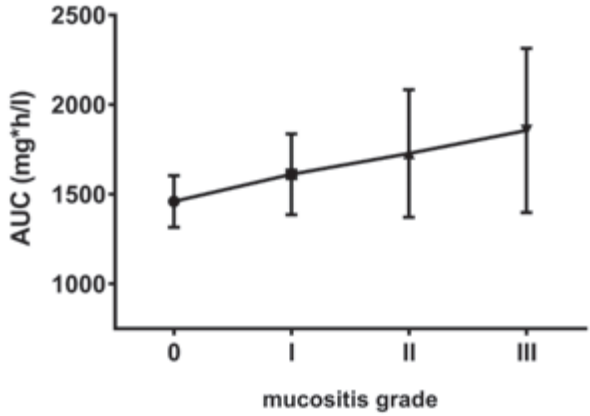

Figure 4. Mean treosulfan AUC with 95% CI according to mucositis grade. The vertical axis represents the (AUC) values in mg*hr/L with 95% confidence interval. Treosulfan AUC is associated with mucositis grade (*P* = .006).

Hepatic toxicity. Hepatic toxicity grade 2 or greater, defined as a >5-fold increase in alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT) levels and more than a 3-fold increase in bilirubin levels, was seen in 24 patients (31.2%). There is no statistically significant association between treosulfan exposure and hepatic toxicity. The odds of developing grade 2 or greater hepatic toxicity are possibly influenced by treosulfan exposure, but no statistically significant association was seen (Table 3). Severe veno-occlusive disease/sinusoidal obstruction syndrome, according to modified Seattle criteria, was not seen in any of the patients [26, 27].

Skin toxicity. Skin toxicity, which includes erythematous rash and skin exfoliation, occurred in 18 (23.2%) patients (Figure 5). Children belonging to the high AUC group (>1,650 mg*hr/L) showed an increased risk of developing skin toxicity (OR 4.51; 95% CI 1.07-18.93, P = .040 and OR 9.96; 95% CI 1.85-53.46, P = .007, adjusted for conditioning regimen and age). An increased risk was also seen in the 1,350-1,650 mg*hr/L group, although the difference with the low exposure group is not statistically significant (Table 3). The addition of thiotepa to the conditioning regimen also showed a trend of increased risk of skin toxicity, however this was not statistically significant.

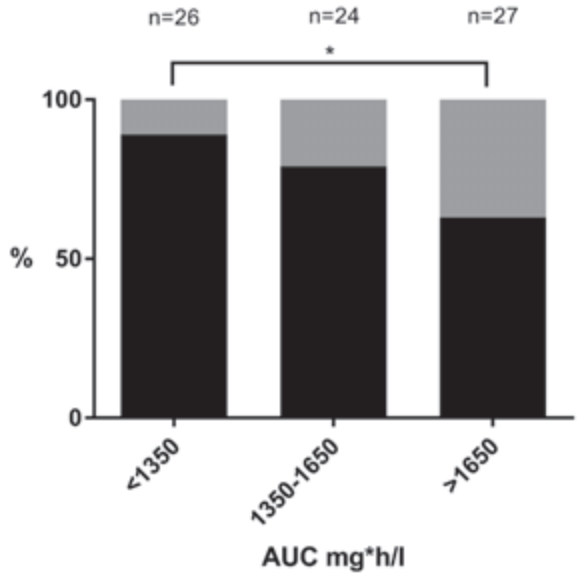

Figure 5. Incidence of skin toxicity in different treosulfan exposure groups. Skin toxicity: gray, no skin toxicity: black (*P = .040).

Neurological toxicity. Five patients (6.5%) experienced neurological symptoms, including convulsions and posterior reversible encephalopathy syndrome (PRES). No significant association was seen between neurological symptoms and treosulfan exposure (Table 3).

Cumulative toxicity. We investigated whether AUC is correlated with the occurrence of multiple toxicities including mucositis, skin and/or liver toxicity. Of all patients, 46.8% experienced no toxicity, 20.8% experienced one toxicity, 23.4% two toxicities and 9.1% experienced three toxicities. The risk of experiencing two or more toxicities is higher when AUC exceeds 1650 mg*hr/L compared to AUC under 1350 mg*hr/L (OR 4.52; 95% CI 1.32-15.53, P = .016 and OR 8.25; 95% CI 1.88-36.13, P = .005, adjusted for conditioning regimen and age).

Table 3. Relationship between treosulfan AUC and early regimen-related toxicity

Treosulfan AUC mg*hr/L	**Mucositis**		**Hepatic toxicity**		**Skin toxicity**		**Neurological toxicity**		**Cumulative toxicity ≥ 2 toxicities**	
<1350										
OR (95%CI)	Reference		Reference		Reference		Reference		Reference	
Adjusted OR (95%CI)										
1350-1650		*p*		*p*		*p*				*p*
OR (95%CI)	3.30 (0.86-12.71)	.083	1.12 (0.33-3.84)	.860	2.02 (0.43-9.55)	.376	-		1.40 (0.37-5.37)	.624
Adjusted OR (95%CI)	3.66 (0.93-14.52)	.065	1.19 (0.34-4.22)	.786	2.38 (0.48-11.78)	.289			1.56 (0.39-6.21)	.531
>1650		*p*		*p*		*p*		*p*		*p*
OR (95%CI)	4.40 (1.19-16.28)	*.026*	1.60 (0.50-5.13)	.432	4.51 (1.07-18.93)	*.040*	1.50 (0.23-9.80)	.672	4.52 (1.32-15.53)	*.016*
Adjusted OR (95%CI)	7.03 (1.60-30.86)	*.010*	2.40 (0.64-8.93)	.193	9.96 (1.85-53.46)	*.007*	1.77 (0.22-14.24)	.659	8.25 (1.89-36.13)	*.005*

AUC: area under the curve, OR: Odds Ratio, CI: Confidence interval. The adjusted OR was adjusted for conditioning regimen and age.

GvHD

Cumulative incidence of grade II-IV acute GvHD was 11% (95% CI 6-21%) with 4 patients experiencing grade II and 4 patients grade III GvHD. None of the patients developed grade IV GvHD. Within the TF group, only 1 of 22 (4.5%) evaluable patients at day +100 developed grade II-III acute GVHD, compared to 7 of 45 (15.5%) in the TFT group. Two of the four patients with grade II and all four with grade III acute GvHD were transplanted from an unrelated donor including two with a T-cell-replete peripheral blood stem cell graft. Mean treosulfan exposure in patients with and without GvHD was 1,365 mg*hr/L and 1,579 mg*hr/L, respectively. There was no significant difference between the two groups (P = .108).

Engraftment, chimerism and survival

Cumulative incidence of neutrophil engraftment was 94% (95% CI 89-100%). Median time to neutrophil and platelet engraftment was 19 (IQR 15-23) days and 22 (IQR 11-34) days, respectively. All the 7 patients experiencing graft failure, three with a T-cell replete and four with T-cell depleted graft, received a total treosulfan dose of 42 g/m^2; 5 patients were conditioned with TFT and 2 patients with TF. Mean AUC was 1,605 mg*hr/L and 1,342 mg*hr/L in the engraftment and non-engraftment group, respectively. No relationship was found between engraftment and treosulfan AUC (P = .750). Four patients with non-engraftment underwent subsequent second transplantation, whereas the other three patients died because of treatment-related complications.

There were 72 patients evaluable for day +30 chimerism, 63 patients for day +100 chimerism and 58 patients at 1 year after HSCT. In 63 patients (87.5%), 44 patients (69.8%) and 38 patients (65.5%) donor chimerism was between 90 and 100% for day +30, +100 and 1 year, respectively. No relationship was seen with treosulfan exposure (P = .857, P = .535 and P = .500 for day +30, +100 and 1 year, respectively). Cumulative incidence of treatment related mortality at day +100 was 10.4% (95% CI 5-20%) and no relationship was found with treosulfan exposure. Overall survival in this cohort was 86%. No relationship was seen with treosulfan exposure (data not shown).

DISCUSSION

Treosulfan-based conditioning is increasingly used in paediatric HSCT and has been demonstrated to be effective and well tolerated in patients with both malignant and non-malignant diseases [9, 10, 12, 13, 28-30]. Despite the increased use of treosulfan in recent years, pharmacokinetic data in children are still limited. To the best of our knowledge, this is the largest paediatric cohort studied so far, covering a broad age range. We demonstrate large inter-patient variability of treosulfan exposure. Furthermore, pharmacokinetic parameters were shown to be age-dependent with higher AUC values in younger children (<1 year old) and corresponding lower treosulfan clearance. This is the first study that reports the relationship between treosulfan exposure and early clinical outcome parameters. We provide evidence that higher treosulfan exposure is associated with a higher risk of drug related toxicity and specifically with moderate to severe mucositis and skin toxicity. Also, a higher exposure is a predictor for experiencing multiple toxicities.

In our study, the mean day 1 AUC of 1,561 mg*hr/L in patients receiving 14 g/m^2 was lower than that reported by Glowka et al. (2,400 ± 1,267 mg*hr/L), but slightly higher than that reported by Koyyalamudi et al. (1,412 ± 215 mg*hr/L) [18, 20]. However, those results were based on small number of patients, i.e. 7 and 3 patients, respectively (age range 2-18 years), which may contribute to explain these differences. When comparing our pharmacokinetic data with previous studies, we found an intra-patient variability of 13.9% (CV), based on measurements in 19 patients, which is much lower than the inter-patient variability (33-56%), and lower than reported by Glowka et al. [18]. In our cohort, a subgroup of 12 children <1 year old received a dose of 10 g/m^2. In these infants, treosulfan exposure was higher (mean of 1,744 mg*hr/L) compared with older patients receiving 14 g/m^2 (mean of 1,561 mg*hr/L) with an inter-patient variability of 56%. In previous studies, PK data in very young children are scarce and point to either an increased AUC or no difference in AUC compared to older children [19, 20, 31]. We found that treosulfan clearance in children under the age of 1 year, receiving 10 g/m^2, was significantly lower than that of older children receiving 14 g/m^2.

It is unclear why younger children, despite an already adjusted lower dose, have higher AUC values. A possible explanation for this phenomenon could be the maturation and development of renal function in children under the age of one year. The glomerular filtration rate increases rapidly during the first two weeks of life, but adult values are not reached until 8-12 months [32]. Because approximately 25% of treosulfan is excreted via a renal route in unchanged form, the not fully matured renal function of these young infants could be an explanation of lower treosulfan clearance [33]. Scheulen et al. hypothesized that this observation could be explained by metabolic acidosis associated with the release of large amount of methanesulfonic acid during treosulfan activation which causes inhibition of pH-dependent treosulfan transformation [34]. We believe this is unlikely to be the explanation in our patients, given the buffering action of blood and the relatively stable clinical situation of the patients prior to administration of treosulfan. Either way, the difference in AUC between infants and older children warrants further investigation. Most conditioning protocols recommend dose adjustment, i.e. 10 g/m^2 or 12 g/m^2, in children <1 years old to limit toxicity. Our PK data show that the dose adjustment in younger children results in comparable treosulfan exposure in children under and above 1 years old. Whether similar exposure as a consequence of current age-adjusted dosing leads to similar clinical outcome in these different age groups remains to be demonstrated.

Treosulfan itself is a pro-drug and converted into its active derivatives (a monoepoxide and diepoxide) in a non-enzymatic and pH-dependent manner. In this study, we focused on the PK of the parent compound, as we thought this to be a good representation of alkylating activity. Recently, the concentrations of the metabolites of treosulfan have been analyzed in patients [18]. In this study the diepoxide derivative could not be detected in patient samples, probably due to fast elimination and the monoepoxide derivative was found in concentrations approximately two-order lower that the parent compound. Whether the metabolite exposure is also in association with early toxicity is yet unclear and requires further investigation.

Our study provides evidence of a relationship between treosulfan exposure and early toxicities (in particular severe mucositis and skin toxicity), whereas we did not yet find

an association with engraftment, occurrence of severe acute GvHD and chimerism at day +30, +100 and 1 year. This may however be due to the heterogeneity of primary diseases in our cohort. PK/PD studies in more homogeneous and single disease patient cohorts with longer follow up will be of great value to study the relationship of AUC and these long-term outcome parameters. These studies are currently ongoing.

Our observation that high treosulfan exposure is associated with a higher risk of moderate to severe mucositis, skin toxicity and a higher risk of experiencing multiple toxicities in the first 28 days after SCT indicates that avoiding high exposure may reduce transplant-related morbidity in individual patients. Because of the great inter-patient variability and different pharmacokinetic parameters in very young children, therapeutic drug monitoring as a personalized approach may be a suitable option to optimise individual outcome in this group. However, before considering such an approach the relationship between treosulfan exposure and long-term disease specific outcome needs to be established as well. Also, longer follow up of these patients is needed to investigate whether the occurrence of late toxicities, particularly those involving gonadal function, is correlated with treosulfan exposure, and how this compares to other myeloablative regimens based on the use of either busulfan or irradiation.

In this study, we provide, for the first time, evidence for high variability in pharmacokinetic parameters of treosulfan in children. There is an inverse relationship between AUC and age, suggesting that the adjusted dose in very young children is justified to achieve an exposure which is similar to older children. High treosulfan exposure is associated with the occurrence of moderate to severe mucositis and skin toxicity. Ongoing studies will reveal whether treosulfan exposure is related to long-term disease outcome and late treatment-related toxicity.

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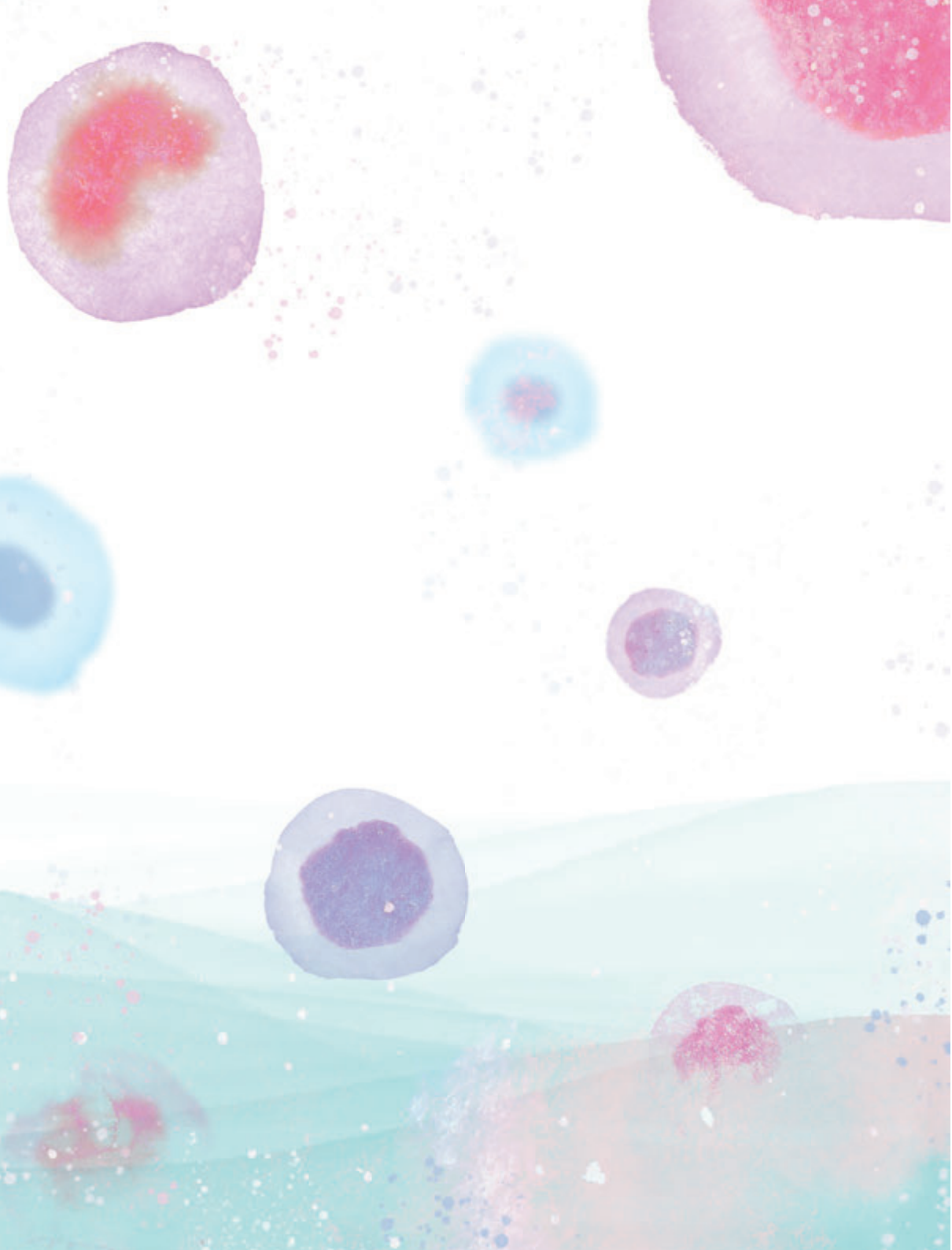

CHAPTER 04

IMPACT OF TREOSULFAN EXPOSURE ON EARLY AND LONG-TERM CLINICAL OUTCOME IN PEDIATRIC ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION RECIPIENTS: A PROSPECTIVE MULTICENTRE STUDY

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ABSTRACT

Treosulfan-based conditioning has gained popularity in pediatric allogeneic hematopoietic stem cell transplantation (HSCT) because of its presumed favourable efficacy and toxicity profile. Treosulfan is used in standardized dosing regimens based on body surface area. The relationship between systemic treosulfan exposure, early and long term clinical outcome in pediatric patients undergoing allogeneic HSCT for non-malignant diseases is as yet unresolved. In this study we assessed the association between treosulfan exposure and early, and in particular, long term clinical outcomes. We conducted a multicentre, prospective observational study and included 110 pediatric patients with non-malignant diseases transplanted between 2011 and 2019 in Leiden, The Netherlands and Rome, Italy. Blood samples were collected and treosulfan area under the curve ($AUC_{0-\infty}$) was estimated as a measure of exposure. Cox proportional hazard survival analyses were performed to assess the relationship between treosulfan exposure, OS and EFS. The predictive value of systemic treosulfan exposure for the occurrence of toxicity within 28 days is evaluated using a multivariable logistic regression analysis. In the overall cohort, overall survival (OS) and event-free survival (EFS) at 2 years were 89.0% and 75.3%, respectively, with an excellent OS of 97% in children under the age of 2 years. The occurrence of grade II-IV aGvHD, the level of 1-year whole blood chimerism, and 2-year OS and EFS were not correlated with treosulfan exposure. The occurrence of skin toxicity (odds ratio (OR) 3.97, 95% confidence interval (CI) 1.26-13.68, p=0.02) and all grade mucositis (OR 4.43, 95%CI 1.43-15.50, p=0.02), but not ≥ grade 2 mucositis (OR 1.51, 95%CI 0.52-4.58, p=0.46) was related to high treosulfan exposure (>1750 mg*h/L). Our study demonstrates that standardized treosulfan-based conditioning results in a favourable OS and EFS in infants and children with non-malignant diseases, independent of interindividual variation in treosulfan exposure. These outcomes can be achieved without the need for therapeutic drug monitoring (TDM), thereby emphasizing the advantage of treosulfan use in this category of patients. Although higher treosulfan exposure increases the risk of skin toxicity, there is no absolute necessity for therapeutic drug monitoring if proper preventive skin measures are taken. More research is needed to assess whether deescalation of treosulfan doses is possible in order to minimize early and long-term toxicity without compromising efficacy.

INTRODUCTION

Over the past decade, treosulfan has been increasingly used as part of conditioning regimens in pediatric allogeneic hematopoietic stem cell transplantation (HSCT) for both malignant and non-malignant diseases [1-4]. Treosulfan (Trecondi®) is a prodrug and a water-soluble alkylating agent. It is non-enzymatically, pH-dependently converted into a monoepoxide- and a diepoxide derivative, which are thought to cause DNA alkylation [5, 6]. Treosulfan has gained popularity, because of its myelo- and immunoablative properties, which are combined with an apparent favourable toxicity profile. This makes treosulfan an interesting backbone of conditioning regimens, particularly in patients with non-malignant diseases. In recent years, pharmacological studies have provided evidence that therapeutic drug monitoring (TDM) is an important tool to optimize efficacy and limit toxicity of chemotherapeutic agents, especially in pediatric patients. Large interindividual variation of busulfan exposure while using uniform dosing regimens and the relationship between exposure and clinical outcome and toxicity have resulted in individualized treatment regimens [7, 8]. Building on this experience, similar approaches have been used to investigate interindividual variability in drug exposure and its impact on clinical outcome for anti-thymocyte globulin, alemtuzumab and fludarabine [9-12]. Treosulfan is used in standardized dosing regimens, both in children and adults, mostly based on body surface area. In a retrospective pediatric study, no correlation was found between total dose and clinical outcome [13]. However, in various single and multicentre studies, large interindividual variability in treosulfan exposure has been reported in patients [14-18]. So far, only three studies, including a study from our group, have analysed the relation between treosulfan exposure, treatment-related toxicity and clinical outcome [19-21]. These studies showed associations between treosulfan exposure, toxicity and survival, although results were not consistent. We previously reported the pharmacokinetic behaviour of treosulfan and its relationship with early toxicity, in a pediatric cohort transplanted for malignant and non-malignant diseases. In the present, so far largest, multicentre prospective observational study in pediatric patients with non-malignant diseases only, we assessed the association between treosulfan exposure and early and, in particular long-term clinical outcomes.

METHODS

Study design and patients

A prospective, observational, multicentre study was conducted between June 2011 and January 2019. Pediatric patients who received conditioning with treosulfan prior to their first allogeneic HSCT for a non-malignant disease in the Willem-Alexander Children's Hospital/Leiden University Medical Center in The Netherlands (n=69) and the Children's Hospital Bambino Gesù (OPBG) in Rome, Italy (n=41) were included in this study. The LUMC institutional Ethics Committee approved the study protocol (P12.267) which was subsequently approved in OPBG. Written informed consent for participation in the study was obtained from the parents or legal guardians, as well as consent from patients when they were older than 12 years according to the Helsinki Declaration (last amended in 2013, Fortaleza Brazil). The short term outcome of 61 patients in this cohort was already described in an earlier paper of a more heterogenous cohort [20]. In this study, 49 new patients were added resulting in this large cohort which exclusively includes non-malignant diseases.

Procedures

Patients received HSCT according to institutional protocols and in line with the EBMT Inborn Errors Working Party recommendations. Patients older than 1 year received treosulfan in a total dose of 42 g/m^2, administered over 3 consecutive days (14 g/m^2 per day). Children under the age of 1 year received 30 g/m^2 per day, administered over 3 consecutive days (10 g/m^2 per day). Treosulfan (day -5 to day -3) was combined with fludarabine (total dose of 150-160 mg/m^2, day -6 to day -2), with or without thiotepa (total dose 8-10 mg/kg, day -6). Serotherapy consisted of anti T-lymphocyte globulin (ATLG), anti-thymocyte globulin (ATG) or alemtuzumab. In patients with a mismatched related donor, peripheral blood stem cell grafts were processed by either CD34-positive selection or selective elimination of αβ+ T and CD19+ B cells [22]. Pharmacological graft versus host disease (GvHD) prophylaxis was given to patients receiving an unmanipulated graft according to institutional guidelines. Granulocyte colony-stimulating factor (G-CSF) was routinely given in cord blood transplants from day +8 onwards. Both transplant units are JACIE accredited and supportive care was according to institutional guidelines.

Pharmacokinetics of treosulfan

Blood sample collection was as previously described [17, 20]. Because intra-variability of treosulfan pharmacokinetics was low, blood samples were only collected on day 1 as a good representation of total exposure, as previously demonstrated [20, 23]. Treosulfan concentrations were measured with two different assays, the first part was measured in serum with an HPLC-UV method as described previously [17, 20]. The second part was measured with a validated LC/MS-MS assay. This assay was developed and validated according to EMA guidelines on bioanalytical method validation [24]. Both methods were cross-validated using a large set of study samples and it was concluded that the methods were interchangeable and therefore it was not necessary to reanalyse all samples with one method. Subsequently, the patients who were included after this validation were measured with the new LC/MS-MS method. Details regarding sample preparation, quantification and cross-validation can be found in Supplemental Material 1. A previously developed treosulfan pharmacokinetic model was used to estimate treosulfan area under the curve ($AUC_{0-\infty}$) as a measure of exposure using the posthoc estimation function in NONMEM with the final model [23].

Outcomes

Event-free survival (EFS) at 2 years was defined as survival without either primary or secondary graft failure, death due to any cause, or extensive chronic GvHD (cGvHD). Secondary outcomes were 2-year overall survival (OS), regimen-related toxicity, engraftment, donor chimerism, acute GvHD (aGvHD) and cGvHD. Overall survival was defined as survival from HSCT to last follow-up with death considered as the only event. Engraftment was defined as the first of three days with a neutrophil count of $\geq 0.5 \times 10^9$/L. Primary graft failure was defined as alive on day +28 with neutrophil count $<0.5 \times 10^9$/L. Secondary graft failure is defined as loss of previously functioning graft resulting in cytopenia involving at least two lineages. For hemoglobinopathies, this is recurrence of transfusion dependency. Acute and cGvHD were classified according to standard criteria [25, 26]. Data on chimerism determined in either whole blood or peripheral blood granulocytes and mononuclear cells by VNTR polymorphism at 1 year after transplantation were used in this analysis. When chimerism was determined in both

granulocytes and mononuclear cells the mean percentage was used for the final analysis. Mixed chimerism was defined as a donor chimerism <90%. Early toxicity endpoints were evaluated until +28 days after HSCT and included mucosal, skin, hepatic and neurological toxicity assessed according to CTCAE criteria and Bearman et al. [27]. The relationship between treosulfan exposure ($AUC_{0-\infty}$) and the outcomes of interest were evaluated.

Statistical analysis

Cox proportional hazard survival analyses were performed to assess the relationship between treosulfan exposure, OS and EFS. The predictive value of systemic treosulfan exposure for the occurrence of toxicity within 28 days is evaluated using a multivariable logistic regression analysis. $AUC_{0-\infty}$ is tested as discrete variable, considering 3 exposure groups based on tertiles: low [<1350 mg*h/L (1st tertile)], medium [1350-1750 mg*h/L (2nd tertile)] and high [>1750 mg*h/L (3th tertile)], age was tested as 2 groups (<2 years and ≥2 years old). This age cut-off point was used, because children under the age of 2 years old have immature renal and metabolic drug elimination pathways, which could influence the pharmacokinetics of treosulfan [28]. All statistical considerations are described in detail in Supplemental Material 2. All *p*-values were 2-tailed and considered significant when $p < .05$. Statistical analyses were performed with R (version 4.0.0) and R studio version 1.2.5042 with packages cmprsk, survival, car and rms.

RESULTS

Patient, donor, and transplantation characteristics

A total of 110 pediatric patients were included in the study between June 2011 and January 2019 with a median follow-up of 41 months (range 12-97 months). Clinical and demographic characteristics are detailed in Table 1. Seventy-one males and 39 females were included. Median age at HSCT was 5.2 years (range 0.2-18.8 years). Underlying disease categories were inborn errors of immunity (IEI) (n=38,35%), hemoglobinopathies (HBP) (n=55, 50%) and bone marrow failure disorders (BMF) (n=17, 15%). Thirty-four patients (31%) were conditioned with treosulfan and fludarabine (TF) and 76 patients (69%) were conditioned with treosulfan, fludarabine and thiotepa.

Table 1. Patient characteristics

	Total (N=110)
Characteristic	
Age (years, median (range))	5.2 (0.2-18.8)
Weight (kg, median (range))	18 (3.8-75.0)
Sex (n: M/F)	71/39
Diagnosis for HSCT	
Inborn errors of immunity (%)	38 (35)
Hemoglobinopathies (%)	55 (50)
Bone marrow failure (%)	17 (15)
Donor	
MSD (%)	32 (30)
MUD (≥ 9/10) (%)	50 (45)
MMFD (haplo) (%)	28 (25)
Stem cell source	
BM (%)	73 (66)
PB	
T cell replete (%)	5 (5)
TCR αβ/CD19 depletion (%)	19 (17)
CD34 enrichment (%)	3 (3)
CB (%)	10 (9)
Conditioning	
TFT (%)	77 (68)
TF (%)	37 (32)
Treosulfan dose	
14 g/m^2 (%)	92 (84)
10 g/m^2 (%)	18 (16)
Treosulfan pharmacokinetics	
$AUC_{0-\infty}$, mg*h/L (10 g/m^2) median (IQR)	1776 (1129-1977)
$AUC_{0-\infty}$, mg*h/L (14 g/m^2) median (IQR)	1562 (1140-1860)
Serotherapy	
Yes	
ATG (%)	55 (50)
ATLG (%)	35 (32)
Alemtuzumab (%)	12 (11)
No (%)	8 (7)
Pharmacological GvHD prophylaxis	
CsA (%)	6 (6)
CsA / MTX (%)	61 (55)
CsA / Pred (%)	5 (4)
Other (%)	4 (4)
None (%)	22 (20)
Post-Cy / CsA / MMF (%)	12 (11)

MSD: matched sibling donor, MMFD: mismatched family donor, MUD: matched unrelated donor, BM: bone marrow, PB: peripheral blood, CB: cord blood, TF: treosulfan-fludarabine, TFT: treosulfan-fludarabine-thiotepa, AUC: Area under the Curve, ATG (Thymoglobulin): Anti thymocyte globulin, ATLG (Grafalon): Anti T lymphocyte globulin, GvHD: Graft-versus-Host Disease, CsA: Cyclosporine A, MTX: methotrexate, Pred: prednisolone, Post-Cy: Post transplantation cyclophosphamide, MMF: mycophenolate mofetil

Treosulfan pharmacokinetics

Eighteen patients (< 1 year old) received a treosulfan dose of 10 g/m^2 and 92 patients (≥ 1 year old) a dose of 14 g/m^2 on three consecutive days. Median day 1 treosulfan $AUC_{0-\infty}$ was 1776 (IQR 1129-1977) and 1562 (IQR 1140-1860) mg*h/L in patients receiving 10 g/m^2 and 14 g/m^2, respectively, and showed large interindividual differences. Treosulfan clearance was lower in younger patients (Figure 1). Median age at transplant was significantly lower in the IEI group (1.5 yrs), compared to HBP (8.5 yrs) and BMF group (7.2 yrs) (p<0.001), therefore treosulfan clearance was also significantly lower in the IEI group (p<0.001). Median age was also significantly lower in the treosulfan-fludarabine (TF) group than the treosulfan-fludarabine-thiotepa (TFT) (3.6 vs 7.6 years (p=0.011)), resulting in corresponding higher treosulfan $AUC_{0-\infty}$ in the TF group (1800 vs 1443 mg*h/L (p<0.001).

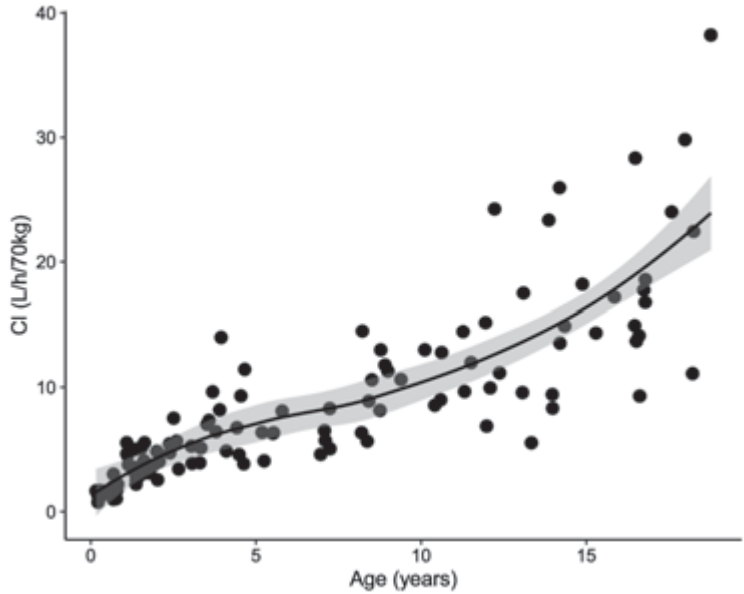

Figure 1. Treosulfan clearance versus age. Each dot represents the clearance of treosulfan (L/h/70 kg) of a patient plotted against age.

Treosulfan exposure and clinical outcome

Engraftment and chimerism

The cumulative incidence of engraftment was 97.1% (95%CI 93.5-100.0), with a median time to neutrophil and platelet engraftment of 20 days (11-43) and 24 days (8-94), respectively. Three patients died before engraftment (on day 0, +11 and +17), 7 patients experienced primary graft failure (3 HBP, 2 IEI, 2 BMF). Mean $AUC_{0-\infty}$ in patients with primary graft failure (1310 mg*h/L) and patients with successful engraftment (1586 mg*h/L) showed no significant difference (p=0.20). Three of the primary graft failure patients subsequently died because of transplant-related complications, 4 patients underwent a second transplantation. Three were successful, one patient rejected again and required autologous reinfusion. Eight patients, all with hemoglobinopathy as underlying disease (14,5% of the hemoglobinopathy group (n=55)), experienced secondary graft failure. Six of them experienced secondary graft failure within 6 months, two patients lost the graft after 2 and 5 years respectively. Four patients received a subsequent transplantation, of which two were successful and two rejected again. The four other patients did not receive a second transplantation or are scheduled for a new transplantation. More detailed information can be found in Supplemental Material 3. Mean $AUC_{0-\infty}$ was 1699 mg*h/L versus 1558 mg*h/L for patients with and without secondary graft failure, respectively (p=0.31).

Eighty-nine patients (81%) were evaluable for 1-year chimerism. Fifty-nine (66%) achieved ≥90% donor chimerism, 14 patients (16%) 50-90% and 16 patients (18%) <50%. Treosulfan $AUC_{0-\infty}$ was not correlated with either donor chimerism at 1-year in whole blood (p=0.87), nor with granulocyte chimerism in a subgroup (n=53) in which these data was available. In contrast, use of TF conditioning (OR 4.96; 95%CI 1.50-18.18, p=0.01) and age < 2 years old (OR 7.69; 95%CI 2.00-35.82, p=0.005) were significantly correlated with mixed chimerism at 1-year.

Graft-versus-host disease

The cumulative incidence of grade II-IV aGvHD was 12.4% (95% CI 7.4-20.7) and 5.1% (95% CI 2.2-12.0) of grade III-IV aGvHD. Eight patients developed grade II (7%), 4 patients grade III (3.6%) and 1 patient grade IV (0.9%).In the TF and TFT groups the cumulative incidence of grade II-IV aGVHD was 8.8% (95%CI 2.9-26.5) and 14.0% (95%CI 7.8-25.0, p=0.36), respectively. No relationship was found between treosulfan $AUC_{0-\infty}$ and the occurrence of aGvHD (p=0.42). Chronic GvHD was reported in 6 patients (CI 5.5% 95%CI 2.5-11.9) of whom three had extensive cGvHD including two patients with bronchiolitis obliterans. Treosulfan $AUC_{0-\infty}$ was not a significant risk factor for cGvHD (p=0.32).

EFS and OS

The cumulative incidence of 2-year OS was 89.0% (95% CI 83.3-95.1) (Figure 2). Nine patients died of TRM (8%) due to severe infections (n=4), toxicity (n=4) and GvHD (n=1). Two patients died because of progressive disease and one patient with TTC7A deficiency died 2.5 years after HSCT because of complications after bowel transplantation. OS in children under 2 years of age was high (97%) and no TRM was seen in this group.

Multivariable Cox regression analysis demonstrated that treosulfan exposure was not correlated with 2-year OS (HR 1.09 (95% CI 0.22-5.46, p=0.92 for treosulfan exposure >1750 mg*h/L) (Table 2; Figure 2). Underlying disease was a significant predictor for OS with the most favourable outcome for HBP (HR 0.13 (95% CI 0.03-0.64, p=0.01).

Estimated 2-years EFS was 75.3% (95%CI 67.6-83.8) (Figure 3). In multivariable Cox regression analysis, treosulfan exposure was not independently correlated with 2-year EFS, nor were any of the other variables (Table 2).

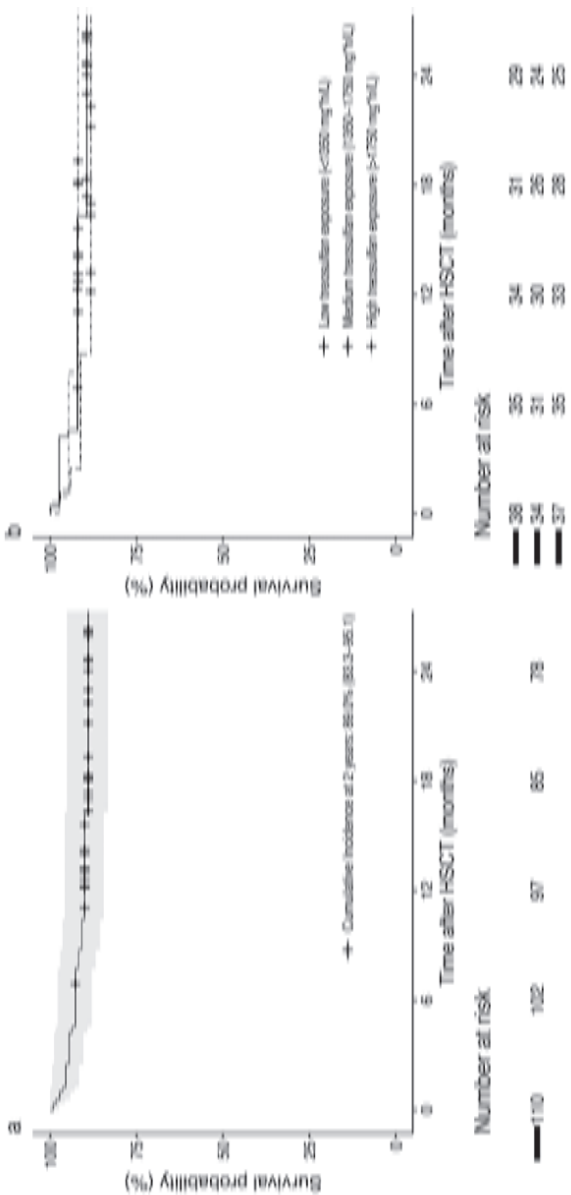

Figure 2. Overall survival. Kaplan Meier-plots of OS (a) and OS, stratified for low (<1350 mg*h/L; solid line), medium (1350-1750 mg*h/L; dashed line) (p=0.36) and high (>1750 mg*h/L; dotdashed line) (p=0.92) treosulfan exposure (b).

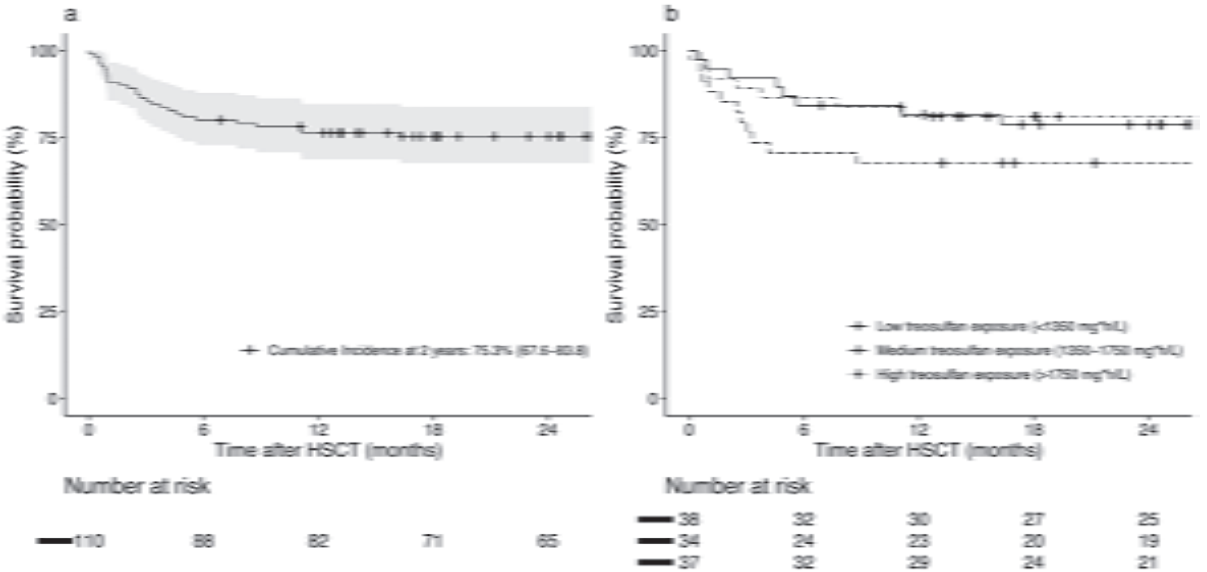

Figure 3. Event free survival. Kaplan Meier-plots of EFS (b) and EFS, stratified low (<1350 mg*h/L; solid line), medium (1350-1750 mg*h/L; dashed line) (p=0.29) and high (>1750 mg*h/L; dotdashed line) (p=0.95) treosulfan exposure (b).

Table 2. Multivariable analysis of event free survival and overall survival

Covariate	EFS 2 years Hazard ratio (95% CI)	P	OS 2 years Hazard ratio (95% CI)	P
Treosulfan $AUC_{0-\infty}$, mg*h/L				
Low (<1350)	1.00 (ref)		1.00 (ref)	
Medium (1350-1750)	1.67 (0.65-4.31)	0.29	2.08 (0.44-9.96)	0.36
High (>1750)	1.03 (0.37-2.89)	0.95	1.09 (0.22-5.46)	0.92
Age				
<2 years old	1.00 (ref)		1.00 (ref)	
≥2 years old	0.83 (0.32-2.18)	0.71	4.81 (0.92-25.28)	0.06
Conditioning regimen				
TF	1.00 (ref)		1.00 (ref)	
TFT	1.17 (0.46-2.97)	0.74	1.77 (0.38-8.30)	0.47
Donor				
MSD	1.00 (ref)		1.00 (ref)	
MUD (≥ 9/10)	1.58 (0.59-4.26)	0.36	1.51 (0.27-8.54)	0.64
MMFD (haplo)	2.65 (0.92-7.69)	0.07	3.24 (0.61-17.34)	0.17
Underlying disease				
Inborn errors of immunity	1.00 (ref)		1.00 (ref)	
Bone marrow failure	1.09 (0.28-4.33)	0.90	0.22 (0.04-1.29)	0.09
Hemoglobinopathies	1.36 (0.44-4.21)	0.60	0.13 (0.03-0.64)	0.01

Adjusted multivariable analyses were done using a Cox proportional hazard model. MSD: matched sibling donor, MMFD: mismatched family donor, MUD: matched unrelated donor, TF: treosulfan-fludarabine, TFT: treosulfan-fludarabine-thiotepa, AUC: Area under the Curve

Early regimen-related toxicity

Mucositis occurred in 50% (n=55) of patients of which 33% (n=36) had grade 2 or higher. In the TF group grade ≥ 2 mucositis occurred in 29% (n=10) versus 34% (n=26) in the TFT group. For the different disease groups this was 37% (n=14) for IEI, 18% (n=3) for BMF and 35% (n=19) for HBP. In multivariable analysis, high treosulfan exposure (>1750 mg*h/L) (OR 4.43 95% CI 1.43-15.50, p=0.01) and age above 2 years (OR 5.69 (95% CI 1.90-19.44, p=0.003) were independent risk factors to develop all grade mucositis while BMF as underlying disease was correlated with significantly less mucositis (OR 0.13 95% CI 0.03-0.57, p=0.01) than IEI and HBP. However, mucositis grade 2 or higher, which is clinically more relevant, was not significantly correlated with high treosulfan exposure (OR 1.51 95%CI 0.52-4.58, p=0.46) (Table 3).

Moderate to severe skin toxicity (≥ grade 2) occurred in 31% of patients, with high treosulfan exposure (>1750 mg*h/L) as risk factor (OR 3.97 95% CI 1.26-13.68, p=0.02). The addition of thiotepa to the conditioning regimen did not significantly increase the risk of skin toxicity (OR 1.85 95% CI 0.61-6.06, p=0.29). Grade 2 or higher hepatic and neurological toxicity occurred in 33% and 6% of patients, respectively, and was not correlated with treosulfan exposure (p=0.67 and p=0.60, respectively), nor with age and conditioning regimen.

Table 3. Early regimen related toxicity

Covariate	Mucositis all grade Odds ratio (95% CI)	P	Mucositis ≥ grade 2 Odds ratio (95% CI)	P	Skin toxicity ≥ grade 2 Odds ratio (95% CI)	P
Treosulfan $AUC_{0-\infty}$, mg*h/L						
Low (<1350)	1.00 (ref)		1.00 (ref)		1.00 (ref)	
Medium (1350-1750)	1.61 (0.56-4.79)	0.38	0.62 (0.20-1.88)	0.40	1.61 (0.53-4.99)	0.40
High (>1750)	4.43 (1.43-15.50)	0.02	1.51 (0.52-4.58)	0.46	3.97 (1.26-13.68)	0.02
Age						
<2 years old	1.00 (ref)		1.00 (ref)		1.00 (ref)	
≥2 years old	5.69 (1.90-19.45)	0.003	4.02 (1.30-14.16)	0.02	2.71 (0.86-9.55)	0.10
Conditioning regimen						
TF	1.00 (ref)		1.00 (ref)		1.00 (ref)	
TFT	2.13 (0.74-6.46)	0.17	1.30 (0.44-4.00)	0.64	1.85 (0.61-6.06)	0.29
Underlying disease						
Inborn errors of immunity	1.00 (ref)		1.00 (ref)		1.00 (ref)	
Bone marrow failure	0.13 (0.03-0.57)	0.01	0.22 (0.04-0.99)	0.06	1.17 (0.26-5.20)	0.84
Hemoglobinopathies	0.66 (0.20-2.05)	0.48	0.59 (0.18-1.85)	0.37	1.66 (0.50-5.93)	0.41

Adjusted multivariable analyses were done using logistic regression. MSD: matched sibling donor, MMFD: mismatched family donor, MUD: matched unrelated donor, TF: treosulfan-fludarabine, TFT: treosulfan-fludarabine-thiotepa, AUC: Area under the Curve

DISCUSSION

In this large prospective multicentre study in children with non-malignant diseases treated with standardized treosulfan-based conditioning, we studied the correlation between treosulfan exposure and both early and long-term clinical outcome after HSCT. The main findings are that treosulfan-based conditioning is safe and results in excellent clinical outcome, despite large interindividual differences in treosulfan exposure. Although treosulfan exposure is correlated with the occurrence of early toxicity, it does not have a significant impact on outcomes such as engraftment, chimerism, GvHD, and OS and EFS.

Treosulfan clearance was correlated with age, thus confirming our initial report developing the population pharmacokinetics model of treosulfan [23]. Clearance increases with age, reflecting maturation of organs and increase in bodyweight. There was a difference in $AUC_{0-\infty}$ between the TF and TFT groups, which could suggest an impact of thiotepa on treosulfan clearance. However, since age was also significantly different between these groups (patients receiving TF were younger), this is the most probable explanation for the observed difference in $AUC_{0-\infty}$.

An important observation in our study was the lack of correlation between the level of donor chimerism at 1-year and treosulfan exposure, while a positive correlation was found for conditioning regimen, i.e. TF versus TFT, and age. Chiesa et al. [19] reported in IEI patients treated with TF a trend toward an association between low level (≤20%) myeloid chimerism and low treosulfan $AUC_{0-\infty}$, but only in univariable analysis. We found a higher risk of mixed donor chimerism (<90%) in the TF compared to the TFT group, however the risk was independent of treosulfan exposure. This information could be of value when deciding between these two regimens in diseases where higher levels of chimerism are preferred. In addition, early toxicity was not significantly increased with the addition of thiotepa to the TF regimen in our patients. However, it has to be noted that the impact of adding thiotepa to TF on long-term toxicity, especially fertility, is currently unresolved.

We demonstrate that high treosulfan exposure is significantly correlated with the risk of skin toxicity, confirming our previous observations [20]. Despite the fact that the use of thiotepa may also lead to skin toxicity [29], similar levels of skin toxicity were observed in the TF and TFT groups, indicating that in this pediatric cohort thiotepa has probably made only a minor contribution to the skin toxicity. Moreover, in multivariable analysis, treosulfan exposure was identified as an independent risk factor. Of note, Chiesa et al. [19] also reported the relationship between treosulfan exposure and skin toxicity in a cohort of 57 children with TF conditioning, thus confirming our observation. While skin toxicity occurs frequently, taking preventive measures can help reduce the incidence of cutaneous complications. Preventive care guidelines for thiotepa-induced skin toxicity, such as suggested by Van Schandevyl and Bauters, could also be implemented for treosulfan [29].

Interestingly, while we previously observed a relationship between high treosulfan exposure and the risk of grade 2 or higher mucositis in a smaller and mixed cohort [20], in the present study on patients with non-malignant diseases exclusively, this correlation was just observed for all grade mucositis, which is clinically less relevant. This difference is probably due to lack of patients with malignant diseases of which 50% experienced ≥ grade 2 mucositis. Our findings are in accordance with Chiesa et al. who did not report a relationship of treosulfan exposure with mucositis. Mohanan et al. [21] reported an incidence of 39% of all grade mucositis and 20% of grade 3-4 mucositis but found no relationship between treosulfan exposure and regimen-related toxicities.

Two-year overall survival was 89.0%, similar to other reports on patients with non-malignant diseases treated with treosulfan-based conditioning [1, 4, 13, 19, 21]. Remarkably, OS of infants under the age of 2 years was 97%, emphasizing the excellent efficacy and safety profile of treosulfan-based regimens in this vulnerable category of patients. Both in the TF and TFT group treosulfan exposure was not correlated with 2-year OS. However, Chiesa et al. [19] found a relationship between treosulfan $AUC_{0-\infty}$ and mortality; in particular a cumulative treosulfan $AUC_{0-\infty}$ >6000 mg*h/L (corresponding with a daily exposure of > 2000 mg*h/L) was associated with higher

transplant-related mortality. Mohanan et al. [21] found that low treosulfan clearance showed a higher risk towards poor OS, however this was not reflected in a similar correlation with $AUC_{0-\infty}$. The differences between our results and those of Chiesa et al. [19] and Mohanan et al. [21] could be explained by the substantial differences in interpatient variation in treosulfan exposure. Chiesa et al. [19] reported daily exposure $AUC_{0-\infty}$ values ranging between 733-4882 mg*h/L and Mohanan et al. [21] reported $AUC_{0-\infty}$ values between 129-4267 mg*h/L. While our patients were treated with similar dosing regimens, the $AUC_{0-\infty}$ values ranged between 366-3368 mg*h/L and thus lacked exposures in the very high region. Therefore, we speculate that the limited interpatient variation and the lack of high levels in our patient cohort, may explain the absence of a correlation between treosulfan exposure and EFS or OS in our study. The other studies did not report whether the patients with high or low $AUC_{0-\infty}$ had specific characteristics (e.g. comorbidities) that could be co-factors explaining the unfavourable outcome.

The EFS rate in our study was very favourable with 75.3% at two years after HSCT, especially if we consider that previous studies (in contrast to ours) did not count cGvHD as an event [1, 4, 13]. An important observation in our study is that EFS was not correlated with treosulfan exposure. This is in accordance with the study of Mohanan et al. [21], who did not find a relationship between treosulfan exposure and EFS in 87 thalassemia patients treated with the same TFT regimen. Our combined results containing more than two hundred patients with non-malignant diseases demonstrate that with current dose regimens treosulfan exposure has no significant impact on EFS, thus supporting the use of these regimens in this category of patients without the need for therapeutic drug monitoring. Whether disease-free survival in children with malignant diseases is similarly independent of treosulfan exposure remains to be demonstrated. Moreover, the correlation between treosulfan exposure and the occurrence of late effects (e.g. growth disorders, gonadal insufficiency and infertility) in children treated with treosulfan-based conditioning has yet to be established.

In the last several years it has become evident that there is a clear relationship between busulfan exposure, clinical outcome and toxicity, resulting in established therapeutic

windows for busulfan exposure. In contrast, our study provides evidence that the impact of treosulfan exposure on clinical outcome is low and, to our opinion, PK-guided dosing is not required to optimize outcome in the majority of children. PK-guided dosing may be instrumental to prevent early toxicity, but since the toxicity profile of treosulfan is relatively mild, the added value and clinical relevance of the introduction of individualized dosing will be limited. Our findings may raise the question whether a lower treosulfan $AUC_{0-\infty}$ can be sufficient to achieve effectiveness. Also, lower treosulfan exposure could be beneficial when it comes to limiting late effects of conditioning, especially gonadal insufficiency. These questions, however, require more (prospective) research and need to be addressed in future studies.

In conclusion, the use of a treosulfan-based conditioning regimen in children with non-malignant diseases translates into very favourable clinical outcomes. Our data demonstrate that standardized dose regimens can be applied in the vast majority of patients to achieve favourable OS and EFS.

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Supplemental Material 1. Sample preparation, quantification and cross-validation

Blood sampling was collected in serum tubes, which were centrifuged at 2500 x *g* for 5 min. The resulting serum samples were stored in the -80°C freezer while waiting for analysis.

For sample preparation, a 50 µl serum aliquot was combined with 200 µl of internal standard (IS) solution in an Eppendorf tube. The IS solution consisted of 10 mg/L treosulfan D4 in acetonitrile. The tube was vortex-mixed (2000 rpm) and centrifuged (13.000 rpm) for 5 and 5 min, respectively. Subsequently, a 100 µl aliquot of supernatant was transferred to an autosampler vial and combined with 500 µl of Mobile Phase A. The final mixture was then vortex-mixed for 5 s, after which 5 µl was injected onto the Thermo LC-MS/MS system.

Quantification of treosulfan with LC-MS/MS was performed using a Thermo Endura UPLC-MS/MS system, consisting of an Ultimate 3000 series UHPLC system, coupled to a TSQ Endura triple stage quadrupole mass spectrometer, all from ThermoFisher Scientific. The UPLC system consisted of a dual gradient pump, autosampler and column heater, also from ThermoFisher Scientific. Data was acquired and processed using ThermoFisher Scientific Chromeleon software version 7.2. Chromatographic separation was achieved using a Zorbax Eclipse Plus C18 5 µm; 2,1 x 12,5 mm precolumn coupled to a Zorbax Eclipse Plus C18 3,5 µm; 2,1 x 100 mm column, both from Agilent. Mobile phase eluents were Mobile Phase A: 0,1% v/v formic acid + 10 mM ammonium acetate in water = 1000 ml water + 1,0 ml formic acid + 0,80 gr. Ammonium acetate and Mobile Phase B: 0,1% v/v formic acid + 10 mM ammonium acetate in methanol = 1000 ml MeOH + 1,0 ml formic acid + 0,80 grams ammonium acetate. The elution gradient was 90%A/10%B from initiation to 0.50 min, followed by 10%A /90%B for 2 min at a constant flow of 0.3 ml min^{-1}, followed by 90%A/10%B for the remaining 1.00 min at a constant flow of 0.5 ml min^{-1} and concluded with the initial settings for the remaining 2.00 min, at a constant flow of 0.3 ml min^{-1}. The injection volume was set to 5 µL, the column temperature was set at 40 °C and sample manager operated at room temperature. The MS was operated in the ESI+ mode. The following mass transitions were used for MRM acquisition (m/z): treosulfan 296-279 and treosulfanD4 300-283.

The HPLC-UV assay and the LC/MS-MS assay were cross-validated using 33 samples divided over the studied concentration range. The obtained mean accuracy by the different methods were within 15% and also meet the specific cross validation requirements described in the EMA guidelines on bioanalytical method validation section 4.3. Furthermore, Passing-Bablok and Bland-Altman analysis showed that both methods were interchangeable in a 1:1 manner.

Supplemental Material 2. Statistical considerations

Normally distributed continuous parameters are shown as mean ± standard deviation, all log-normally continuous distributed parameters as median (IQR) and categorical variables as frequency (percentage). Differences in exposure between different groups was tested with the Kruskall-Wallis or Wilcoxon rank test. The predictive value of systemic treosulfan exposure for the occurrence of toxicity within 28 days is evaluated using a multivariable logistic regression analysis for mucosal, skin, hepatic and neurological toxicity events, with age, conditioning regimen (treosulfan-fludarabine and treosulfan-fludarabine-thiotepa) and underlying disease (inborn errors of immunity (IEI), bone marrow failure disorders (BMF) and hemoglobinopathies (HBP)) as other possible predictors. $AUC_{0-\infty}$ was tested as discrete variable, considering 3 exposure groups low [<1350 mg*h/L (1st tertile)], medium [1350-1750 mg*h/L (2nd tertile)] and high [>1750 mg*h/L (3th tertile)], age was tested as 2 groups (<2 years and ≥2 years old). This age cut-off point was used, because children under the age of 2 years old have immature renal and metabolic drug elimination pathways, which could influence the pharmacokinetics of treosulfan.

The cumulative incidence of engraftment and acute GvHD (aGvHD) was estimated using the method of Fine and Gray for censored data subject to competing risks, taking into account graft failure, death without engraftment and subsequent HSCT as competing risk for engraftment and death before day +100 as competing risk for aGvHD. The association between treosulfan exposure and aGvHD and engraftment was tested with the Gray test. The relationship between treosulfan exposure and chimerism at 1 year after HSCT (≥90% chimerism) was determined

with multivariable logistic regression analysis with age, conditioning regimen and underlying disease as other possible predictors.

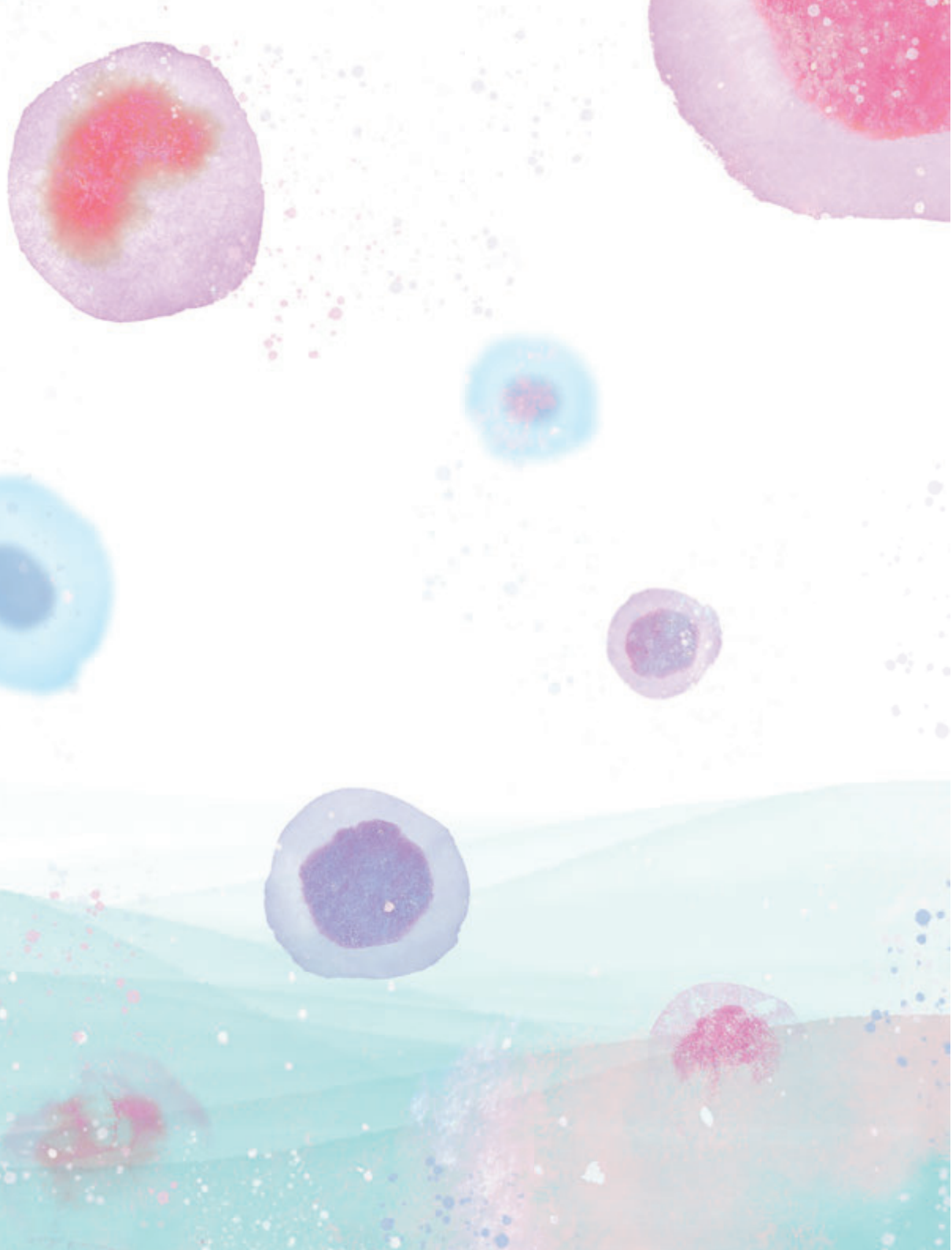
Survival curves were drawn by the Kaplan-Meier method. Duration of follow-up was defined as time from HSCT to last contact or death. Patients were censored at the date of last contact. For the endpoints overall survival and event-free survival (EFS), Cox proportional hazard survival analyses were performed. Factors considered as predictors for outcome were treosulfan $AUC_{0-\infty}$, age, conditioning regimen (treo-flu and treo-flu-thiotepa), donor source and HLA matching (MSD, MUD or MMFD) and underlying disease (IEI, BMF and HBP) in multivariable analysis. $AUC_{0-\infty}$ was tested as discrete variable, considering the 3 exposure groups mentioned above, age was tested as 2 groups (<2 years and ≥2 years old).

All *p*-values were 2-tailed and considered significant when $p < .05$. Statistical analyses were performed with R (version 4.0.0) and R studio version 1.2.5042 with packages cmprsk, survival, car and rms.

Supplemental Material 3. Characteristics of patients with primary and secondary graft failure

Patient	Underlying disease	Sex	Age at HSCT (years)	Donor	Stem cell source	Conditioning	Treosulfan dose (g/m2)	Treosulfan AUC (mg*h/L)	Outcome	Comments
1	Beta thalassemia	F	13	MMFD	PB	TFT	14	1567	Primary graft failure	TRM
2	Beta thalassemia	M	1.4	MUD	BM	TFT	14	1396	Primary graft failure	second transplant unsuccesful
3	Bare lymphocyte syndrome	M	1.1	MUD	CB	TFT	14	1318	Primary graft failure	second transplant successful
4	SAA	M	4.4	MMFD	PB	TFT	14	1482	Primary graft failure	second transplant successful
5	Bone marrow failure	M	4.6	MMFD	PB	TF	14	1893	Primary graft failure	second transplant successful
6	SCID	F	3.6	MMFD	PB	TFT	14	1145	Primary graft failure	TRM
7	Beta thalassemia	F	16.3	MUD	BM	TFT	14	366	Primary graft failure	TRM
8	Sickle cell disease	F	5.2	MMFD	BM	TF	14	1726	Secondary graft failure	second transplant is considered/planned
9	Beta thalassemia	M	1.9	MUD	BM	TFT	14	2110	Secondary graft failure	second transplant unsuccesful
10	Beta thalassemia	F	2.4	MUD	BM	TFT	14	1628	Secondary graft failure	second transplant unsuccesful
11	Sickle cell disease	M	5.5	MMFD	BM	TF	14	1656	Secondary graft failure	second transplant is scheduled
12	Beta thalassemia	F	1.1	MSD	BM	TFT	14	1851	Secondary graft failure	second transplant successful
13	Beta thalassemia	M	1.5	MUD	BM	TFT	10	846	Secondary graft failure	second transplant is considered/planned
14	Beta thalassemia	M	1.5	MSD	BM	TFT	14	1573	Secondary graft failure	second transplant successful
15	Beta thalassemia	F	5.3	MSD	CB	TFT	14	2205	Secondary graft failure	second transplant is considered/planned

SAA: Severe Aplastic Anemia, SCID: Severe Combined Immunodeficiency, MSD: matched sibling donor, MMFD: mismatched family donor, MUD: matched unrelated donor, BM: bone marrow, PB: peripheral blood, CB: cord blood, TF: treosulfan-fludarabine, TFT: treosulfan-fludarabine-thiotepa, AUC: Area under the Curve, TRM: transplant-related mortality



CHAPTER 05

IDENTIFICATION OF TREOSULFAN-INDUCED MYALGIA IN PEDIATRIC HEMATOPOIETIC STEM CELL TRANSPLANTATION USING AN ELECTRONIC HEALTH RECORD TEXT MINING TOOL

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ABSTRACT

Introduction:

Treosulfan is increasingly used as myeloablative agent in conditioning regimen prior to allogeneic hematopoietic stem cell transplantation (HSCT). In our pediatric HSCT program, myalgia was regularly observed after treosulfan-based conditioning, which is a relatively unknown side effect.

Objective:

Using a natural language processing and text-mining tool (CDC), we investigated whether treosulfan compared with busulfan was associated with an increased risk of myalgia. Furthermore, among treosulfan users, we studied the characteristics of given treatment of myalgia, and studied prognostic factors for developing myalgia during treosulfan use.

Methods:

Electronic Health Records (EHRs) until 28 days after HSCT were screened using the CDC for myalgia and 22 synonyms. Time to myalgia, location of pain, duration, severity and drug treatment were collected. Pain severity was classified according to the WHO pain relief ladder. Logistic regression was performed to assess prognostic factors.

Results:

114 patients received treosulfan and 92 busulfan. Myalgia was reported in 37 patients; 34 patients in the treosulfan group and 3 patients in the busulfan group (p=0.01). In the treosulfan group, median time to myalgia was 7 days (0-12) and median duration of pain was 19 days (4-73). 44% of patients needed strong acting opiates and adjuvant medicines (e.g. ketamine). Hemoglobinopathy was a significant risk factor, as compared to other underlying diseases (OR 7.16 95%CI 2.09-30.03, p=0.003).

Conclusion:

Myalgia appears to be a common adverse effect of treosulfan in pediatric HSCT, especially in hemoglobinopathy. Using the CDC, EHRs were easily screened to detect this previously unknown side effect, proving the effectiveness of the tool. Recognition of treosulfan-induced myalgia is important for adequate pain management strategies and thereby for improving the quality of hospital stay.

INTRODUCTION

Treosulfan, a bifunctional alkylating agent, was originally registered for the palliative treatment of ovarian carcinoma in the mid-90s (Ovastat®) [1-4]. More recently in 2019, it was also registered as part of conditioning treatment prior to allogeneic hematopoietic stem cell transplantation (alloHSCT) in adult and pediatric patients (Trecondi®) [5]. In the past decade, several studies have reported the efficacy and tolerability of treosulfan-based conditioning regimens in pediatric alloHSCT for both non-malignant and malignant diseases [6-12]. Treosulfan-based conditioning has gained popularity, particularly in children with non-malignant diseases, because of its favourable toxicity profile. Common side effects are gastrointestinal, mucosal and skin disorders and elevation of liver enzymes, but they are usually limited and mild. In our pediatric HSCT program, some patients experienced myalgia and arthralgia after conditioning with treosulfan, side effects which are not mentioned in the Summary of Product Characteristics (SmPC) of Ovastat® [13]. In the SmPC of Trecondi®, that has become recently available, pain in extremities is mentioned in the undesirable effects in the pediatric population with unknown frequency [14]. In the European pharmacovigilance database (EudraVigilance, www.adrreports.eu), there are nine reports within the group 'musculoskeletal and connective tissue disorders', of 304 reports up to the end of 2020. The majority of these reports are in adult patients.

Real world data might contribute to the knowledge on adverse events and the electronic health record (EHR) is an important source of data and contains valuable information collected during routine clinical practice, including side effects of drugs that the patient experiences during treatment. Unfortunately, this information is often stored in the EHR as free-text notes and therefore less suitable for automated extraction. Manual chart review is the gold standard for collection of data from EHRs, but this is laborious and very time-consuming [15]. Natural language processing (NLP) and text mining techniques in the EHR can provide additional information about drugs that has not been discovered in clinical development. Recently, the NLP and text-mining tool Clinical Data Collector (CDC; CTcue B.V., Amsterdam, The Netherlands), has

proven to be a helpful tool for retrieving real world data (RWD) from EHRs in a validation study in patients with metastatic renal cell carcinoma, compared to manual chart review [16]. With the use of CDC, we investigated whether treosulfan compared with busulfan was associated with an increased risk of myalgia. Furthermore, among treosulfan users, we studied the characteristics of given treatment of myalgia, and studied prognostic factors for developing myalgia during treosulfan use.

METHODS

Study population and design

A retrospective cohort study was conducted at the Pediatric Hematopoietic Stem Cell Transplantation unit of the Leiden University Medical Centre (LUMC) from May 2011 until May 2019. The study was approved by the Medical Ethics Review Committee of the LUMC, Leiden. Informed consent was waived by the Medical Ethics Review Committee of the LUMC. All methods were carried out in accordance with relevant guidelines and regulations. Pediatric patients (≤ 18 years) who received treosulfan (TREO)- or busulfan (BU)-based conditioning prior to HSCT were included and divided in two cohorts. Treosulfan was given in a dose of 42 g/m^2 and 30 g/m^2 in children above or under the age of 1 year old, respectively. Busulfan was initially dosed as 120 mg/m^2 and then targeted to a total exposure (as area under the concentration curve, $AUC_{0-\infty}$) of 75-95 mg*h/L.

Data retrieval

Electronic Health Records (EHRs) were screened anonymously, using the intelligent search engine CTcue Clinical Data Collector (CTcue B.V., Amsterdam, The Netherlands), a software package that can be used to search through Electronic Health Records [16]. The EHR includes records from nurses, physicians, physical therapists, dieticians, social workers and pharmacists. The patient population and data points are defined using CDC queries. Two queries were created; in one patients were included ≤ 18 years of age that have received treosulfan and in the other patients received busulfan

between May 2011 and May 2019. Using myalgia and 22 synonyms as keywords (see Supplemental Material 1), patients with one of these keywords mentioned in the EHR until 28 days after HSCT were highlighted. Subsequently, highlighted EHRs were manually checked for validity.

Measurement of myalgia and pain severity

The presence of myalgia within 28 days after HSCT (i.e. discomfort originating from a muscle or group of muscles) was scored as an event. Time of onset, location and duration of pain were derived manually from the EHR. Duration of pain was derived from the use of pain medication. Additionally, pain severity was categorized according to the World Health Organization (WHO) pain relief ladder: paracetamol (PCM) (step 1), PCM and tramadol (step 2), addition of strong acting opiate (step 3) and addition of adjuvant medicines (e.g. ketamine, clonidine, pregabalin) (step 4). When mucositis was present, EHRs were thoroughly screened to confirm that pain medication was given for myalgia and not for mucositis only.

Collection of other variables

Patient characteristics such as gender, age at SCT, underlying disease and transplant characteristics such as donor, graft, match and graft versus host disease (GvHD) prophylaxis were collected from the EHRs. Creatine kinase (CK) levels and treosulfan exposure (as $AUC_{0-\infty}$) were collected if available.

Endpoints

The incidence and course (timing, location of pain and duration) of myalgia after conditioning were the primary endpoints. Secondary endpoint was pain severity according to the WHO pain relief ladder. Other variables noted above were collected to evaluate potential predisposing factors.

Statistical analysis

Descriptive statistics, such as median and frequency, were used to summarize baseline characteristics and outcomes. Odds ratios (OR) were estimated by means of logistic regression to examine the association between conditioning regimen (TREO-based vs. BU-based) and myalgia and adjusted for possible confounding (hemoglobinopathies versus other indications). In the TREO cohort, univariable and multivariable logistic regression was performed to assess whether baseline- or transplant characteristics (age, underlying disease, conditioning regimen, treosulfan exposure) were prognostic for the development of myalgia. All P-values were 2-tailed and considered significant when $P<0.05$. Statistical analyses were performed using R version 3.6.1 and RStudio version 1.2.5019.

RESULTS

Patients and baseline characteristics

A total of 206 patients were included in the study, 114 patients were treated with treosulfan-based conditioning (TREO cohort) and 92 with busulfan-based conditioning (BU cohort). The median age was 5.4 and 8.5 years old in the TREO and BU cohort, respectively. There were 64 patients under 3 years of age. The majority of patients with hemoglobinopathy (i.e. beta-thalassemia or sickle cell disease (SCD)) received a TREO-based conditioning regimen, whereas patients with a hematological malignancy were mostly treated with a BU-based regimen. The most common combination of conditioning agents within the TREO cohort was treosulfan combined with fludarabine and thiotepa (66.7%), followed by treosulfan with fludarabine alone (31.6%). Within the BU cohort this was busulfan with fludarabine and clofarabine (58.7%), followed by busulfan and fludarabine (30.4%) and busulfan combined with fludarabine and thiotepa (7.6%). Serotherapy and GvHD prophylaxis were comparable among the two groups, except that post-transplant cyclophosphamide (PTCy) was used in a subgroup of the TREO cohort namely when a patient was transplanted with a mismatched donor. Baseline characteristics are summarized in Table 1.

Table 1. Patient characteristics

Characteristic	Treosulfan (n=114)	Busulfan (n=92)
Age at SCT (years, median (range))	5.4 (0.2-18.2)	8.5 (0.4-17.8)
Sex (M/F) (%)	62/38	58/42
Diagnosis for HSCT		
Beta-thalassemia (%)	35 (30.7)	6 (6.5)
Sickle cell disease (%)	20 (17.5)	0 (0)
Inborn errors of immunity (%)	32 (28.1)	10 (10.9)
Hematological malignancy (%)	18 (15.8)	63 (68.5)
Bone marrow failure (%)	9 (7.9)	13 (14.1)
Donor		
MSD (%)	36 (31.6)	18 (19.6)
MUD (≥ 9/10) (%)	62 (54.4)	69 (75.0)
MMFD (haplo) (%)	16 (14.0)	5 (5.4)
Stem cell source		
BM (%)	85 (74.6)	63 (68.5)
PBSC (%)	14 (12.3)	14 (15.2)
CB (%)	15 (13.2)	15 (16.3)
Conditioning		
Treo-Flu-Thiotepa (%)	76 (66.7)	-
Treo-Flu (%)	36 (31.6)	-
Treo-Other (%)	2 (1.7)	-
Bu-Flu-Clo (%)	-	54 (58.7)
Bu-Flu (%)	-	28 (30.4)
Bu-Flu-Thiotepa (%)		7 (7.6)
Bu-Cy-Mel (%)	-	3 (3.3)
Serotherapy		
ATG (%)	77 (67.5%)	71 (77.2)
Alemtuzumab (%)	27 (23.7)	7 (7.6)
No (%)	10 (8.8)	14 (15.2)
GvHD prophylaxis		
CsA + MTX(%)	60 (52.6)	57 (62.0)
PTCy + MMF + CsA (%)	16 (14.0)	0 (0)
CsA + Pred (%)	9 (7.9)	11 (12.0)
CsA (%)	9 (7.9)	3 (3.3)
Other (%)	13 (11.4)	16 (17.4)
None (%)	7 (6.1)	5 (5.4)

MSD: matched sibling donor, MUD: matched unrelated donor, MMFD: mismatched family donor, BM: bone marrow, PBSC: peripheral blood stem cells, CB: cord blood, Treo: treosulfan, Flu: fludarabine, Thio: thiotepa, Bu: busulfan, Clo: clofarabine, ATG: Anti thymocyte globulin, GvHD: Graft-versus-Host Disease, CsA: Cyclosporine A, MTX: methotrexate, PTCy: Post-transplant cyclophosphamide, MMF: mycophenolate mofetil, Pred: prednisolone.

Incidence, course and duration of myalgia

Myalgia or one of the synonyms were found in 46 of 114 EHRs (40.4%) in the TREO cohort, using the CDC of which 34 patients (29.8%) were confirmed after manual check. In the BU-cohort, 15 out of 92 EHRs (16.3%) were selected using the CDC. Three (3.3%) were confirmed after manual check (Figure 1). Manual check prevented a denial of an adverse event from being scored as an event. Patients in the TREO cohort were more likely to experience myalgia than patients in the BU cohort (crude OR 12.61, 95% CI 4.32-53.78, p<0.001; adjusted OR 5.36, 95% CI 1.63-24.19, p=0.01). In addition, the 3 patients who experienced myalgia in the BU group, experienced this myalgia during or directly after infusion of clofarabine. Characteristics of patients with myalgia are summarized in Table 2. In the TREO cohort, median time to myalgia was 7 days (range 0-12), calculated from the first day of TREO infusion. The most reported locations of pain were legs (97%) and arms (82%), often combined with pain in knees (47%) and elbows (26%). Other locations in which myalgia was reported were feet (44%), neck (26%), back (24%), hands (24%) and shoulder (21%). In patients under 3 years of age unwillingness to stand, pain and/or crying when stretching or bending arms or legs were considered as a report of pain in legs and arms. Duration of pain varied greatly, ranging from 4 to 73 days, with a median period of 19 days. Almost half of patients (44%) experienced pain for more than 3 weeks. CK-levels were measured in 6 patients during the period of myalgia, which were all within the normal range.

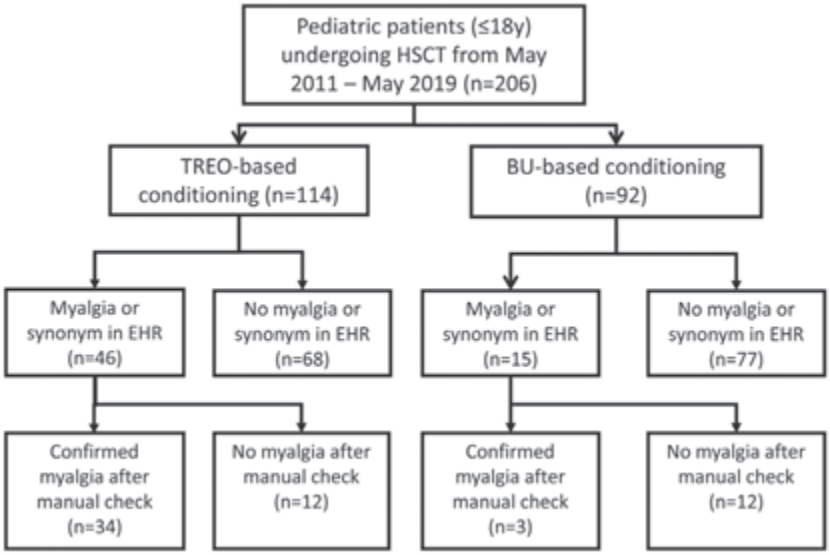

Fig. 1 Study design (HSCT: hematopoietic stem cell transplantation, TREO: treosulfan, BU: busulfan, EHR: Electronic Health Record)

Table 2. Myalgia characteristics in the treosulfan cohort and busulfan cohort

Characteristic	Treosulfan (n=34)	Busulfan (n=3)
Age at SCT (years, median (range))	12.2 (1.8-18.2)	11.5 (10.9-17.2)
Sex (n: M/F)	20/14	67/33
Diagnosis for HSCT		
Beta-thalassemia (%)	15 (44)	-
Sickle cell disease (%)	13 (38)	-
Inborn errors of immunity (%)	1 (3)	-
Hematological malignancy (%)	4 (12)	3 (100)
Bone marrow failure (%)	1 (3)	-
Conditioning		
Treo-Flu-Thiotepa (%)	28 (82)	-
Treo-Flu (%)	5 (15)	-
Treo-Other (%)	1 (3)	-
Bu-Flu-Clo (%)	-	3 (100)
Location of pain		
Leg (%)	33 (97)	3 (100)
Arm (%)	28 (82)	3 (100)

Characteristic	Treosulfan (n=34)	Busulfan (n=3)
Knee / ankle (%)	16 (47)	-
Elbow (%)	9 (26)	-
Neck (%)	9 (26)	-
Back (%)	8 (24)	-
Shoulder (%)	7 (21)	-
Foot (%)	15 (44)	-
Hand (%)	8 (24)	-
Duration of pain		
≤ 7 days (%)	4 (12)	1 (33)
8-14 days (%)	7 (20)	2 (67)
15-21 days (%)	8 (24)	-
>21 days (%)	15 (44)	-
Medical intervention		
Paracetamol / acetaminophen	34 (100)	3 (100)
NSAIDs	4 (12)	1 (33)
Tramadol	32 (94)	2 (67)
Opiate, oral	5 (15)	1 (33)
Opiate, intravenous	21 (62)	1 (33)
Antiepileptics (e.g. gabapentin, pregabalin)	8 (24)	-
Other (e.g. ketamine, clonidine, benzodiazepines)	13 (38)	1 (33)
Pain severity		
Step 1 (paracetamol)	2 (6)	
Step 2 (paracetamol + tramadol)	11 (32)	1 (33)
Step 3 (addition of strong acting opiate)	6 (18)	1 (33)
Step 4 (addition of adjuvant medicines)	15 (44)	1 (33)

Treo: treosulfan, Flu: fludarabine, Thio: thiotepa, Bu: busulfan, Clo: clofarabine

Pain severity

Pain severity is shown in Figure 2. Two patients were treated with PCM only, all other patients required stronger acting agents to relieve pain. In the TREO cohort, 11 out of 34 patients (32%) were treated with additional tramadol and 6 patients (18%) needed strong acting opiates (morphine, fentanyl). Strikingly, 15 patients (44%) needed adjuvant medicines (e.g. ketamine, clonidine, pregabalin or gabapentin, benzodiazepines) in order to manage pain adequately. Twelve patients (11%) experienced both myalgia and mucositis. In eight patients, mucositis was not severe (grade 2) and pain medication was intended for relieving myalgia only. In the other four patients, a strong acting opiate with one or more adjuvant medicines was started to relieve both mucositis and myalgia.

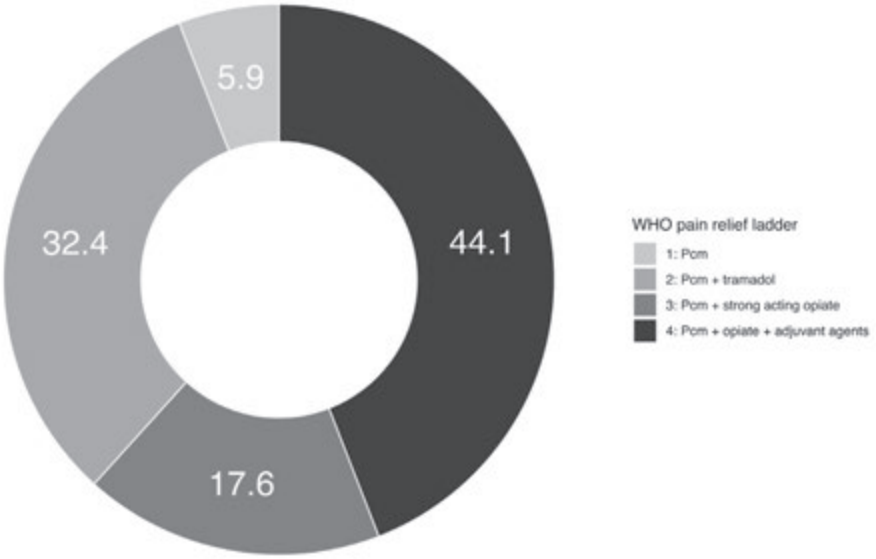

Fig. 2 Distribution (in %) of pain severity (classified as steps in the WHO pain relief ladder) in patients with myalgia in the TREO cohort (Pcm: paracetamol)

Prognostic factors for development of myalgia

Prognostic factors were explored in the TREO cohort (Table 3). In univariable analysis, conditioning regimen, age, underlying disease and treosulfan exposure ($AUC_{0-\infty}$) were explored as possible factor. Multivariable logistic regression showed that underlying disease was a significant prognostic factor for the development of myalgia. Patients with hemoglobinopathy, especially patients with SCD, had a higher risk of experiencing myalgia than patients with other underlying diseases (OR 7.16 95%CI 2.09-30.03, p=0.003). Thirteen of 20 patients (65%) with SCD experienced myalgia, half of them experiencing severe pain. It is important to note that the pain described by SCD patients was different from what they had previously experienced as disease specific pain (vaso-occlusive crises). Frequencies of pain severity per disease category are shown in Figure 3. Furthermore, age proved a significant factor. Children above 3 years of

age had a higher risk of experiencing myalgia than infants under 3 years old (OR 8.98 95%CI 2.04-64.54, p=0.01). Conditioning regimen was not a significant covariate (OR 0.64 95%CI 0.14-2.92, p=0.57 and OR 1.73 95%CI 0.05-71.17, p=0.75 for treosulfan with fludarabine only or treosulfan with other agents, respectively). Treosulfan exposure ($AUC_{0-\infty}$) in bloodserum was available in 93 of 114 patients (82%). Treosulfan $AUC_{0-\infty}$ was not related with the occurrence of myalgia (p=0.23).

Table 3. Univariable and multivariable analysis of the treosulfan cohort

Variable	OR 95% CI	p-value	Adjusted OR 95% CI*	p-value
Underlying disease				
Hemoglobinopathy	9.16 (3.58-26.97)	<0.001	7.16 (2.09-30.03)	0.003
Age				
> 3 years of age	10.3 (3.34-45.50)	<0.001	8.98 (2.04-64.54)	0.01
Conditioning regimen				
Treo-Flu	0.28 (0.09-0.74)	0.02	0.64 (0.14-2.92)	0.57
Treo-Other	1.71 (0.07-44.50)	0.71	1.73 (0.05-71.17)	0.75
Treosulfan exposure ($AUC_{0-\infty}$) (for every 500 mg*hr/L increase in $AUC_{0-\infty}$)	0.34 (0.17-0.64)	0.002	0.61 (0.26-1.33)	0.23

* Adjusted for underlying disease, age, conditioning regimen and treosulfan exposure. Treo: treosulfan, Flu: fludarabine, AUC: Area under the concentration curve.

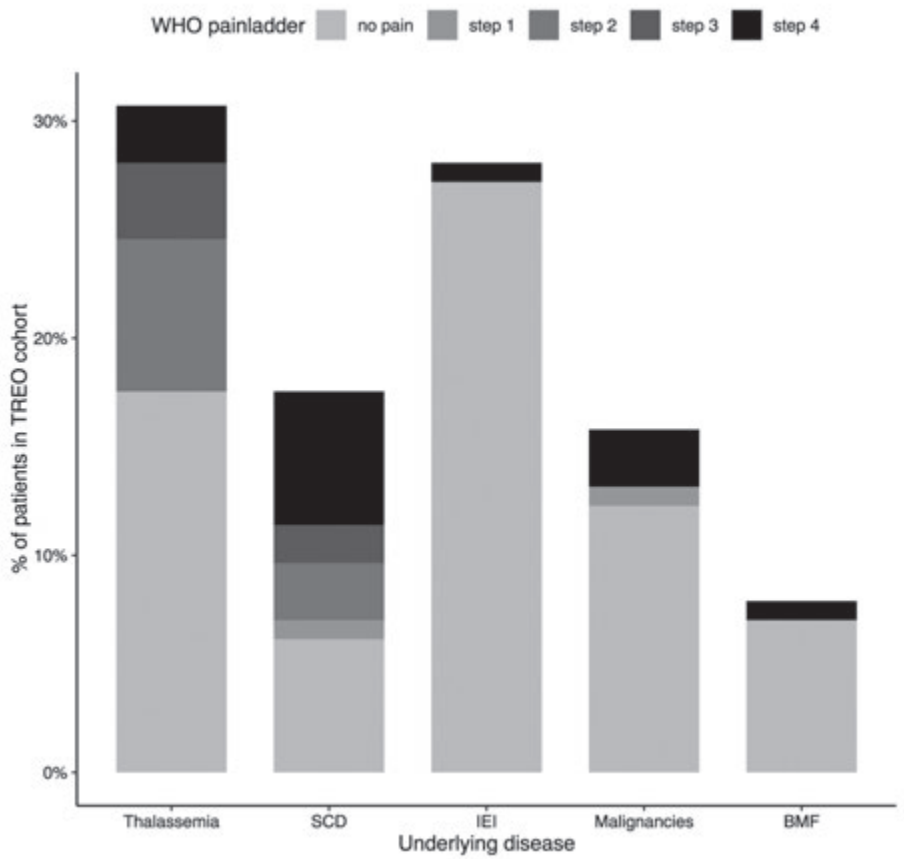

Fig. 3 Pain severity (classified as steps in the WHO pain relief ladder) according to underlying disease in the TREO cohort (SCD: sickle cell disease, IEI: inborn errors of immunity, BMF: bone marrow failure)

DISCUSSION

This study provides an insight into the incidence, duration and severity of myalgia after treosulfan-based conditioning prior to pediatric HSCT. A substantial amount of the patients in the TREO-based cohort developed severe myalgia, especially in patients treated for hemoglobinopathy. This was not observed after BU-based conditioning.

The comparison was made with a BU cohort, so that potential confounding of other factors, such as concomitant agents in the conditioning regimen (namely fludarabine, but also thiotepa), could be ruled out as much as possible.

To our knowledge, this is the first study addressing this serious side effect of treosulfan. In the original SmPC, myalgia was not mentioned as one of the side effects [13]. In the updated SmPC, pain in extremities is mentioned in the undesirable effects in the pediatric population with unknown frequency. For the adult population, myalgia and arthralgia are listed as common (1%-10%) side effects. In our patients we found a higher incidence of 30%, predominantly in the hemoglobinopathy group. A possible explanation could be the difference in the height of the dosages: for ovarian cancer, the dose is 5-8 g/m^2 every 3-4 weeks, whereas for conditioning prior to HSCT a higher dose of 30-42 g/m^2 is applied, divided over three consecutive days.

It is known that particular chemotherapeutic agents are associated with significant muscle and joint pains. These are agents that inhibit microtubular function (antimitotics, i.e. the vinca alkaloids, particularly vinblastine, vincristine and vindesine, and the taxanes, paclitaxel and docetaxel). The pathophysiologic mechanisms remain unclear, but it is thought that disruption of the microtubules, which are critical for maintenance of cell shape, motility and anchorage, mediation of signals between surface receptors and the nucleus and intracellular transport, cause cell death [17]. CK elevation is reported sometimes, while other case reports do not [18-21]. Treosulfan is not an antimitotic agent and its mechanism of action is different. In a study in rats done by Romanski et al., the disposition of treosulfan and its active monoepoxide in different organs (bone marrow, liver, lungs, brain and muscle) was investigated [22]. The study shows a comparable exposure of treosulfan in muscle and plasma, but a higher exposure of the active metabolite in muscle than in plasma. The authors mention that these findings may be explained by lower molecular weight (182 Da) and higher lipophilicity (logP -1.18) of the metabolite. It is possible that this higher exposure ratio in muscle to plasma is responsible for damage in myocytes, causing myalgia. Busulfan can also cause myalgia, as reported in the adverse effects section

of the SmPC [23]. However, in our study we found a significantly lower incidence in patients treated with busulfan-based conditioning compared to treosulfan-based conditioning. A possible explanation is that the standard daily doses of treosulfan that is applied in conditioning prior to HSCT (10-14 g/m^2) are much higher than the doses used for busulfan (max. 3.2 mg/kg). The concentrations of treosulfan and its active monoepoxide are expected to be much higher than busulfan. In our study, we had data of treosulfan exposure in a majority of patients. A relationship between treosulfan exposure in serum and the occurrence of myalgia could not be found, in contrast to other early adverse events, such as mucositis and skin toxicity [24]. However, the $AUC_{0-\infty}$ in serum might not reflect the concentration of the metabolite in muscle, which could be different. The concentration of active monoepoxide could be more interesting, as this seems relatively high in muscle. CK elevation was not found. However, myalgia without CK elevation is not uncommon and is described in many drug-induced myopathies, such as statins [25].

We found that underlying disease was a prognostic factor for the development of myalgia. Patients transplanted for beta-thalassemia and SCD are seven times more likely to develop myalgia than patients transplanted for other diseases. In the literature, no specific reports on myalgia are found. This could be due to lack of awareness of this (transient) adverse event as well as the low incidence in some categories of patients as shown in this study. The underlying cause of this correlation is currently unknown, but several hypotheses can be considered. Most SCD patients have a complex pre-transplant disease history, with systemic vasculopathy causing different complications involving pain. Their pain perception could be altered and this could lead to myalgia being perceived as more painful than in other diseases. It is also possible that there is a genetic predisposition factor associated with the occurrence of myalgia after treosulfan administration. Wonkam et al. found pain-related genes correlated with vaso-occlusive crises (CACNA2D3-rs6777055, *P=0.025*; DRD2-rs4274224, *P=0.037*; and KCNS1-rs734784, *P=0.01*) in patients with sickle cell disease [26]. Thalassemia, and hemoglobinopathies in general, are more common in certain ethnic groups. It would be interesting to investigate if genes may be associated with this adverse effect. This could be addressed in future research.

There are some limitations to our study. We performed a retrospective database study and the validity of the data is dependent on the observations of the nursing staff and accuracy of reporting in the medical records. In the pediatric population, recognition and assessment of myalgia in babies and infants (< 3 years old) is difficult, because they are (mostly) unable to indicate the type of pain they are experiencing. This may have contributed to our observation that higher age appeared to be a significant prognostic factor. Also, it is possible that we underestimated the number of patients with myalgia and its severity, because the medical records were initially screened using the CDC. However, a recent study has shown that the use of this search engine is reliable and accurate [27]. Overestimation is highly unlikely, because all EHRs with a positive hit were screened and confirmed manually. Furthermore, due to the fact that myalgia was reported more frequently over time after treosulfan was introduced as a conditioning agent, it is possible that the nursing staff recognized and classified this type of pain better over time. This means that there is a possibility of underreporting in the first years after introduction of treosulfan. However, since the majority of patients required heavy pain medication to alleviate the pain, the risk of underreporting can be considered small. Another limitation is the imbalance in underlying diseases between the TREO and BU cohort. We found that hemoglobinopathies have a significantly higher risk to develop myalgia and this group is underrepresented in the BU cohort. As there were no SCD patients in the BU cohort, we cannot definitively rule out the possibility that myalgia would have occurred as well when SCD patients were conditioned with a BU-based regimen. However, the majority of published literature on transplantations of SCD patients are with BU-based regimens [28, 29]. In those studies, myalgia has not been reported as an adverse event. Moreover, in our study there were no reports of myalgia in patients with beta-thalassemia in the BU cohort, whereas in the TREO cohort 15 out of 34 patients (44%) reported myalgia.

This study provides important new knowledge about treosulfan and its adverse events. The impact of myalgia on the patient's experience during the SCT treatment can be significant. Patient and nursing staff education is an essential part of the nursing care plan to manage drug- or disease related arthralgias and myalgias. If patients and the

nursing staff - in particular for babies and infants - recognize these symptoms and a plan is available to deal with the discomfort, therapeutic approaches can be initiated earlier and the quality of life of the transplanted patients is minimally affected. Furthermore, the use of an electronic health record text mining tool has proven to be helpful in tracking adverse events. More studies have been published using CTcue or a comparable tool to retrieve data from the EHR, including a validation study [27, 30, 31]. In the future, in case of a suspicion of the occurrence of a specific adverse event, a text mining tool can efficiently extract data from EHRs and can therefore quickly provide clarity on the relationship with the use of a particular drug.

CONCLUSION

Myalgia is a common adverse effect in treosulfan-based regimens in pediatric patients in the setting of HSCT, particularly in hemoglobinopathies. This study shows that retrospective studies can make an important contribution to the knowledge and recognition of adverse events. It provides valuable information, that can be included in the Summary of Product Characteristics of treosulfan. A text mining tool such as the CDC can help to detect adverse events more efficiently. More research is needed to learn more about the mechanism of action and factors that influence the development of myalgia.

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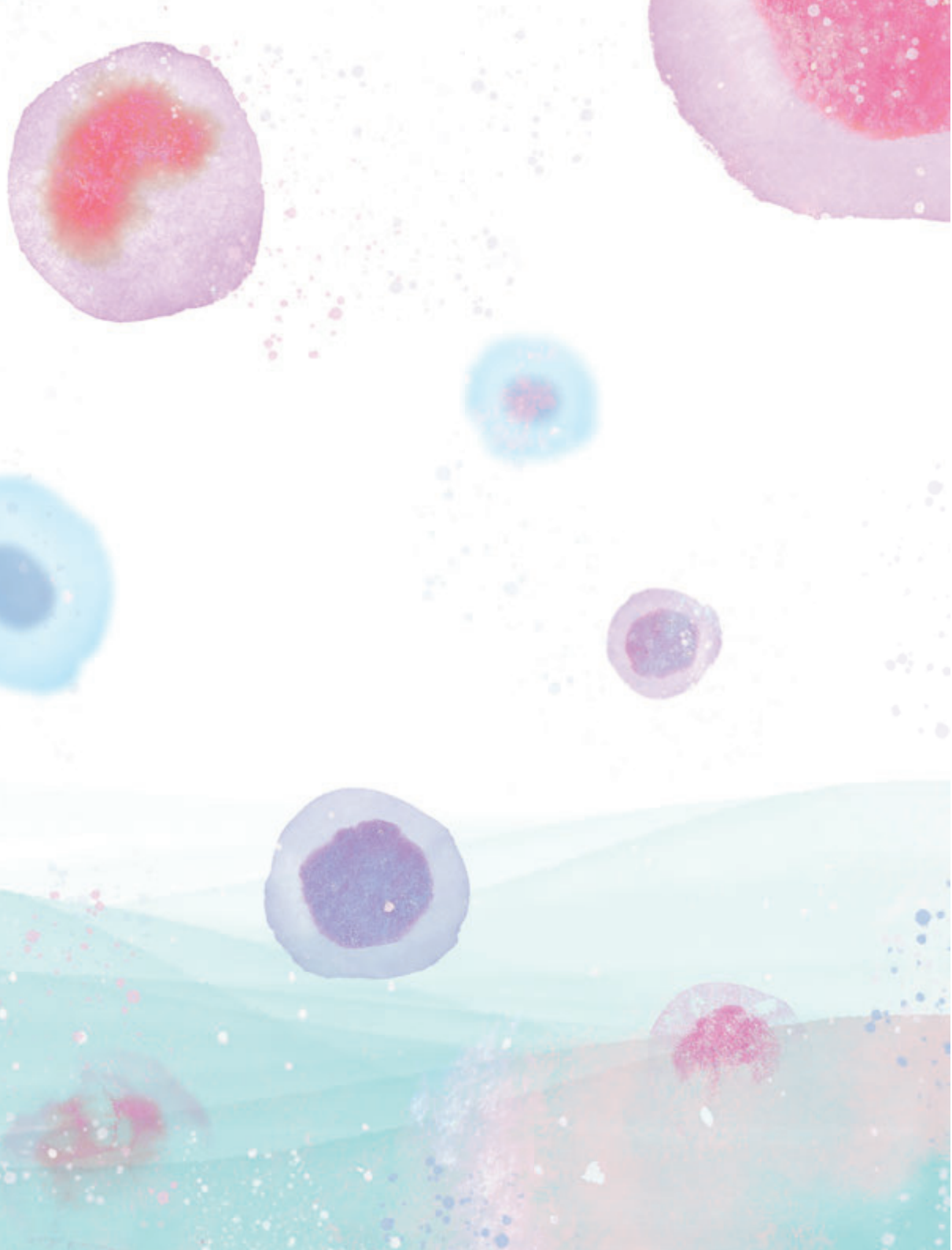
Supplemental Material 1. Synonyms of myalgia used for screening EHRs

Dutch	
Myalgie	Spierpijn
Spier pijn	Pijn spier
Spierpijnen	Spier pijnlijk
Spieren pijn	Pijn spieren
Pijnlijke spier	Pijnlijk spieren
Spieren pijnlijk	Pijnlijke spieren
Gegeneraliseerde spierpijn	Gegeneraliseerd spierpijn
Spierpijnen gegeneraliseerd	Gegeneraliseerde spierpijnen
English	
Myalgia	Myodynia
Muscle pain	Muscle aches
Muscular pains	Muscle soreness



CHAPTER 06

EFFECT OF BUSULFAN AND TREOSULFAN ON GONADAL FUNCTION AFTER ALLOGENEIC STEM CELL TRANSPLANTATION IN CHILDREN AND ADOLESCENTS WITH NONMALIGNANT DISEASES IS NOT EXPOSURE-DEPENDENT

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Submitted

ABSTRACT

With an increasing number of young patients surviving into adulthood after hematopoietic stem cell transplantation (HSCT), gonadal dysfunction becomes an important late effect with significant impact on quality of life. In this retrospective single-center study, we evaluated the exposure of busulfan (BU) and treosulfan (TREO) in relation to gonadal function in pediatric patients transplanted for a nonmalignant disease between 1997 and 2018. In the BU cohort, 56 patients could be evaluated and gonadal dysfunction occurred in 35 (63%) patients. Lower BU exposure (cumulative area under the curve cAUC <70 mg*h/L) was not associated with a reduced risk of gonadal dysfunction (OR 0.92 95% confidence interval (CI) 0.25-3.49, p=0.90). In the TREO cohort, 32 patients were evaluable and gonadal insufficiency occurred in 9 patients (28%). Lower TREO exposure (AUC <1750 mg*h/L on day 1) was not associated with a reduced risk of gonadal dysfunction (OR 1.6 95%CI 0.16-36.6, p=0.71). Our data do not support the premise that reduced intensity BU-based conditioning lowers the risk for gonadal toxicity and it is unlikely that TDM-based reduced treosulfan exposure will further reduce the risk for gonadal dysfunction.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment option for a growing number of nonmalignant indications in childhood. Increased safety and effectiveness of the transplant procedures and particularly conditioning regimens have contributed to this rise in transplants in the last decade [1]. The focus, when improving the conditioning regimen, has mainly been on decreasing acute toxicity, while trying to maintain efficacy. Using less toxic agents, less toxic combinations, dose optimization and personalized dosing with the help of therapeutic drug monitoring (TDM) or model informed precision dosing (MIPD) have shown to be successful strategies to achieve this goal [2-4]. The late effects of the transplant procedure, such as gonadal dysfunction and growth impairment, become more important as an increasing number of (very) young patients are transplanted, that benefit from the curative potential of HSCT and survive into adulthood [5]. In a recent study, we reported a high prevalence of endocrine complications in survivors of pediatric HSCT in nonmalignant diseases [6]. Female patients were more likely to develop gonadal dysfunction after busulfan-based (BU) conditioning compared to treosulfan-based (TREO) conditioning. To date, it is unknown if drug exposure is of influence on the prevalence of endocrine complications. In this study we retrospectively evaluated if the exposure of busulfan and treosulfan was related to the risk of gonadal dysfunction in pediatric patients transplanted for a nonmalignant disease.

METHODS

Study population and design

This retrospective non-interventional single-center study included patients with a nonmalignant disease who received BU- or TREO-based conditioning prior to HSCT in line with the respective EBMT Working Party and institutional guidelines at the department of Pediatrics at the Leiden University Medical Center in the Netherlands between 1997 and 2018. Exclusion criteria were re-transplantation, no data available on the outcome measures of the study and death within two years post-transplant. The

study protocol was assessed by the local medical ethical committee who determined that the Medical Research Involving Human Subjects Act (WMO) does not apply to this study. The need for informed consent was waived.

Data collection

All patients underwent a clinical and laboratory endocrine evaluation prior to HSCT. At annual follow-up visits after HSCT, pubertal stage was evaluated and laboratory investigations including FSH, LH, testosterone and estradiol, were performed. Patient and transplant characteristics were collected from the medical files including sex, age, underlying disease and conditioning regimen. Plasma serum concentrations of BU and TREO were collected if available. Indications for HSCT were classified as inborn errors of immunity or metabolism (IEI/IEM), hemoglobinopathies (HBP) and bone marrow failure (BMF). Data on gonadal dysfunction were collected up until last follow-up.

Busulfan and treosulfan pharmacokinetics

Validated analytical methods were used to quantify BU and TREO in serum and described earlier [7-9]. TREO area under the curve (AUC) on day 1 as a measure of total exposure was estimated with a pharmacokinetic model using the posthoc estimation function in NONMEM [10]. BU cumulative AUC (cAUC) was estimated using a validated limited sampling model [7]. Empirical Bayesian PK parameter estimates at steady state (clearance and volume of distribution) were generated for all individual children using the PK software package MwPharm, University of Groningen, The Netherlands [11]. The AUC was calculated from the expression dose/clearance (CL).

Outcomes

Gonadal dysfunction was defined as gonadotropins above the reference range, i.e. FSH ≥ 21.5 U/L and/or LH ≥ 60 U/L for females and FSH ≥ 12.5 U/L and/or LH ≥ 9.0 U/L for men. If elevated gonadotropins had normalized at subsequent visits gonadal dysfunction was classified as transient; if they remained elevated at last visit it

was classified as permanent [12]. Patients at Tanner stage ≥G2 or ≥B2 were classified as (post)pubertal and were included in the analysis [13, 14]. Patients diagnosed with gonadal dysfunction before HSCT were excluded from this analysis.

Statistical analysis

Descriptive statistics were performed on the data. Normally distributed continuous parameters are shown as mean ± standard deviation, all log-normally continuous distributed parameters as median and interquartile range (IQR) and categorical variables as frequency (percentage). BU and TREO exposure was divided in 2 exposure groups; low (< 70 mg*h/L for BU and < 1750 mg*h/L for TREO) and high (≥ 70 mg*h/L for BU and ≥ 1750 mg*h/L for TREO). For BU this is based on recommended targets for myeloablative (85-95 mg*h/L) and reduced intensive conditioning (60-70 mg*h/L) [15]. For TREO, this is based on results published earlier on exposure and acute toxicity and clinical outcome [9]. Univariate logistic regression analyses were performed to evaluate BU and TREO exposure as a risk factor for outcome. BU and TREO exposure was tested as discrete variable, considering low and high exposure groups. All *p*-values were 2-tailed and considered significant when $p < .05$. Statistical analyses were performed with R (version 4.1.0) and R studio version 1.4.1717.

RESULTS

Patient characteristics

A total of 157 patients were included, 90 were conditioned with BU and 67 with TREO. Of the 90 patients in the BU cohort, 56 patients were eligible for analysis; 27 patients were still prepubertal and data of 7 patients were incomplete or were excluded from the analysis because of gonadal dysfunction prior to HSCT. Of the 67 patients in the TREO cohort, 32 patients were eligible for analysis; 34 patients were still prepubertal and data of 1 patient was incomplete. Patient and transplant characteristics are shown in Table 1. In the BU group, the majority of patients were conditioned with BU in combination with cyclophosphamide (48%), followed by BU and cyclophosphamide in combination

with another agent (melphalan, etoposide or fludarabine) (23%). In the TREO group, the majority was conditioned with TREO in combination with fludarabine and thiotepa (59%), followed by TREO with fludarabine (25%). Exposure data of 41 (68%) and 19 (59%) patients was available in the BU and TREO group, respectively.

Table 1. Patient and transplant characteristics

	Eligible BU patients (N=56)	**Eligible TREO patients (N=32)**
Characteristic		
Sex (n: M/F)	39/17	16/16
Age (years, median (IQR))	5.6 (3.2-11.3)	13.5 (8.7-15.0)
Age at last follow up (years, median (IQR))	18.2 (15.4-20.6)	16.6 (15.3-18.6)
Length of follow up (years, median (IQR))	11.4 (8.3-17.1)	4.0 (2.5-8.3)
Diagnosis for HSCT		
Inborn errors of immunity (%)	31 (55)	5 (16)
Hemoglobinopathies (%)	15 (27)	24 (75)
Bone marrow failure (%)	10 (18)	3 (9)
Donor		
MSD (%)	22 (39)	15 (47)
MUD (%)	27 (48)	13 (41)
MMFD/Haplo (%)	7 (12)	3 (9)
ORD (%)	0 (0)	1 (3)
Pubertal status at HSCT		
Prepubertal (%)	43 (77)	15 (47)
(Post)pubertal (%)	13 (23)	17 (53)
Combining agents		
Fludarabine (%)	10 (18)	8 (25)
Fludarabine + Thiotepa/Melphalan (%)	6 (11)	19 (59)
Cyclophosphamide (%)	27 (48)	0
Cyclophosphamide + Melphalan/Etoposide/Flu (%)	13 (23)	5 (16)
Exposure measured (%)	41 (68)	19 (59)
Low (<70 mg*h/L for BU, <1750 mg*h/L for TREO) (%)	27 (66)	5 (26)
High (≥70 mg*h/L for BU, ≥1750 mg*h/L for TREO) (%)	14 (34)	14 (74)

MSD: matched sibling donor, MUD: matched unrelated donor, MMFD: mismatched family donor, ORD: other related donor.

Gonadal dysfunction in BU-treated patients

At time of HSCT, 43 (77%) of 56 patients were prepubertal and 13 (23%) were (post) pubertal (Table 2). Median age at HSCT was 5.6 years (IQR 3.2-11.3) and median

age at last visit was 18.2 years (IQR 15.4-20.6). Gonadal dysfunction occurred in 35 (63%) patients, 19 male and 16 female patients. In 4 patients (2 male and 2 female), gonadal dysfunction was transient of whom one female patient needed temporary hormonal substitution. When comparing BU + Cyclophosphamide and BU + Fludarabine, permanent gonadal dysfunction occurred in 50% of evaluable patients in both groups. BU exposure data was available of 41 patients. Lower BU exposure (cAUC < 70 mg*h/L) was not associated with a reduced risk of gonadal dysfunction (OR 0.92 95% confidence interval (CI) 0.25-3.49, p=0.90). The distribution of BU exposure in relation to gonadal function is shown in Figure 1A.

Table 2. Gonadal dysfunction in BU-treated patients

	No (N = 21)	**Yes (N=31)**	**Transient (N=4)**
Characteristic			
Sex (n: M/F)	20/1	17/14	2/2
Age (years, median (IQR))	5.5 (3.2-7.7)	7.4 (3.5-14.0)	2.9 (1.1-6.2)
Age at last follow up (years, median (IQR))	16.6 (13.7-20.6)	19.7 (16.6-23.1)	18.9 (16.8-21.7)
Length of follow up (years, median (IQR))	13.3 (8.9-16.9)	10.9 (7.8-18.4)	15.2 (12.4-18.0)
Diagnosis for HSCT			
Inborn errors of immunity (%)	14 (67)	14 (45)	3 (75)
Hemoglobinopathies (%)	3 (14)	11 (35)	1 (25)
Bone marrow failure (%)	4 (14)	6 (19)	0 (0)
Donor			
MSD (%)	8 (38)	13 (42)	1 (25)
MUD (%)	12 (57)	13 (42)	2 (50)
MMFD/Haplo (%)	1 (5)	5 (16)	1 (25)
Pubertal status at HSCT			
Prepubertal (%)	20 (95)	20 (65)	4 (100)
(Post)pubertal (%)	1 (5)	11 (35)	0 (0)
Combining agents			
Fludarabine (%)	5 (24)	5 (16)	0 (0)
Fludarabine + Thiotepa/Melphalan (%)	0 (0)	5 (16)	1 (25)
Cyclophosphamide(%)	12 (57)	12 (39)	3 (75)
Cyclophosphamide + Melphalan/Etoposide/ Flu (%)	4 (19)	9 (29)	0 (0)
Exposure measured (%)	17 (81)	21 (68)	3 (75)
Low (<70 mg*h/L) (%)	11 (52)	15 (48)	1 (25)
High (≥70 mg*h/L) (%)	6 (29)	6 (20)	2 (50)

MSD: matched sibling donor, MUD: matched unrelated donor, MMFD: mismatched family donor.

Gonadal dysfunction in TREO-treated patients

At time of HSCT, 15 (47%) of 32 patients were prepubertal and 17 (53%) were (post) pubertal (Table 3). Median age at HSCT was 13.5 years (IQR 8.7-15.0) and median age at last visit was 16.6 years (IQR 15.3-18.6). Gonadal dysfunction occurred in 9 (28%) patients, 3 male and 6 female patients. In 5 patients (3 male, 2 female), gonadal dysfunction was transient and 3 patients (1 male, 2 female) needed temporary hormonal substitution. TREO exposure data was available of 19 patients. Lower TREO exposure (<1750 mg*h/L on day 1) was not associated with a reduced risk of gonadal dysfunction (OR 1.6 95%CI 0.16-36.6, p=0.71). The distribution of TREO exposure in relation to gonadal function is shown in Figure 1B.

Table 3. Gonadal dysfunction in TREO-treated patients

	No (N = 23)	**Yes (N=4)**	**Transient (N=5)**
Characteristic			
Sex (n: M/F)	13/50	0/4	3/2
Age (years, median (IQR))	11.5 (8.3-14.1)	15.9 (12.3-16.7)	14.9 (14.3-15.7)
Age at last follow up (years, median (IQR))	16.3 (14.5-18.3)	17.2 (15.5-19.0)	18.9 (17.4-21.5)
Length of follow up (years, median (IQR))	5.1 (2.7-8.3)	2.4 (1.7-4.9)	3.5 (3.3-6.6)
Diagnosis for HSCT			
Inborn errors of immunity (%)	4 (17)	0 (0)	1 (20)
Hemoglobinopathies (%)	17 (74)	3 (75)	4 (80)
Bone marrow failure (%)	2 (9)	1 (25)	0 (0)
Donor			
MSD (%)	13 (57)	1 (25)	1 (20)
MUD (%)	6 (26)	3 (75)	4 (80)
MMFD/Haplo (%)	3 (13)	0 (0)	0 (0)
ORD (%)	1 (4)	0 (0)	0 (0)
Pubertal status at HSCT			
Prepubertal (%)	13 (57)	1 (25)	0 (0)
(Post)pubertal (%)	9 (43)	3 (74)	5 (100)
Combining agents			
Fludarabine (%)	8 (35)	0 (0)	0 (0)
Fludarabine + Thiotepa/Melphalan (%)	12 (52)	3 (75)	4 (80)
Cyclophosphamide + Melphalan/Etoposide/Flu (%)	3 (13)	1 (25)	1 (20)
Exposure measured (%)	14 (61)	2 (50)	3 (60)
Low (<1750 mg*h/L) (%)	4 (17)	0 (0)	1 (20)
High (≥1750 mg*h/L) (%)	10 (43)	2 (50)	2 (40)

MSD: matched sibling donor, MUD: matched unrelated donor, MMFD: mismatched family donor, ORD: other related donor.

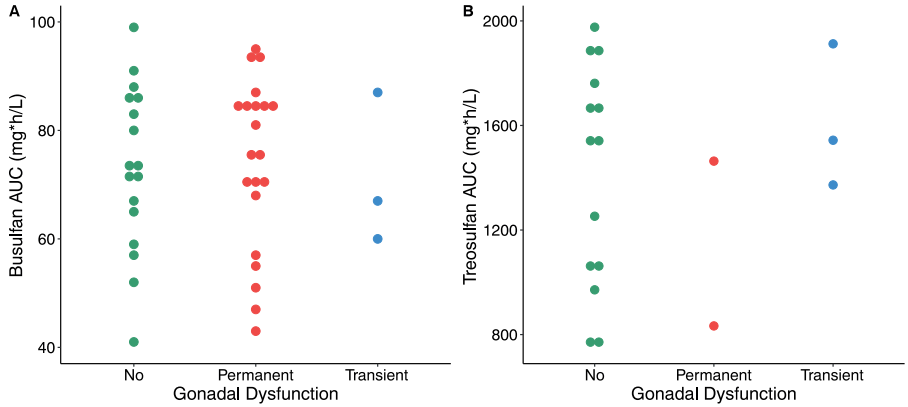

Figure 1. A) Busulfan cumulative exposure and B) treosulfan day 1 exposure in relation to gonadal dysfunction.

DISCUSSION

Both busulfan- and treosulfan-based conditioning have been demonstrated to be effective in patients transplanted for a nonmalignant disease [2, 15, 16]. With an increasing number of transplant survivors, the late effects of the transplant procedure become more important. We studied a cohort of 157 patients, transplanted for a nonmalignant disease, conditioned with a BU- or TREO-based regimen. Previous studies looked at different conditioning regimens in relation to gonadal function, pointing to a more favorable outcome in TREO-based conditioning [6, 17-19]. However, these studies did not take BU and TREO exposure into account. To the best of our knowledge, this is the first time BU and TREO exposure was studied in relation to late endocrine complications. While low BU exposure (reduced intensity conditioning) is known to result in less acute toxicity, it was not associated with a lower risk of gonadal dysfunction as compared to high exposure (myeloablative conditioning). Combining BU with either cyclophosphamide or fludarabine made no difference for the occurrence of gonadal insufficiency. Together, our data do not support the hypothesis that the use of reduced intensity BU-based conditioning lowers

the risk of gonadal dysfunction compared to high dose regimens. Gonadal dysfunction occurred at a much lower frequency in the TREO group in comparison to the BU group. No evidence was found for a correlation between TREO exposure and gonadal dysfunction, but numbers in our study were low, therefore probably lacking statistical power. Various studies in a variety of nonmalignant and malignant diseases have indicated that BU and TREO-based conditioning in general result in similar overall and event-free survival [20-23]. While reduced intensity BU-based conditioning has been reported to be beneficial in patients with co-morbidity to limit acute toxicity and improve outcome, our data do not support the premise that low exposure also lowers the risk for gonadal toxicity, either permanent or transient [24, 25]. Similarly, although the a priori risk of gonadal dysfunction is lower compared to BU-based conditioning, our data indicate that it is unlikely that TDM-based reduced treosulfan exposure will further reduce the risk for gonadal dysfunction.

Our study has some limitations. While the initial cohort consisted of 157 patients, the number of evaluable patients was lower, because a subgroup of patients was still prepubertal and were therefore not available for evaluation. Also, exposure data was not available for every patient. Future research should preferably be conducted in larger groups of former HSCT patients reaching adolescence and adulthood, so that other covariates, such as age at HSCT, sex and underlying condition can also be taken into account.

To conclude, in this first study on the association between BU- and TREO exposure and gonadal dysfunction after HSCT for nonmalignant diseases in childhood we demonstrate a higher incidence of gonadal dysfunction in the BU-conditioned group while no correlation was found with either BU or TREO exposure.

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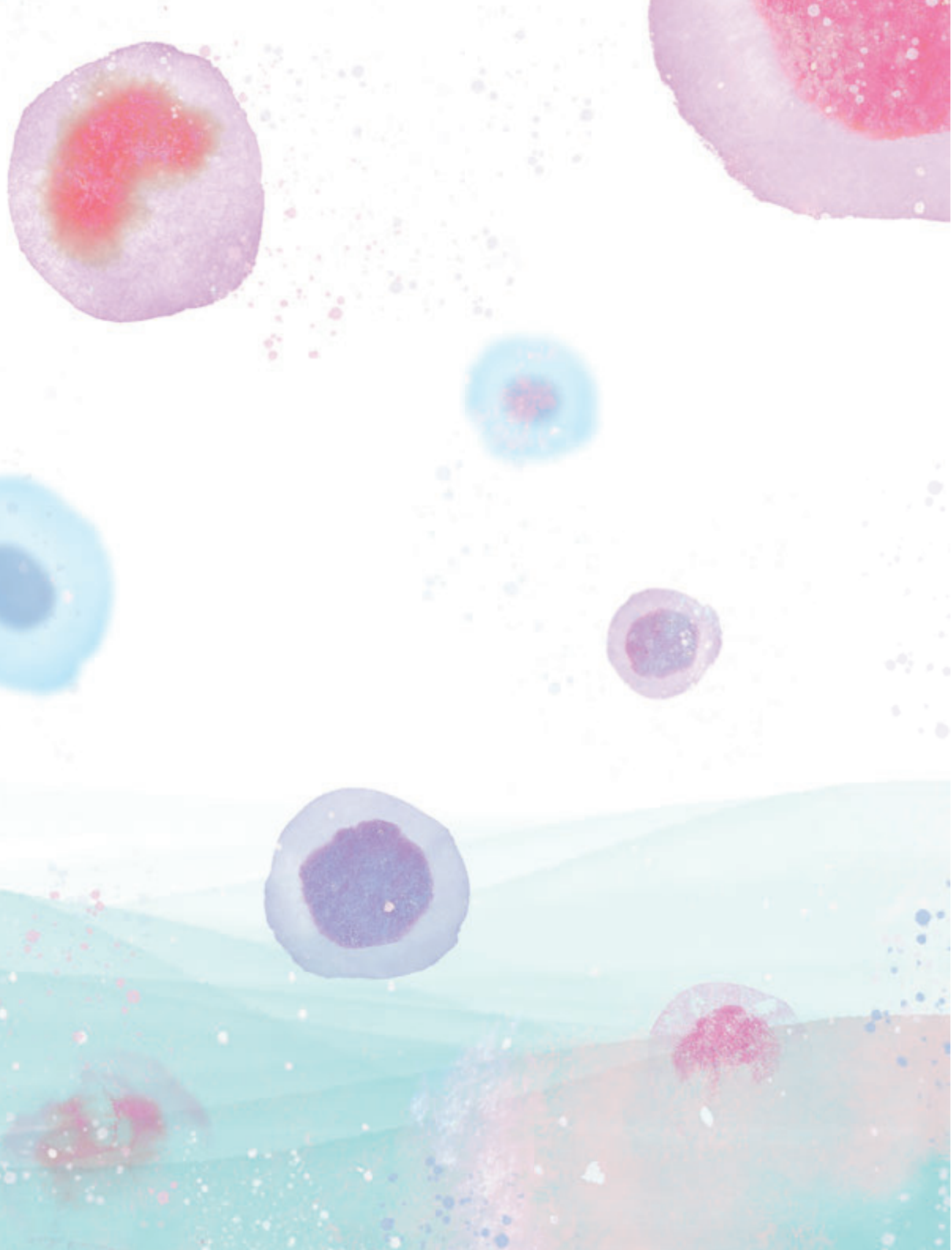

CHAPTER 07

THERAPEUTIC DRUG MONITORING OF CONDITIONING AGENTS IN PEDIATRIC ALLOGENEIC STEM CELL TRANSPLANTATION; WHERE DO WE STAND?

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established curative treatment that has significantly improved clinical outcome of pediatric patients with malignant and non-malignant disorders. This is partly because of the use of safer and more effective combinations of chemo- and serotherapy prior to HSCT. Still, complications due to the toxicity of these conditioning regimens remains a major cause of transplant-related mortality (TRM). One of the most difficult challenges to further improve HSCT outcome is reducing toxicity while maintaining efficacy. The use of personalized dosing of the various components of the conditioning regimen by means of therapeutic drug monitoring (TDM) has been the topic of interest in the last decade. TDM could play an important role, especially in children who tend to show greater pharmacokinetic variability. However, TDM should only be performed when it has clear added value to improve clinical outcome or reduce toxicity. In this review, we provide an overview of the available evidence for the relationship between pharmacokinetic parameters and clinical outcome or toxicities of the most commonly used conditioning agents in pediatric HSCT.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is an established curative treatment for malignant and non-malignant disorders in both adult and pediatric patients. In HSCT, the hematopoiesis of the host (i.e. the patient) is eliminated by a conditioning regimen in order to allow donor (i.e. healthy individual) stem/progenitor cell engraftment in the bone marrow and thymic niches. Furthermore, prevention of immune-mediated rejection is an important goal of conditioning regimens that should facilitate a successful HSCT outcome [1]. Depending on the underlying disease, the conditioning regimen usually consists of agents that have myeloablative (MA) properties to create 'space' in the bone marrow of the patient and eradicate the primary disease [2]. Immunoablative/-suppressive agents are applied to prevent rejection (host-versus-graft) as well as graft-versus-host disease (GvHD). After the infusion of the donor stem cells containing graft, immunosuppressive agents are usually used as prophylaxis to ensure engraftment and prevent the development of GvHD [3].

The choice for the optimal conditioning regimen is dependent on different factors. The required intensity of the conditioning regimen, particularly the immunosuppressive component, is usually greater when an unrelated or mismatched family donor is used. Myeloablative regimens are associated with a high likelihood to result in full donor chimerism, a situation where the newly developed hematopoietic system is of donor origin only [4]. For malignant diseases, MA regimens are often required to eradicate all malignant cells, whereas in patients with non-malignant diseases less intense protocols can also be sufficient, depending on the specific disease and required level of chimerism. These less intense, non-MA protocols are often referred to as reduced intensity (RIC) regimens, in which the use of reduced doses of myeloablative drugs (or radiotherapy) is more likely to result in mixed chimerism, a state where donor and recipient hematopoiesis coexist within the recipient [2, 4]. In addition, patient specific factors, such as age, immune status, DNA repair disorders, tumor load, disease activity and comorbidities, play a role in requirement for and tolerability to the various conditioning agents and therefore the choice for the preferred regimen [5]. Nowadays, more emphasis is placed

on the immunosuppressive aspect of the regimen to prevent rejection and GvHD in the case of unrelated or mismatched donors [6]. While an effective conditioning regimen is necessary prior to the infusion of the HSCs, it may also be accompanied with acute toxicity which can even be life-threatening. Complications related to toxicity of the conditioning regimen are still a major cause of transplant-related mortality (TRM). Besides the risk of acute toxicity, late toxicities, such as infertility, are also a major problem [7]. One of the main challenges to improve HSCT outcome is reducing toxicity caused by the conditioning regimen while maintaining efficacy.

In the last decade, significant improvements have been made to optimize efficacy and safety of conditioning regimens. These include the use of less toxic agents, less toxic combinations and dose optimization. Personalized dosing of several components of the conditioning regimen by means of therapeutic drug monitoring (TDM) has contributed to more favourable HSCT outcome. Therapeutic Drug Monitoring is the clinical practice of individualization of dosage by measuring plasma or blood drug concentrations and maintaining it within a therapeutic range or window. TDM is considered useful when the following criteria are met [8]: 1) There should be a clear relationship between concentration and effect (either efficacy or toxicity or both), 2) drug concentrations cannot be predicted from a given dose, because of high interindividual variability in pharmacokinetic (PK) parameters, 3) the drug has a narrow therapeutic index, 4) the dose cannot be easily optimized by clinical observation and 5) a bioanalytical assay should be available. TDM in combination with the use of mathematical models (such as population PK models), and other patient and disease characteristics, such as genotype, organ function, and age, is now increasingly being used to personalize dosing right at the start of treatment; a dosing paradigm that is now often referred to as 'model-informed precision dosing (MIPD)' [9].

Especially in children, TDM/MIPD can be of value. Because of the development and maturation of organ systems, in general children have greater pharmacokinetic variability than adults due to age-related differences in drug metabolism [10]. Also, the developing organ systems may lead to different susceptibility to toxicity. Moreover, pharmacokinetic studies in children are sparse which makes it challenging to establish evidence based

TDM recommendations. In this review, the focus lies on providing an overview of the available evidence for the relationship between pharmacokinetic parameters and clinical outcome or toxicities of the most commonly used conditioning agents given prior to pediatric HSCT and discuss whether TDM could be a useful tool to improve outcome.

LITERATURE SEARCH METHODS

Literature searches in PubMed were conducted using the generic names of the conditioning agents and the terms 'pharmacokinetics' and 'pediatric' (e.g. 'treosulfan AND pharmacokinetics AND pediatric). The results were screened and studies were included if the majority of patients were ≤ 18 years of age and if PK parameters of the drug were studied in relationship to toxicities and/or outcome. For busulfan, only the studies that report either a hazard ratio (HR), odds ratio (OR) or relative risk (RR) were selected to limit results and keep the review concise. For a detailed overview of busulfan PK studies we refer to two recent reviews [11, 12]. Studies were described in chronological order.

CHEMOTHERAPY

Busulfan

Busulfan (Bu) is a widely used and established chemotherapeutic agent in conditioning regimens prior to HSCT. It is a bifunctional alkylating agent that diffuses into cells, where it is hydrolyzed to produce highly reactive carbonium ions that alkylate and damage DNA [13]. Its metabolism is complex and not yet completely understood. It is primarily metabolized by the liver through conjugation with glutathione, mainly by glutathione-S-transferase A1 (GSTA1). The glutathione conjugate is then further oxidized before it is excreted into the urine. Intravenous (i.v.) Bu has widely replaced oral Bu when this formulation became available, which was expected to reduce pharmacokinetic variability [14]. However, interpatient variability in clearance of i.v. Bu is still reported to be up to 30% [15, 16]. Factors explaining this interpatient variability in children are age, body weight and GSTA1 genotype, among others [12]. In the past decades, many studies have shown that Bu exposure is related to clinical outcome. In Table 1, the studies that report either a hazard ratio (HR), odds ratio (OR) or relative risk (RR) are shown.

Bartelink et al. reported the results of a retrospective study of 102 pediatric patients (median age 3.1 years (range 0.2-21.0)) undergoing allogeneic HSCT for malignant (45%) and non-malignant (55%) indications. Patients received conditioning with busulfan, cyclophosphamide and melphalan (BuCyMel) (43%) or in other combinations. A once daily regimen was given in 63% of the patients, the rest received Bu 4 times daily. OS, EFS and toxicity were associated with Bu exposure. In multivariate analysis, a cumulative Bu exposure between 72 and 80 mg*h/L was associated with the most favourable EFS and OS. Higher AUC was associated with a lower incidence of graft failure and relapse. Higher Bu exposure was also a significant predictor for aGVHD, but not for veno-occlusive disease (VOD) or mucositis [17].

Ansari et al. performed a prospective study to examine the association between i.v. Bu exposure and clinical outcome in a pediatric cohort of 75 patients (median age 3.2 years (range 0.1-20.0)). Patients were included with malignant (64%) and non-malignant diseases (36%). The majority of patients received a conditioning regimen consisting of BuCy (89%) and Bu was given 4 times daily over 4 days. They found that an average Bu concentration of the first dose ($C_{ss,day1}$) > 600 ng/mL (corresponding with a daily AUC of 14.4 mg*h/L or cumulative AUC of 57.6 mg*h/L) was associated with higher incidence of aGvHD and higher risk of non-relapse mortality (NRM). In multivariate analysis, $C_{ss,day1}$ > 600 ng/mL was associated with lower EFS and lower OS [18].

A landmark study done by *Bartelink* and colleagues in 2016 included 674 patients (median age 4.5 years (range 0.1-30.4)) from 15 different pediatric transplantation centers. Malignant (41%) and non-malignant (59%) indications were included and the majority received a conditioning regimen with BuCy (52%), followed by BuFlu (37%) and BuCyMel (10%). The main outcome of interest was EFS; secondary outcomes were graft failure, relapse, TRM, acute toxicity, cGvHD, OS and cGvHD free survival. They defined that a target of 90 mg*h/L (range 78-101 mg*h/L) gave the highest probability of EFS. Compared with the low AUC group (< 78 mg*h/L), the optimal AUC decreased the probability of graft failure or disease relapse and a high AUC (> 101 mg*h/L) increased the risk of TRM and acute toxicities [19].

Benadiba et al. conducted a study with 36 pediatric patients (median age 5.9 years (range 0.6-19.3)) receiving a umbilical cord blood (UCB) transplantation for a myeloid malignancy. All patients received Bu in a regimen of 4 times daily in combination with Cy (91.7%), Mel (6%) or Cy plus etoposide (2.3%). In multivariate analysis, $C_{ss,day1}$ > 600 ng/mL (daily AUC of 14.4 mg*h/L or cumulative AUC of 57.6 mg*h/L) was a significant risk factor for OS and EFS. Furthermore, neutrophil and platelet recovery and non-relapse mortality were significantly higher in patients with $C_{ss,day1}$ < 600 ng/mL than $C_{ss,day1}$ > 600 ng/mL [20].

Philippe et al. specifically looked at the occurrence of VOD in relationship with Bu exposure. In this retrospective study, 293 pediatric patients with a median age of 6.2 years (0.2-21) were included of whom 75 (25.6%) developed VOD. There was a 6-fold increased risk of VOD in patients with a maximum drug concentration level (C_{max}) of ≥ 1.88 ng/mL. Also, weight < 9 kg and age < 3 years were independent predictors of VOD [21].

Together, these data suggest that overexposure to Bu (either on day one, or overall AUC) has a negative effect on OS and EFS. A cumulative AUC of 78-101 mg*h/L or $C_{ss,day1}$ < 600 ng/mL are suggested as possible targets. A target value for the first dose below 600 ng/mL (= 14.4 mg*h/L per day and 57.6 mg*h/L in total) seems rather low, but adequate overall exposure over the course of the treatment could still be achieved because of decreased clearance of Bu over time [22-24]. On the other hand, the target suggested by *Bartelink* et al. is higher than the historical target of 56-86 mg*h/L (C_{ss} 600-900 ng/mL), which seems to be in contrast with the results of *Ansari* et al. Also, the study done by *Bartelink* et al. shows that low cAUC (< 78 mg*h/L) gave a higher risk of graft failure or disease relapse. However, the considerable variability in Bu dosing (once, twice or four times daily), difference in exposure targets (C_{ss}, cAUC, AUC_{dose}), difference of exposure units (mg*h/L, μM*min), the method of exposure estimation and co-medication (cyclophosphamide versus fludarabine) makes comparison of all these results difficult and complex. Also, optimal exposure may differ between groups based on factors, such as underlying disease, age and comorbidities [25]. A proposal of harmonizing Bu exposure unit to mg*h/L has been done and will hopefully lead to more accurate assessment of exposure and thereby evaluation of outcomes in multicentre studies [26].

Table 1. Reported associations of pharmacokinetic parameters of busulfan and clinical outcomes

First author, year	N	Age, median (range)	Diagnosis	Regimen	Dose interval	Major Findings
Bartelink, 2009	102	3.1 (0.2-21.0)	Malignant: 46 (45%) Non-malignant: 56 (55%)	BuCyMel: 43 (42%) Other: 59 (58%)	Once daily: 64 (63%) 4 times daily: 38 (37%)	Bu exposure of 72-80 mg*h/L was associated with the highest OS and EFS (P=0.021 and P=0.028). Increased AUC was associated with less graft failure and relapse (HR 0.047; P=0.004), but more aGVHD (HR 1.56; P=0.019).
Ansari, 2014	75	6.2 (0.1-20.0)	Malignant (ALL/AML/MDS): 48 (64%) Non-malignant: 27 (36%)	BuCy: 67 (89%) BuCyVP16: 6 (8%) BuMel: 2 (3%)	4 times daily	$C_{ss,day1}$ > 600 ng/mL (daily AUC of 14.4 mg*h/L or cumulative AUC of 57.6 mg*h/L) was associated with lower EFS (HR 5.14; 95%CI 2.19–12.07; P<0.001) and lower OS (HR 7.55; 95%CI 2.20–25.99; P=0.001).
Bartelink, 2016	674	4.5 (0.1-30.4)	Malignant: 274 (41%) Non-malignant: 400 (59%)	BuCy: 352 (52%) BuFlu: 252 (37%) BuCyMel: 70 (10%)	Once daily: 267 (40%) 4 times daily: 324 (48%) Other: 83 (12%)	Optimum cAUC of 78-101 mg*h/L decreased the probability of graft failure or relapse (HR 0.57; 95%CI 0.39-0.84, P=0.0041). High cAUC increased the risk of TRM (HR 2.99; 95%CI 1.82-4.92, P<0.0001) and toxicities (HR 1.69; 95%CI 1.12-2.57; P=0.013).
Benadiba, 2018	36	5.9 (0.6-19.3)	AML: 23 (63.9%) MDS: 13 (36.1%)	BuCy: 33 (91.7%) BuMel: 2 (6%) BuCyVP16: 1 (2.3%)	4 times daily	$C_{ss,day1}$ > 600 ng/mL (daily AUC of 14.4 mg*h/L or cumulative AUC of 57.6 mg*h/L) was a significant risk factor for OS (HR 5.2; 95%CI 1.26-21.5, P=0.02) and EFS (HR 3.83; 95%CI 1.33-11.05, P=0.01).
Philippe, 2019	293	6.2 (0.2-21.0)	Malignant: 170 (58%) Non-malignant: 123 (42%)	Not clearly specified	4 times daily: 282 (96%) Once daily: 10 (3.4%0 Twice daily: 1 (0.6%)	The incidence of VOD was 25.6%. Patients with C_{max} of ≥ 1.88 ng/mL were 6 times more likely to develop VOD (63.3 vs. 21.3%, RR6.0 p < 0.001).

ALL: acute lymphoblastic anemia, AML: acute myeloid leukemia, MDS.: myelodysplastic syndrome, Mel: melphalan, Cy: cyclophosphamide, VP16: etoposide

Treosulfan

In the last decade, treosulfan (Treo) has gained popularity as a chemotherapeutic agent in conditioning regimens prior to HSCT for malignant and non-malignant disorders. It is a water-soluble bifunctional alkylating agent and a structural analogue of busulfan. Although Treo has structural similarities with Bu, its mechanism of alkylation is different. As a pro-drug, it undergoes non-enzymatic and pH-dependent conversion into active mono- and diepoxide derivatives under physiological conditions. These derivatives cause DNA alkylation and interstrand DNA crosslinking, leading to DNA fragmentation and apoptosis [27]. Approximately 25-40% of Treo is excreted renally in unchanged form [28]. Interpatient variability of clearance in children is high; between 30% and 68% have been reported in population pharmacokinetic studies [29-31]. Age, bodyweight and renal clearance are covariates that were found to (partially) explain the large interindividual variability. More recently, the relationship between Treo exposure and clinical outcome has been explored in several studies with pediatric patients undergoing HSCT. Table 2 summarizes the reports of Treo PK associated with outcome in pediatric patients.

Van der Stoep et al. described a pediatric cohort of 77 patients transplanted for non-malignant (84.4%) and malignant (15.6%) diseases (median age 4.8 years (range 0.2-18.3)). Patients received Treo with fludarabine only (35.5%) or with additional thiotepa (67.5%). Twelve patients < 1 year of age received a total dose of 30 g/m^2 and 65 patients ≥ 1 year of age received 42 g/m^2. Patients were divided into three exposure groups (on day 1); low (<1350 mg*h/L, medium (1350-1650 mg*h/L) and high (>1650 mg*h/L). Patients in the high exposure group had an higher risk for mucosal and skin toxicity compared to the low exposure group. The risk of experiencing two or more toxicities was also higher in the high exposure group compared with the low exposure group. No relationship was found between exposure and aGvHD, engraftment, chimerism and survival [32].

In a study done by *Mohanan* et al., 87 patients with thalassemia major undergoing HSCT were included to study the PK of Treo in relationship with outcome. The majority of included patients were children, although some adults up to 25 years

of age were also included (median age 9.0 years (range 1.5-25)). Treo was given in combination with fludarabine and thiotepa in a total dose of 42 g/m^2. The influence of Treo PK on rejection, toxicities, OS, EFS and TRM was evaluated and no association was found with these outcome parameters. A trend was seen towards better OS with high Treo clearance (> 7.97 L/h/m2) and low day 1 AUC (< 1828 mg*h/L). In a *post-hoc* analysis they found that lower Treo clearance (< 7.97 L/h/m^2) was significantly associated with poor OS and EFS [30].

Chiesa et al. investigated the relationship between Treo PK and OS and donor engraftment in 87 children (median age 1.6 years (range 0.2-16.7)), transplanted mainly for an inborn error of immunity (91%). All patients received Treo with fludarabine with a total dose of 42 g/m^2 in children aged > 12 months, 36 g/m^2 in children aged 3-12 months and 30 g/m^2 in children ≤ 3 months. A higher Treo cumulative AUC (the sum of Treo AUC on three days, cAUC) showed a higher risk of mortality in multivariable analysis. Also, children with cAUC > 6000 mg*h/L had higher TRM than children with cAUC < 6000 mg*h/L (39% vs. 3%). A trend was seen for low AUC to be associated with poor donor engraftment (≤ 20%), but this was observed only in univariable analysis. The authors propose a therapeutic target of cAUC 4800 mg*h/L, corresponding with 1600 mg*h/L daily [29].

Very recently, *Van der Stoep* et al. published results on Treo PK in a cohort of 110 pediatric patients with non-malignant diseases (median age 5.2 years (range 0.2-18.8)). The influence of Treo PK on early and long-term clinical outcome was evaluated. The main outcome of interest was 2-year EFS and secondary outcomes were 2-year OS, toxicities, engraftment, donor chimerism and GvHD. No association was found between Treo PK and 2-year EFS, nor with 2-year OS, engraftment, donor chimerism and GvHD. High Treo exposure (> 1750 mg*h/L) on day 1 was associated with all grade mucositis, but not with mucositis ≥ grade 2. High Treo exposure was also associated with ≥ grade 2 skin toxicity [33].

While there seems to be a relationship with Treo PK and mucositis in the first study of *Van der Stoep* et al., this was not confirmed by *Mohanan* and *Chiesa* et al. Furthermore,

in a more recent study of *Van der Stoep* et al., only a relationship between exposure and all grade mucositis was seen, but not with grade 2 or higher, which is clinically more relevant. However, in both studies of *Van der Stoep* et al. as well as the study of *Chiesa* et al., high Treo exposure was related to the risk of ≥ grade 2 skin toxicity. In terms of survival, *Chiesa* et al. showed a relationship between exposure and OS, while *Mohanan* et al. hinted towards a trend and *Van der Stoep* et al. did not observe a relationship. These differences could possibly be explained by interindividual variability in exposure between the studies, which was higher in the studies of *Chiesa* et al. and *Mohanan* et al. Also, no relationship with EFS was found [30, 33] and overall, it is noticed that Treo is well tolerated, with limited regimen-related toxicities, while still achieving good results when it comes to clinical outcome. Together, these results indicate a moderate exposure-toxicity relationship, but a relationship with survival is not evident and consistent. The clinical value of TDM could be investigated to prevent skin toxicity, although implementation of preventive care guidelines could possibly reduce the incidence of cutaneous complications as well. The current evidence do not justify the use of TDM in routine patient care, but can be useful in specific cases and subgroups and warrants further investigation.

Table 2. Reported associations of pharmacokinetic parameters of treosulfan and clinical outcomes

First author, year	N	Age, median (range)	Diagnosis	Regimen	Dose	Major Findings
Van der Stoep, 2017	77	4.8 (0.2-18.3)	HBP: 31 (40.3%) Hem. malig: 12 (15.6%) IEI: 22 (28.5%) BMF: 11 (14.3%) Other: 1 (1.3%)	TreoFlu: 25 (35.5%) TreoFluThio: 52 (67.5%)	3 x 10 g/m^2: 12 (15.6%) 3 x 14 g/m^2: 65 (84.4%)	High Treo $AUC_{0-\infty}$ (>1650 mg*h/L per day) was associated with a higher risk of ≥ grade 2 mucositis (OR 7.03; 95%CI 1.60-30.86, P=0.01). There is also an increased risk of skin toxicity (OR 9.96; 95%CI 1.85-53.46, P=0.007).
Mohanan, 2018	87	9.0 (1.5-25)	TM: 87	TreoFluThio	3 x 14 g/m^2: 87 (100%)	In a *post-hoc* analysis, lower Treo clearance (<7.97 L/h/m^2) was associated with poor overall survival (HR 2.7; 95%CI 1.09-6.76, P=0.03) and event free survival (HR 2.4; 95%CI 0.98-5.73, P=0.055). No association with toxicity.
Chiesa, 2020	87	1.6 (0.2-16.7)	IEI: 79 (91%) IBD: 5 (5%) JMML: 2 (2%) IEM: 1 (1%)	TreoFlu	3 x 10 g/m^2: 4 (5%) 3 x 12 g/m^2: 23 (26%) 3 x 14 g/m^2: 60 (69%)	Higher cumulative Treo $AUC_{0-\infty}$ showed higher risk of mortality in multivariable analysis (HR 1.32; 95%CI 1.07-1.64, P=0.0093), a trend was seen for low $AUC_{0-\infty}$ associated with poor engraftment (HR 0.61; 95%CI 0.36-1.04, P=0.072) in univariable analysis. TRM was higher in patients with AUC>6000 mg*h/L than <6000 mg*h/L (39% vs. 3%, P=0.00001). A cumulative $AUC_{0-\infty}$ of 4800 mg*h/L is proposed as target.
Van der Stoep, 2021	110	5.2 (0.2-18.8)	IEI: 38 (35%) HBP: 55 (50%) BMF: 17 (15%)	TreoFlu: 37 (32%) TreoFluThio: 77 (68%)	3 x 10 g/m^2: 18 (16%) 3 x 14 g/m^2: 92 (84%)	All grade mucositis was associated with high Treo $AUC_{0-\infty}$ (OR 4.43; 95%CI 1.43-15.50, P=0.01), but not mucositis ≥2 or higher (OR 1.51; 95%CI 0.52-4.58, P=0.46). Skin toxicity ≥ grade 2 was associated with high $AUC_{0-\infty}$ (OR 3.97; 95%CI 1.26-13.67, P=0.02). No association with 1-year donor chimerism, 2-year OS and EFS.

HPB: hemoglobinopathies, hem. malig.: hematological malignancies, IEI: inborn errors of immunity, BMF: bone marrow failure, TM: thalassemia major, IBD: inflammatory bowel disorder, JMML: juvenile myelomonocytic leukemia, IEM: inborn errors of metabolism

Fludarabine

The purine analogue fludarabine (Flu) has become an alternative for cyclophosphamide (Cy) in the classical myeloablative conditioning regimen BuCy, because of the lower risk of NRM without compromising efficacy [34]. Flu is currently being used as part of various different conditioning regimens, whether it be myeloablative, reduced-intensity or non-myeloablative. Fludarabine phosphate is a prodrug that is rapidly converted into F-ara-A in the systemic circulation. Subsequently, F-ara-A is phosphorylated in the cell into the active metabolite fludarabine triphosphate, F-ara-ATP, which is responsible for the inhibition of DNA synthesis and RNA production, leading to apoptosis [35]. Flu is predominantly excreted renally. Interpatient variability in clearance is high and bodyweight and renal clearance were found to be contributing factors to this variability [36-38]. Table 3 summarizes the reports of Flu PK associated with outcome in pediatric patients.

Ivaturi et al. reported a prospective PK study of 133 pediatric patients transplanted for malignant (44%) and non-malignant (56%) indications (median age 5.0 years (range 0.2-17.9)). Patients received Flu in various different conditioning regimens and in different dosages. No association was found between Flu exposure and the primary endpoint TRM. The highest 1-year OS rate was seen in patients with a cumulative AUC (cAUC) between 15 and 19 mg*h/L, however this was not statistically significant. In the malignant subgroup, 1- year disease free survival (DFS) was higher in patients with a cAUC between 15-19 mg*h/L than < 15 mg*h/L (82.6% vs. 52.8%). Based on the data in their study, the authors propose a minimum exposure threshold of 15 mg*h/L to achieve the best possible outcome [37].

Mohanan et al. studied the pharmacokinetics of Flu in 53 patients with aplastic anemia (75%) and Fanconi anemia (25%). They included both children and adults, however the number of children was not specified (median age 17 years (range 3-57)). The majority of patients received a regimen with Flu and Cy (55%), others received Flu and Cy in combination with TBI (38%) or anti-thymocyte globulin (ATG) (7%). All patients received a dose of 30 mg/m^2 daily for 6 days. There was no association

between the PK parameters of Flu and engraftment, mixed chimerism, rejection, OS or TRM. In multivariate analysis, a cAUC of > 29.4 μM*h was associated with a higher risk of aGVHD [39].

Chung et al. described the pharmacokinetics of Flu in 43 Korean pediatric patients (median age 11.8 years (range 1.3-18.5)). The majority of patients received a transplantation for a malignant disease (72.1%). Flu was given in combination with various different agents, but the majority received a regimen with Bu and etoposide (55.8%) with a daily dose of 40 mg/m^2 for 6 days. In their exploratory analyses, they did not find any relationship between Flu cAUC and toxicities, GVHD, relapse, EFS and survival [36].

The most recent study is from *Langenhorst* and colleagues, who conducted a retrospective cohort analysis in 192 patients (119 adults and 73 children, median age 36.2 years (range 0.23-74)). All patients received a conditioning regimen of BuFlu (4 x 40 mg/m^2), mostly for malignant diseases (65%). They found an increased incidence of NRM with higher Flu cAUC and more graft failures were observed with lower Flu cAUC. No influence on relapse was seen. Based on these results, they calculated that a cAUC of 15-25 mg*h/L was the optimal target window for Flu to minimize the chance of an event. When considering three exposure groups (below-optimal, optimal and above-optimal), the optimal exposure group had a significantly higher EFS compared with the above-optimal exposure group and (non-significantly) higher than the below-optimal group. NRM was the main cause of an event in the above-optimal group and immune reconstitution was significantly lower, whereas the risk of graft failure and NRM was increased in the below-optimal group [40].

The abovementioned studies show variable results. *Langenhorst* et al. showed that Flu exposure within the optimal target (cumulative AUC of 15-25 mg*h/L) had significant higher EFS than the above-optimal group and *Ivaturi* et al. showed better DFS with a cumulative Flu exposure > 15 mg*h/L in a subgroup of 59 children with malignancy. However, *Mohanan* et al. and *Chung* et al. failed to show associations with EFS and OS. Patient cohorts in the last two studies were small (53 and 43, respectively), so

it is possible that a statistically significant relationship could not be detected. Also, in all studies except *Langenhorst* et al. various different conditioning regimens were included with various Flu dosage schemes, which makes comparison of the results difficult. Currently, a randomized phase II study is ongoing to study the influence of individualized fludarabine conditioning on the incidence of severe viral infections and other transplant-related outcomes in adult patients with hematological malignancies (Clinicaltrialsregister.eu: TARGET study 2018-000356-18)). Whether these results can be extrapolated to children remains to be determined. Ideally, a randomized study in children is done to address whether individualized dosing improves clinical outcome. For now, the evidence for TDM for Flu is growing, but more studies are needed to explore whether a single optimal target can be defined. In the meantime, the use of TDM in routine patient care remains limited.

Table 3. Reported associations of pharmacokinetic parameters of fludarabine and clinical outcomes

First author, year	N	Age, median (range)	Diagnosis	Regimen	Dose	Major Findings
Ivaturi, 2017	133	5.0 (0.2-17.9)	Hem. malig: 59 (44%) IEI: 18 (14%) HBP: 8 (6%) Metabolic: 22 (16%) BMF: 22 (16%) Epidermolysis bullosa: 4 (4%)	BuFlu: 40 (30%) FluCy: 45 (34%) BuFluClo: 18 (14%) FluThioMel: 15(11%) Other: 15 (11%)	3-5 x 40 mg/m^2: 55 (41%) 3-5 x 12.5-35 mg/m^2: 40 (30%) 3-5 x 0.9-1.22 mg/m^2: 38 (29%)	No association with Flu and TRM (P=0.35). In the malignancy group DFS was highest at 1 year post HSCT in patients with a cumulative AUC > 15 mg*h/L compared to < 15 mg*h/L (82.6 vs. 52.8%, P=0.04). A cumulative AUC of > 15 mg*h/L is considered as a minimum exposure threshold.
Mohanan, 2017	53 (no. of children not specified)	17 (3-57)	AA: 40 (75%) FA: 13 (25%)	FluCy: 29 (55%) FluCyTBI: 20 (38%) FluCyATG: 4 (7%)	6 x 30 mg/m^2	AUC > 29.4 μM*h was a significant factor associated with aGVHD in multivariate analysis (P=0.02). None of the PK parameters showed any association with engraftment, mixed chimerism, rejection, overall survival or TRM.
Chung, 2018	43	11.8 (1.3-18.5)	Acute leukemia: 29 (67.4%) Other malig: 2 (4.7%) Non-malignant: 12 (28%)	BuFluVP16: 24 (55.8%) BluFlu: 12 (27.9%) BuFluMel: 4 (9.3%) FluCy: 2 (4.7%) BuFluCy: 1 (2.3%)	6 x 40 mg/m^2: 40 (93%) 5 x 40 mg/m^2: 3 (7%)	No significant association was found between AUC and toxicities, GvHD, relapse and survival.
Langenhorst, 2019	192 (119 adults, 73 children	36.2 (0.23-74)	Benign: 68 (35%) Leukemia/ lymphoma: 71 (37%) MDS: 30 (16%) Plasma cell disorder: 23 (12%)	BuFlu	4 x 40 mg/m^2	Flu exposure is a predictor for EFS. NRM was increased with high Flu exposure (P<0.001) and more graft failure was seen with low exposure (P=0.04). An optimal cumulative AUC of 20 mg*h/L (±5) is suggested. The optimal exposure group had a significantly higher EFS compared with the above-optimal exposure group (HR 2.0; 95%CI 1.1-3.5, P=0.01) and (non-significantly) higher than the below-optimal group (HR 1.8; 95%CI 0.72-4.5, P=0.21).

AA: aplastic anemia, FA: Fanconi anemia, TBI: total body irradiation

Clofarabine

The addition of clofarabine (Clo) to the conditioning regimen with Bu and Flu prior to HSCT in pediatric hematological malignancies has proven to be a safe and promising strategy [41, 42]. Similar as fludarabine, clofarabine is a purine analogue and a prodrug that is converted intracellularly to its active metabolite clofarabine-5'-triphosphate. This metabolite inhibits DNA polymerase-α, resulting in inhibition of DNA synthesis and repair. Furthermore, it disrupts mitochondrial membrane integrity, leading to apoptosis [43]. Excretion is predominantly through the kidneys. Very recently, the pharmacokinetics of Clo in pediatric HSCT recipients have been characterized by two groups [44, 45]. Bodyweight, age and renal function were covariates influencing clofarabine variability in clearance. Exposure-response relationships between clofarabine and clinical outcome have not been published so far.

Thiotepa

Thiotepa is an alkylating drug that is often combined with Treo and Flu or Bu and Flu in a myeloablative regimen. It is given in a dose of 8-10 mg/kg, (usually 8 mg/kg once or 5 mg/kg for two days). Because of its highly lipophilic nature and therefore its ability to cross the blood brain barrier, the addition of thiotepa not only adds myeloablative ability, but may also be beneficial in diseases with central nervous system involvement [46]. Thiotepa is quickly metabolized in the liver into the active metabolite triethylene phosphoramide (TEPA), which has a comparable alkylating activity as thiotepa. By cross-linking of DNA strands, these compounds inhibit DNA, RNA and protein synthesis. Thiotepa and TEPA are eliminated in urine, but also dermally via sweat [47]. The pharmacokinetics of thiotepa has been studied in adults and children, but not in the allogeneic HSCT setting [48-50].

SEROTHERAPY

ATG

Serotherapy with rabbit anti-thymocyte globulin (ATG) or anti-T lymphocyte globulin (ATLG) is often added to the conditioning regimen in pediatric allogeneic HSCT for prophylaxis against GvHD and graft rejection. ATG is a rabbit polyclonal IgG that is

produced by the immunization of rabbits with human thymocytes (Thymoglobulin®, Sanofi Genzyme), whereas ATLG is generated upon immunization with the Jurkat T-cell line (Grafalon®, Neovii Pharmaceuticals AG). Both ATG and ATLG contain antibodies recognizing antigens expressed on the surface of many immune and non-immune cells, and several mechanisms by which ATG/ATLG eliminates these targeted cells are described, including inducing apoptosis, complement-dependent lysis or NK-cell mediated lysis [51]. Due to the differences in the manufacturing of both products, the lymphodepleting capacity of both brands is not the same. This is reflected in the total dosage given, which varies in the pediatric setting for ATG between 4.5-10 mg/kg while for ATLG it is much higher (15-45 mg/kg). The fraction that is capable of lymphocyte binding is also described as active ATG/ATLG and is only a minor part of the total rabbit IgG (total ATG/ATLG) dosage. The lympholytic level of active ATG/ATLG is 1 AU/mL [52]. ATG/ATLG is given i.v. and the total dosage is often divided over 3 to 4 days. As for all antibodies, target binding is besides the main mechanism of action also one of the main clearance mechanisms of ATG/ATLG together with non-specific degradation. A third clearance method, leading to rapid elimination of ATG/ATLG, may occur when anti-drug-antibodies (anti-ATG/ATLG) are developed [53]. The pharmacokinetics and –dynamics (PD) of ATG in the pediatric HSCT setting have been described, however only in a limited number of studies [54-57]. Interindividual variability for linear clearance is reported to be between 50% and 86%, with body weight and absolute lymphocytes number pre-ATG as important covariates [56, 57]. For ATLG, no population PK models have been published so far, and knowledge about its PK and PD is only obtained from a few studies investigating concentration-time curves [58, 59]. Table 4 summarizes the reports of ATG PK associated with outcome in pediatric patients.

Call et al. evaluated the pharmacokinetics of total and active ATG Thymoglobulin in a prospective trial with 13 children (median age 10 years (range 2-16)) who underwent an unrelated donor HSCT with non-T-cell–depleted bone marrow grafts for hematologic malignancies. There were no occurrences of grade III–IV acute GvHD and none of the patients had serious infections following transplantation. *Call* et al. concluded that the use of a 10 mg/kg dose of ATG in children with hematologic malignancies can be administered without increasing the risk of rejection, or serious

infection in pediatric patients with a low rate of GvHD [57].

Admiraal et al. described in 2015 the pharmacokinetics of ATG in a much larger patient cohort, including 267 HSCT patients from two study centers [56]. With the use of a population PK model, pharmacokinetic endpoints (i.e., AUC) were calculated and studied in relation to the clinical outcome measures of the patients, to determine the therapeutic window and the optimal active Thymoglobulin exposure. The results of this analysis were published in a separate publication. Successful immune reconstitution, defined as CD4+ T cells > $0.05x10^9$ cells/L within 100 days, was lower in patients with a higher AUC post-HSCT (for patients receiving a cord blood graft ≥ 20 AU x day/mL, and for patients with a bone marrow or peripheral blood stem cell graft ≥ 100 AU x day/mL) and correlated with TRM and viral reactivations. A lower risk for graft failure and acute GvHD was seen in patients with an AUC pre-HSCT of ≥ 40 AU x day/mL compared to patients with an AUC less than 40 AU x day/mL [55].

Based on these two publications, *Admiraal* et al. developed an individualized dosing regimen taken body weight, baseline lymphocytes pre-ATG and stem cell source for each patient into account. The effectiveness of this individualized dosing regimen was assessed in a cohort of 137 children receiving a cord blood graft and in a prospective, open-label, phase II clinical trial including 58 patients and 110 historical controls. Chance of successful immune recovery was significantly increased in the individualized dosing group in both studies, but no differences were seen between patients with low or high ATG exposure for severe acute GvHD (grade III-IV) and failure of the graft [54, 60].

Concluding from the above-mentioned publications, using an individualized dosing regimen for ATG could improve patient outcome. Both ATG population PK models described so far showed large interpatient variability, which could be minimized by applying TDM. However, TDM for ATG at this moment is time-consuming, expensive and the assays to measure active ATG are to our knowledge performed only at a few centers worldwide. For ATLG, both studies assessing the PK/PD mentioned differences in the pharmacological and immunological impact between ATLG and ATG [58, 59]. The next step would be to assess whether there is a relationship between ATLG drug concentrations and clinical and immunological outcome in order to determine if TDM could be useful.

Table 4. Reported associations of pharmacokinetic parameters of ATG and clinical outcomes

First author, year	N	Age, median (range)	Diagnosis	Regimen	Dose ATG	Major Findings
Call, 2009	13	10 (2-16)	AML: 4 (31%) ALL: 3 (23%) CML: 3 (23%) JCML: 2 (15%) MDS: 1 (8%)	TBI/Thio/CY	Thymoglobulin 10 mg/kg, administered as 1 mg/kg on day -4 and 3 mg/kg/day on days -3 to -1	Weight-based dosing regimen (total dose 10 mg/kg) of Thymoglobulin was effective and well tolerated by all patients. None of the patients developed grade III-IV aGvHD.
Admiraal, 2015	251	6.2 (0.2-22.7)	Malignancy: 116 (46%) IEI: 51 (20%) BMF:15 (6%) Non-malignant: 69 (27%)	RIC MAC – chemo MAC - TBI	Thymoglobulin < 9 mg/kg 4% 9-11 mg/kg 94% >11 mg/kg 2% Day start ATG -5, dose divided over 4 days	Individualized dosing of ATG could result in improved outcomes. For the CB group, AUC ≥20 AU × day/mL decreased immune reconstitution in CB, but decreased immune reconstitution was noted only if AUC ≥ 100 AU × day/mL in BM and PB. Successful immune reconstitution by day 100 was associated with increased OS. An AUC before HSCT of ≥40 AU × day/mL resulted in a lower incidence of aGvHD, cGvHD and graft failure compared with an AUC < 40 AU × day/mL.
Admiraal, 2016*	137	7.4 (0.2 – 22.7)	ALL: 22 (16%) AML: 30 (22%) Lymphoma: 4 (3%) IEI: 33 (24%) BMF: 7 (5%) Benign non-IEI (41 (30%)	Bu-Flu Bu-Flu-Clo TBI based Cy-Flu	Thymoglobulin Before 2010: 10 mg/kg Day start ATG -5, dose divided over 4 days After 2010: < 40 kg: 10 mg/kg > 40 kg: 7.5 mg/kg Day start ATG -9, dose divided over 4 days	Low ATG exposure (AUC < 16 AU*day/mL) was the best predictor for CD+ T cell recovery in CB transplant. Patients with a high AUC had a significantly lower EFS compared to low exposure or without ATG. Every 10-point increase in ATG exposure resulted in 5% lower survival probability. Patients receiving ATG had a significantly lower incidence of aGvHD (III-IV) compared with those not receiving ATG (HR, 0.27; 95% CI, 0.08-0.86; P = .027)

First author, year	N	Age, median (range)	Diagnosis	Regimen	Dose ATG	Major Findings
Oostenbrink, 2019	58: 42 Thymoglobulin 16 Grafalon	9 (1-18) 6 (1-17)	ALL: 33 (57%) AML: 25 (43%)	Chemo + TBI Chemo	Thymoglobulin: 8.7 (6.0-10.5) mg/kg Grafalon: 53 (45-60) mg/kg	Active ATG of both ATG products was cleared at different rates, more variability in the Thymoglobulin treated group. Patients treated with Grafalon had a median level of 27.9 AU/mL and with Thymoglobulin 10.6 AU/mL at day 0. Three weeks after HSCT, 15/16 Grafalon patients had an active ATG level <1 AU/mL while 17/42 Thymoglobulin patients had still active ATG levels above this threshold. For Thymoglobulin, exposure to ATG was significantly higher with 10 mg/kg compared to 6-8 mg/kg and was associated with delayed immune recovery. Occurrence of aGvHD (grade III–IV) was highest in the Thymoglobulin low dosage group.
Vogelsang, 2020	32: 22 Thymoglobulin 10 Grafalon	5.3 (0.1–17.3) 13.7 (1.5–17.2)	Non-malignant: 22 (69%) Malignant: 10 (31%)	TreoFluThio NMA TBI/VP-16	Thymoglobulin: 4.5-10 mg/kg Grafalon: 30-60 mg/kg	Grafalon and Thymoglobulin show different pharmacological and immunological impact in children. Active plasma levels for Grafalon were less variable compared to Thymoglobulin. Median active peak plasma levels were 77.9 μg/ml for Grafalon and 8.11 μg/ml for Thymoglobulin. Incidence of GvHD was similar for patients with high (above the median) or low (below the median) exposure. Immune recovery of total leucocytes and T cells was delayed in patients with high ATG exposure. No significant difference was found for overall survival.

*66 patients (48%) were included in the previous analysis of 2015. (J)CML: (juvenile) chronic myeloid leukemia, RIC: reduced intensity conditioning, MAC: myeloablative conditioning

Alemtuzumab

Besides ATG/ATLG, an alternative lymphodepleting drug that is often used as serotherapy is Alemtuzumab (Campath®). Alemtuzumab is a humanized monoclonal antibody targeting CD52, which is expressed on the surface of various hematopoietic cells. Alemtuzumab can be given subcutaneously or intravenously for in vivo depletion of immune cells, but the use of alemtuzumab for in vitro T-cell depletion, by adding alemtuzumab to the graft before infusion, has also been described [61, 62]. The total dose given in children usually varies between 0.5-1.5 mg/kg, however for some diseases (such as hemophagocytic lymphohistiocytosis (HLH)) much higher dosages are being used. The lytic level of alemtuzumab in humans is presumed to be near 0.1 to 0.16 μg/mL [63, 64]. Based on the few studies analysing alemtuzumab PK and PD in the pediatric HSCT setting (see for an overview Table 5), a difference between ATG and alemtuzumab PK is clearance, both linear and saturable, which is lower for alemtuzumab. Furthermore, the interindividual variability for alemtuzumab clearance is described to be much higher than for ATG [65].

In 2016 *Marsh* et al. reported their recommended therapeutic range of alemtuzumab at the day of transplantation of 0.2-0.4 μg/mL. They investigated the relation between alemtuzumab concentrations at day HSCT with several clinical outcome parameters in 105 (mainly) pediatric patients (median age 4.7 years (range 0.3-27.2)). A level ≤ 0.15 μg/mL at the day of transplantation was associated with a lower incidence of mixed chimerism, however also led to a higher probability of acute GvHD. For T-cell recovery at day 100 after transplantation, day 0 alemtuzumab levels ≥ 0.57 μg/mL were correlated with lower T-cell counts [63].

Bhoopalan et al. described the pharmacokinetics of alemtuzumab in 13 patients (median age 15.5 years (range 3-21)) with haploidentical HSCT. Alemtuzumab was given subcutaneous from days -14 to -11 using a BSA-based dosing, except for 5 patients who received intravenous dosing for their last two doses. Patients received a test dose of 2 mg on day -4 followed by a total dose of 45 mg/m^2 in escalating doses of 10, 15 and 20 mg/m^2 on days -13, -12 and -11. Ten of 13 patients had detectable

alemtuzumab levels at week 4 after HSCT. Median AUC was 117.1 (range 28.1-165.4) μg*day/mL. No significant correlation was found between AUC and clinical outcome parameters such as overall survival, engraftment, lymphocyte counts and GvHD [66].

The publication of *Dong* et al. described the results of a patient cohort of 29 patients with non-malignant disease undergoing HSCT (median age 6.4 years (range 0.28-21.4)), who were enrolled in 2 different studies [67, 68]. Alemtuzumab was given as a total dose of 1 mg/kg divided over days -14 to -10 in study 1 (n=17) and in study 2 as a total dose of 0.5-0.6 mg/kg. For patients in study 2 who were expected to clear alemtuzumab by day of HSCT to ≤ 0.15 μg/mL, a top up dose was calculated and given either on day -3 or day -1. The authors concluded that the currently used dosing per kilogram strategy causes uneven exposure of alemtuzumab across different weight and age cohorts. They propose an allometric- or body surface area- based starting dosing regimen in combination with TDM to achieve a recommended therapeutic range of 0.15-0.6 μg/mL on the day of transplantation, which is associated with better HSCT outcomes (less aGVHD and improved lymphocyte recovery) [69].

Altogether, based on the above-mentioned publications, it can be concluded that, as for ATG, a more individualized dosing strategy of alemtuzumab could improve HSCT outcomes of patients. Since there are only a few studies published about alemtuzumab PK and PD in pediatric patients, the need for further PK&PD analyses is urgent. Currently, an international multicentre observational trial (ARTIC study) is open for patient inclusion. The aim of this study is to evaluate current clinical practice and develop a population PK model and explore the exposure response for alemtuzumab in children with non-malignant diseases. This model will be used to provide important additional information on alemtuzumab treatments and might support the need for therapeutic drug monitoring.

Table 5. Reported associations of pharmacokinetic parameters of alemtuzumab and clinical outcomes

First author, year	N	Age, median (range)	Diagnosis	Regimen	Dose Alemtuzumab	Major Findings
Marsh, 2016	105	4.7 (0.3-27.2)	HLH: 54 (51%) BMF: 13 (12%) (S)CID: 17 (17%) CGD: 5 (5%) Metabolic: 4 (4%) SCD: 2 (2%) Other: 10 (10%)	FluMel	Distal dosing: 3/10/15/20 mg over days -22 to -19 <10 kg: 3/10/10/10 mg Intermediate dosing: 1 mg/kg over days -14 to -10 Proximal dosing: 3/10/15/20 mg or 1 mg/kg starting at day -12 or closer to HSCT	Peritransplant alemtuzumab levels have impact on the incidence of aGvHD, mixed chimerism and lymphocyte recovery. 18% developed GvHD with alemtuzumab levels ≥0.16 μg/mL, 68% in patients with levels ≤0.15 μg/mL. Mixed chimerism occurred in 21% of the patients with ≤0.15 μg/mL, in 42% with levels between 0.16 and 4.35 μg/mL and in 100% if levels were above 4.35 μg/mL. Patients with levels ≥0.57 μg/mL had lower T-cell counts at day 100. A therapeutic range at day 0 of 0.2-0.4 μg/mL is recommended.
Bhoopalan, 2020	13	15.5 (3-21)	ALL: 8 (61.5%) AML: 3 (23.1%) CML: 1 (7.7%) Therapy-related MDS: 1 (7.7%)	FluThioMelRitux	Subcutaneous n=8 Subcutaneous and intravenous n=5 Test dose of 2mg/m^2 plus total dose of 45mg/m^2 Dose given from days -14 to -11.	BSA-based dosing of alemtuzumab is feasible in pediatric haplo-transplantation patients. AUC of alemtuzumab did not have a significant relation with OS, engraftment, IR and GvHD.
Dong, 2021	29	6.4 (0.28-21.4)	HLH: 13 (45%) CGD: 2 (7%) IPEX: 2 (7%) SAA: 5 (17%) (S)CID: 3 (10%) Other: 4 (14%)	FluMel	Subcutaneous 0.5-0.6 mg/kg 1 mg/kg Dose given days -14 to -10 or -14 to -12. Top-up dose was given either on day -3 or -1.	Proposed therapeutic range of 0.15-0.6 μg/mL on the day of transplantation is associated with better HSCT outcomes (less aGVHD and improved lymphocyte recovery). To achieve this optimal level allometric or BSA-based dosing is advised. Top-up dose on day -3 for patients who, based on individualized PK estimation, will have a concentration <0.15 μg/mL on the day of transplantation is recommended.

HLH: hemophagocytic lymphohistiocytosis, (S)CID: (severe) combined immune deficiency, CGD: chronic granulomatous disease, SCD: sickle cell disease, IPEX: immunodysregulation polyendocrinopathy enteropathy X-linked syndrome, SAA: severe aplastic anemia, Ritux: rituximab

DISCUSSION

There is a certain set of criteria to determine which drugs are suitable for TDM. The drug should have a narrow therapeutic index and considerable pharmacokinetic variability between patients. Importantly, there should be a reasonable relationship between plasma concentrations and clinical effects, e.g. efficacy or toxicity. The dose cannot be easily optimized by clinical observation and last, but certainly not less important, a bioanalytical assay should be available. The main focus of this review was to assess whether there is a reasonable relationship between plasma concentrations of the most commonly used conditioning agents prior to pediatric HSCT and clinical effects. Taken all the available evidence into account, Bu fulfils all criteria for TDM at the moment which is also reflected in various study protocols and guidelines [70, 71]. Refinement of exposure targets could further improve results for specific subgroups. For Treo, there seems to be some relationship with clinical outcome, however contrasting results are reported. High exposure increases the risk of skin toxicity and, in one study, an association with mortality is seen [29, 32, 33]. A cumulative target concentration of 4800 mg*h/L is suggested in this particular study. In two other studies, no relationship was seen with EFS and OS. For now, the evidence is not convincingly enough to implement Treo TDM for all pediatric patients undergoing HSCT. For Flu, the same arguments can be made. Although a large retrospective study showed a relationship of Flu exposure and EFS, and suggested an optimal target of 15-25 mg*h/L, other studies did not find a clear relationship [36, 37, 39, 40]. More research on Flu PK/PD may provide further evidence whether Flu TDM is of added value in routine clinical practice. For ATG, almost all studies that studied clinical outcome in relation to exposure (pre- and post HSCT) are done with Thymoglobulin. Delayed immune reconstitution was seen in patients with high (post-HSCT) exposure in several studies, which means that patients could potentially benefit from individualized dosing of ATG [54, 55]. For ATLG, this still needs to be determined, but a correlation between exposure and outcome seems most likely. High alemtuzumab levels also seem to be correlated with delayed immune reconstitution, but data is still scarce, and more research is needed in order to define a therapeutic target and an optimal dosing regimen [63, 66, 67, 69].

While most of the transplant community is convinced that Bu TDM is necessary, it is still not implemented in every transplantation centre, since not every centre has a bioanalytical assay available, and logistics of shipping samples is challenging or costly. The available population PK models with identified covariates could help centres define individual Bu doses to achieve exposure within the target range without using TDM. However, given the narrow therapeutic index of Bu and the fact that there is still considerable unexplained variability in Bu PK, the use of population PK models in combination with TDM would be the best option. For ATG, the evidence is suggesting that individualized, PK-guided dosing of ATG improves patient outcome, but due to the time-consuming and expensive assays that are currently available, TDM will probably not be an option for most centres at short notice [72]. Easy to operate and less expensive bioanalytical assays are warranted to overcome this hurdle. Because the therapeutic index of ATG is likely to be much wider, using population PK models to estimate individual ATG doses would be a feasible option for most transplant centres.

When interpreting the data of all these studies, there are some limitations that must be kept in mind. Some studies only report small patient numbers, which makes it difficult to assess the possible influence of transplantation related covariates on outcome. Also, different combinations of conditioning agents and the addition of serotherapy to the regimen are major factors that influence outcome. Furthermore, due to the constantly evolving field and improvement of the transplant procedures over time, some of the regimens and procedures in the reported studies are already amended or revised/renewed.

As much as we can limit the toxicities of chemo-based conditioning, not all side effects are avoidable. Serious concerns about the long-term effects have driven the search for alternative conditioning regimens. Leukocytolytic monoclonal antibodies can provide a potential alternative to achieve myelosuppression and immunosuppression without the concomitant non-hematological toxicity of chemotherapy. Anti-CD45 monoclonal antibodies target CD45 that are selectively expressed on all leukocytes and hematopoietic progenitors. In a study with high-risk pediatric patients with different inborn errors of immunity (IEI), conditioning with anti-CD45 antibodies

in combinations with alemtuzumab, fludarabine and cyclophosphamide resulted in myeloid and lymphoid engraftment [73]. Antibodies conjugated with radionuclides, known as radioimmunotherapy, can deliver radiotherapy directly to the surface of the targeted cells. Normal tissue gets spared, making this kind of conditioning a potentially less toxic alternative [74]. Promising results with anti-CD117 monoclonal antibody as an alternative for traditional conditioning can possibly change the way we prepare patients for HSCT in the future [75]. For an increasing number of pediatric diseases, alternative treatment strategies have become available, such as gene therapy. However, while these therapies are very promising, allogeneic HSCT with the use of 'regular' conditioning regimens will still be the first (and sometimes only) option in many diseases. More research regarding the late effects of conditioning is therefore crucial to further optimize combinations and dosing of conditioning regimens.

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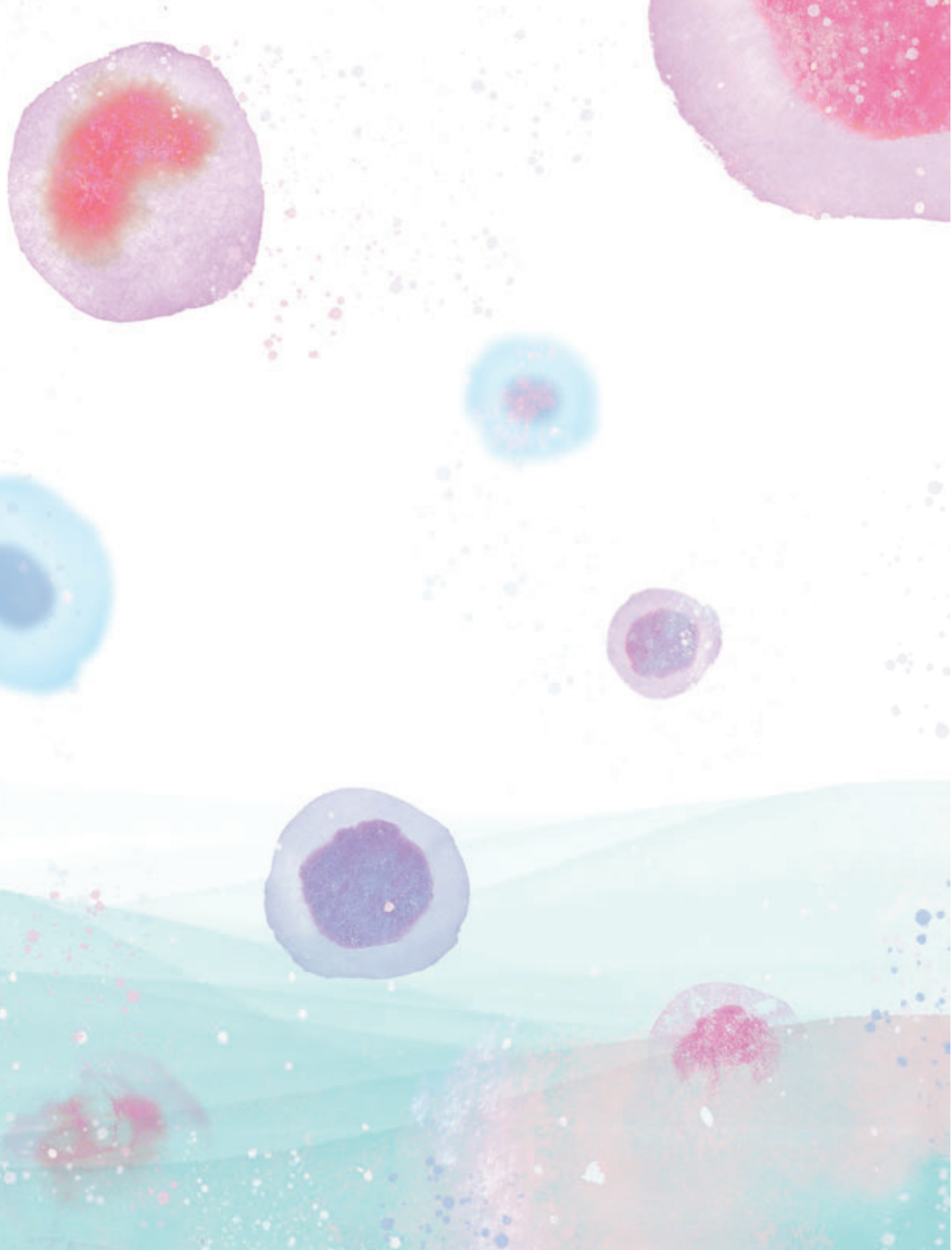

CHAPTER 08

DISCUSSION AND FUTURE PERSPECTIVES

INTRODUCTION

Over the past decade, treosulfan has gained popularity as a conditioning agent prior to pediatric allogeneic hematopoietic stem cell transplantation (HSCT) for both malignant and nonmalignant diseases because of its apparent favourable efficacy and toxicity profile. Unlike its structural analogue busulfan, little was known about its pharmacokinetic (PK) behaviour and its relationship with outcome parameters such as acute toxicity and event free or overall survival. Furthermore, knowledge of late side effects using treosulfan in the setting of HSCT, is limited. The aim of this thesis was three-fold:

1. to investigate the pharmacokinetic behaviour of treosulfan and develop a population pharmacokinetic model,
2. to investigate the relationship between treosulfan exposure, early toxicity and clinical outcome and
3. to acquire knowledge about the acute and late side effects of treosulfan.

PHARMACOKINETICS OF TREOSULFAN

Previous studies have reported great interpatient variability in treosulfan exposure in children [1-4]. However, these studies included only small numbers of patients and therefore factors influencing treosulfan pharmacokinetics could not be assessed properly. Pharmacometrics, which uses mathematical models based on physiology, pharmacology and disease for quantitative analysis of interaction between drugs and patients was used to build a population pharmacokinetic (PopPK) model of treosulfan in pediatric patients in **Chapter 2**. Potential factors influencing pharmacokinetics (covariates) were explored and a limited sampling model was developed. We found that the pharmacokinetic behavior of treosulfan in pediatric patients was best described by a two-compartmental model with first order elimination. Bodyweight with allometric scaling and a maturation function of treosulfan clearance based on postmenstrual age (PMA) were significantly associated with treosulfan clearance. Other covariates,

such as estimated glomerular function (eGFR), sex, underlying disease, conditioning regimen did not improve the model. Current dosing recommendations of treosulfan are based on body surface area (BSA) [5]. It is known that BSA-based dosing can lead to overestimation, especially in younger children. Allometric dosing, with a maturation component accounting for age, is a better way to predict drug doses [6]. Dosing of treosulfan based on bodyweight and age can be used to achieve more comparable exposures throughout the whole age range. This is also shown in a study with pediatric patients that simulated different dosing schemes, including BSA-based according to the Summary of Product Characteristics, an age-based scheme, dosing based on a PopPK model with age and weight as covariate, and a PopPK model with age, weight and creatinine [7]. Dosing according to the PopPK model with weight and age achieved better predictable treosulfan exposures across all ages, while BSA-based and age-based dosing led to higher exposures in very young children (<2 years old). The addition of creatinine did not improve target attainment. With that being said, there still is unexplained variability of ~30% in treosulfan clearance that could not be attributed to one of the explored covariates. Uncovering covariates can further optimize treosulfan (initial) dosing. Possible interesting covariates mentioned by others are blood pH and body temperature, because of the pH- and temperature-dependent conversion of treosulfan to its metabolites [8].

TREOSULFAN PHARMACOKINETICS AND THE RELATIONSHIP WITH EARLY TOXICITY AND CLINICAL OUTCOME

Building on the experience with personalized dosing of busulfan using therapeutic drug monitoring (TDM), we hypothesized that pharmacokinetic parameters of treosulfan, in particular area under the concentration-time curve (AUC), could also have a relationship with toxicity and efficacy. In **Chapter 3**, we studied the relationship between treosulfan AUC and early toxicity in a cohort of 77 pediatric patients, transplanted for nonmalignant or malignant diseases. In **Chapter 4**, we studied treosulfan exposure in relationship to long term clinical outcome (2-year event free survival, EFS), in a cohort

of 110 pediatric patients with nonmalignant diseases. The results of these studies are summarized and discussed in **Chapter 7.** Briefly, high interindividual variability was observed for day 1 treosulfan AUC. High day 1 treosulfan AUC (>1750 mg*h/L) was associated with an increased the risk of ≥ grade 2 skin toxicity. Although a relationship was found with ≥ grade 2 mucositis in the study described in **Chapter 3**, this could not be confirmed in **Chapter 4**. Only a relationship with all grade mucositis was found, probably because of the lack of patients with malignant diseases, of whom 50% experienced grade ≥2 mucositis. More importantly, no associations were found between treosulfan AUC and 2-year EFS and other outcome parameters, such as 2-year overall survival (OS), engraftment, chimerism (at 1 year) and graft-versus-host disease (GvHD). Two other studies investigated the relationship between treosulfan exposure and outcome in pediatric stem cell transplantation [7, 9]. While one study reported an association of high treosulfan exposure with transplant-related mortality [7], the other reported only a trend towards such an association, but not with EFS [9]. These differences could possibly be explained by interindividual variability in exposure between the studies, which was much higher in the two aforementioned studies [7, 9]. Taken all these results into account, a moderate exposure-toxicity relationship is seen, but this is not evident and consistent for (event free) survival. While TDM could be used to prevent skin toxicity, the use of other measures, such as preventive skin care, could also reduce the incidence of ≥ grade 2 skin complications [10, 11]. We think that the current evidence does not justify the use of TDM in routine patient care, but can be useful in specific cases and subgroups - such as infants, certain disease types or patients with comorbidities - and warrants further investigation.

ACUTE AND LATE SIDE EFFECTS OF TREOSULFAN

In general, it is noticed that treosulfan is well tolerated in pediatric patients. Common (but moderate) side effects are gastrointestinal, mucosal and skin related. Transient elevation of liver enzymes are also commonly reported [5]. Because treosulfan is relatively new in the field of HSCT it is possible that some less known acute side effects have not been observed or registered yet, possibly because of lack of awareness.

In the pediatric HSCT program of the Willem Alexander Children's Hospital, clinical observations of myalgia and arthralgia after conditioning were reported increasingly by both nurses and physicians in patients treated with treosulfan-based conditioning. In **Chapter 5** we investigated the incidence, duration, location and severity of myalgia after treosulfan-based conditioning using a natural language processing (NLP) and text mining tool to search through Electronic Health Records. In a cohort of 114 patients conditioned with treosulfan, myalgia occurred in 30% of patients. Of this group, 44% needed strong opiates and adjuvant medicines such as pregabalin, gabapentin or ketamine. Patients transplanted for sickle cell disease or beta-thalassemia had a higher risk of experiencing myalgia than patients transplanted for other underlying diseases. The cause of this higher incidence is unknown. Pre-transplant disease history, altered pain perception and genetic predisposition are factors that could be of influence and warrants further investigation. This study has provided important new knowledge about treosulfan and its adverse events and this information has led to a more standardized (early) pain management approach when patients experience myalgia after conditioning in the pediatric HSCT program of the Willem Alexanders Children's Hospital. This study also shows the huge potential of NLP and text mining tools in healthcare applications. With the increasing amount of physician- and nurse-reported information being stored in Electronic Health Records, validated text mining tools can help to extract medical information more efficient in order to assess treatment effectiveness and safety in clinical practice [12].

As a result of the growing popularity of treosulfan as a conditioning agent prior to HSCT for nonmalignant diseases, the need for information on the late effects of treosulfan is growing. More pediatric patients survive into adulthood and complications of the transplant procedure, especially endocrine complications such as gonadal dysfunction, could have a great impact on the quality of life. Only a few studies have reported on the endocrine complications of busulfan and treosulfan-based conditioning [13-16]. These studies indicate a more favourable toxicity profile for treosulfan. However, it is unknown if drug exposure influences the prevalence of endocrine complications. In **Chapter 6,** we evaluated the exposure of busulfan and

treosulfan in relation to gonadal dysfunction in pediatric patients transplanted for a nonmalignant disease in a retrospective study. In the busulfan cohort, gonadal dysfunction occurred in 63% of patients and low busulfan exposure (i.e. reduced intensity conditioning) was not associated with a reduced risk of gonadal dysfunction. In the treosulfan group, gonadal dysfunction occurred less frequently (28%) and we found no association with exposure. Future research should preferably include larger patient numbers with sufficient follow-up time, so that other covariates, such as age at HSCT and underlying condition, can also be taken into account.

FUTURE PERSPECTIVES

Finding a conditioning regimen that is efficacious, but has minimal side effects is very challenging. Significant improvements have been made to optimize conditioning regimens by using less toxic agents, less toxic combinations and dose optimization. Treosulfan has been introduced as a less toxic alternative for busulfan, now a little over 10-15 years ago. Still, knowledge about the pharmacokinetics and dynamics of treosulfan in the pediatric HSCT setting is limited, as are results on long term clinical outcome. This thesis has provided important new insights in the pharmacokinetics and dynamics of treosulfan, but are we there yet? There are still some questions that remain unanswered and can be addressed in future research.

Treosulfan exposure in specific disease types and patient groups

Our research mainly focused on nonmalignant pediatric patients. Treosulfan is also used as a conditioning agent for malignant diseases and the relationship between treosulfan exposure and clinical outcome parameters, such as relapse, have not been investigated in the pediatric setting. It is not known if the currently available data can also be applied to malignant diseases, such as acute lymphoblastic leukemia (ALL). Recently, the first results of the For Omitting Radiation Under Majority age (FORUM) study have been published; a prospective, randomized, controlled trial in which busulfan- and treosulfan-based conditioning regimens are directly compared

to a traditional total body irradiation (TBI)-based regimen in pediatric patients with ALL [17]. The randomization study was prematurely stopped when the relapse incidence in both chemotherapy arms was found to be significantly higher compared to the TBI-based arm. No difference in relapse rate was found between the busulfan- and treosulfan-based arms. However, a difference between the two chemo-based arms in the FORUM study is that a significant proportion of patients in the busulfan arm had PK analysis performed, with subsequent TDM. For treosulfan, TDM-adjusted dosing was not performed. We have conducted an add-on study in the FORUM trial focused on the PK of treosulfan and its relationship with clinical outcome. The data of this add-on study are currently being collected and the final analysis has to be awaited, but so far preliminary data do not point to a clear correlation between exposure and relapse [18]. Furthermore, identifying specific patient groups that could benefit from TDM of treosulfan should ideally be performed. Such a study requires large number of patients and can be difficult to establish. Collaboration of centers all over the world is needed to answer these questions. Currently, a study to perform a patient-level meta-analysis on treosulfan PK and outcome is being set up with centers participating worldwide, which will investigate the relationship between treosulfan drug exposure and disease type and the extent of donor chimerism post-conditioning as well [19].

Treosulfan in combination with other agents

Pharmacological research in the field of HSCT is usually focused on one agent at a time to optimize the studied drug. However, in the case of conditioning agents, these are almost never given alone, but are combined with both other chemotherapeutic agents and/or serotherapy and concomitant drugs. Together with other transplantation related covariates, varying combinations of these agents can have different effects on clinical outcome. Since PK data of more agents have become available, such as fludarabine, and the serotherapy agents anti-thymocyte globulin (ATG), anti-T lymphocyte globulin (ATLG) and alemtuzumab, an integrated approach may eventually be required to achieve optimal results regarding clinical outcome and immune reconstitution [20-26].

Clinical outcome of HSCT with treosulfan-based versus busulfan-based conditioning

With treosulfan being used more often as the backbone in the conditioning regimen, similarities and differences in outcome between treosulfan-based and busulfan-based conditioning are becoming more clear. In general, it seems that there are no major differences in overall survival (OS) between treosulfan-based and busulfan-based myeloablative conditioning. This is shown in both malignant as nonmalignant pediatric cohorts [17, 27-30]. In the FORUM study with pediatric ALL patients, both the busulfan and treosulfan arm show an 2-year overall survival of 77% [17]. In a study with thalassemia major patients, the 2-year OS rates were 92.7% and 94.7% for busulfan and treosulfan, respectively [28]. In a very recent study in patients with Wiskott-Aldrich syndrome (WAS), the OS rates at last follow up were 89.3% and 89.4% for busulfan and treosulfan, respectively [27]. Looking at other outcome parameters, such as event-free survival (EFS), relapse, treatment-related mortality (TRM), GvHD, donor chimerism and the need for secondary procedures, differences can be seen. Although no differences in EFS, relapse, TRM and GvHD were reported in the FORUM study and similar results were observed in studies performed in chronic granulomatous disease (CGD), severe combined immunodeficiency (SCID) and leukocyte adhesion deficiency (LAD) type I and II [17, 29-31], the study in WAS patients reported a higher incidence of graft failure, mixed donor chimerism and more frequently received secondary procedures (e.g. 2nd HSCT, stem cell boost or donor lymphocyte infusion) in patients receiving treosulfan-based conditioning [27]. The necessity of a 2nd HSCT was also higher for treosulfan conditioned patients with thalassemia major compared to busulfan conditioned patients [28]. It is difficult to interpret these data, because it is possible that there is some kind of bias introduced in these retrospective studies. The underlying disease and the need to use a fully myeloablative regimen can play a role. Also, administration of serotherapy and stem cell source can influence the degree of engraftment as well. In **Chapter 4** we found a higher incidence of mixed donor chimerism in patients conditioned with treosulfan and fludarabine compared to treosulfan, fludarabine and thiotepa. The addition of thiotepa might attribute to

a higher donor chimerism rate. Difficult as it is, it would be of great value to try to investigate which factors influence the level of donor chimerism in future research. Still, treosulfan-based conditioning is an excellent alternative for busulfan-based conditioning with good clinical outcome in a large variety of diseases, especially with more data becoming available regarding the favourable late effects of treosulfan.

Late effects of treosulfan

As mentioned before, research on late effects of treosulfan has become more and more important. Research should not only focus on endocrine complications, but should also include other late effects such as dental, neurocognitive, hair, ocular and pulmonary problems. This would be preferably studied in a single disease group, as a heterogeneous cohort is more difficult to analyze. However, such studies are difficult to perform and input from multiple centers is needed to gain a sufficient number of well documented patients. Different initiatives are currently being set up, for instance in RAG1-SCID within the RECOMB consortium [32].

CONCLUSION

Treosulfan has shown to be an effective and safe conditioning agent in pediatric HSCT for malignant and nonmalignant diseases. This thesis has shown that there is considerable interpatient variability in treosulfan exposure. While there is a (moderate) exposure-toxicity relationship, no relationship with clinical outcome is found which makes treosulfan (compared to busulfan) an easy to use conditioning agent without requirement of TDM in the majority of patients. The information from the increased use of treosulfan has added to the knowledge of acute and late side effects, although more research on the late effects with longer follow up is still needed and eagerly awaited.

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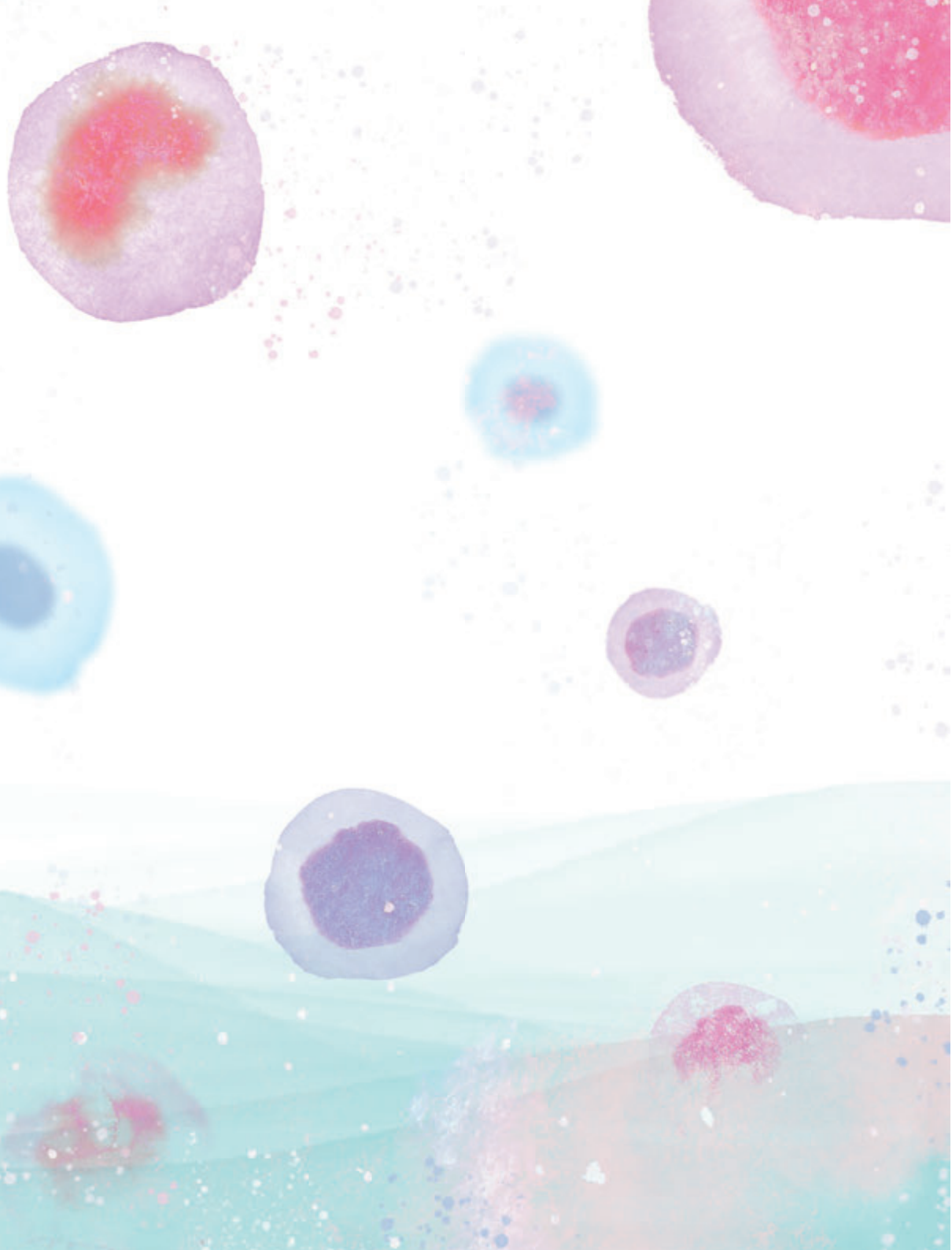

APPENDICES

ENGLISH SUMMARY

Treosulfan is an alkylating agent and is increasingly used as part of the conditioning regimen in pediatric allogeneic hematopoietic stem cell transplantation (HSCT) for malignant and nonmalignant diseases. In the last decade, treosulfan has gained popularity, because of its myeloablative and immunosuppressive properties, together with a relatively mild toxicity profile. This is advantageous, because complications related to toxicity of the conditioning regimen are still a major cause of transplant-related morbidity and mortality. From its structural analogue busulfan, we have learned that personalized dosing with the help of therapeutic drug monitoring (TDM), can reduce the risk of acute toxicity and graft failure in pediatric patients undergoing HSCT. But - in contrast to busulfan - only a few studies were performed to investigate the pharmacokinetics of treosulfan in pediatric patients. Furthermore, there are no studies that investigate the relationship with treosulfan exposure and clinical outcome. Also, treosulfan is relatively new in the field of HSCT and knowledge of acute and late side effects using treosulfan in the setting of HSCT with the currently recommended dose range (30-42 g/m^2) is limited. This thesis aims to answer questions regarding pharmacokinetics and pharmacodynamics of treosulfan in pediatric HSCT.

In **Chapter 2**, we have developed a population pharmacokinetic model of treosulfan in pediatric patients and predictive factors for pharmacokinetics such as patient- and transplant characteristics were explored. Treosulfan clearance was significantly influenced by bodyweight and age, but other factors such as underlying disease or estimated glomerular function as a measure of renal function did not. Also, we developed a limited sampling model with 3 sampling moments, which minimizes the burden of sampling.

In **Chapter 3**, the relationship between treosulfan exposure and acute toxicity is described in a multicentre pediatric cohort of 77 patients. We observed high interindividual variability in day 1 treosulfan exposure, which accurately reflects total exposure. The risk of ≥ grade 2 mucositis and skin toxicity was higher in patients with high treosulfan exposure, compared to patients with low exposure. This study

provides the first evidence that there is a possible exposure-toxicity relationship. In **Chapter 4**, we focused on investigating the relationship between treosulfan exposure and long-term clinical outcome in a pediatric cohort of 110 patients, transplanted for a nonmalignant disease. No associations were found with 2-year event-free survival and other outcomes, such as overall survival, engraftment, donor chimerism and graft-versus-host disease (GvHD). Just like in **Chapter 3**, we found a relationship with moderate to severe skin toxicity, but the relationship with moderate/severe mucositis was not as profound. Together, these results indicate a moderate exposure-toxicity relationship, but a relationship with event-free and overall survival could not be found. The clinical value of TDM could be in the prevention of skin toxicity, although implementation of preventive care guidelines could possibly reduce the incidence of cutaneous complications as well. The current evidence do not justify the use of TDM in routine patient care, but can be useful in specific cases and subgroups, such as infants, and warrants further investigation.

While treosulfan is a relatively old drug, originally registered for the palliative treatment of ovarian carcinoma in the mid-90s, only recently it was also registered as part of conditioning treatment prior to allogeneic HSCT in adult and pediatric patients. Treosulfan is given in 3 consecutive doses with a total dose up to 42 g/m^2, a different dosing scheme than in ovarian carcinoma. In the pediatric HSCT program of the Willem Alexander Children's Hospital, some patients experienced myalgia and arthralgia after conditioning with treosulfan, side effects which are not mentioned in the original Summary of Product Characteristics (SmPC). In **Chapter 5** we investigated the incidence, duration, location and severity of myalgia after treosulfan-based conditioning using a natural language processing (NLP) and text mining tool to search through Electronic Health Records and compared this with a cohort of busulfan-treated patients. In a cohort of 114 patients conditioned with treosulfan, myalgia occurred in 30% of patients. Of this group, 44% needed strong opiates and adjuvant medicines such as pregabalin, gabapentin or ketamine. Patients transplanted for sickle cell disease or beta-thalassemia had a higher incidence of myalgia than patients transplanted for other underlying diseases. It is not known why this particular

disease group is at higher risk for this side effect. This can be addressed in future research. This study has provided important new knowledge about treosulfan and its adverse events and also shows the great potential of NLP and text mining tools in health care applications.

With more pediatric patients that survive into adulthood after HSCT, the late effects of the transplant procedure become more important. Endocrine complications, such as gonadal dysfunction, could have a great impact on the quality of life. It is not known if drug exposure influences the prevalence of gonadal dysfunction. In **Chapter 6**, we evaluated the exposure of busulfan and treosulfan in relation to gonadal dysfunction in pediatric patients transplanted for a nonmalignant disease in a retrospective study. In the busulfan cohort, gonadal dysfunction occurred in 63% of patients and low (reduced intensity) busulfan exposure was not associated with a concomitant reduced risk of gonadal dysfunction. In the treosulfan group, gonadal dysfunction occurred less frequently (28%) and we found no association with exposure.

In **Chapter 7**, we have provided an overview of the available evidence for the relationship between pharmacokinetic parameters and clinical outcome or toxicities of the most commonly used conditioning and serotherapy agents in pediatric HSCT and discuss whether TDM of each agent is useful.

In **Chapter 8**, all study results are discussed with perspectives for future research. Although this thesis has provided important new insights in the pharmacokinetics and dynamics of treosulfan, future research is needed to further investigate the possible added value of treosulfan TDM in specific disease categories or patient groups. Also, integrating PK data of other conditioning and serotherapy agents can possibly further optimize results regarding clinical outcome and immune reconstitution. Furthermore, research regarding the late complications of treosulfan, such as endocrine, dental, neurocognitive, hair, ocular and pulmonary problems should be conducted as this aspect becomes increasingly important with more (very young) patients receiving HSCT with a treosulfan-based conditioning regimen.

NEDERLANDSE SAMENVATTING

Inleiding

Op dit moment is een stamceltransplantatie met bloedvormende stamcellen van een gezonde donor, een zogeheten allogene stamceltransplantatie, de enige curatieve behandeling voor bepaalde maligne en niet-maligne ziekten bij kinderen. Voordat de stamcellen kunnen worden toegediend, wordt meestal eerst chemotherapie gegeven (conditionering) om de lichaamseigen stamcellen en immuun cellen van de patiënt te vernietigen en zo de innesteling van de toegediende stamcellen in het beenmerg mogelijk te maken. Daarnaast wordt vaak ook een extra immuun onderdrukkend middel gegeven (serotherapie), om te zorgen dat het eigen immuunsysteem van de patiënt de donorcellen niet aan zal vallen wat tot afstoting zou kunnen leiden. Daarnaast wordt serotherapie gebruikt om de schadelijke reactie van het donor immuunsysteem tegen het lichaam van de patiënt, graft-vs-host ziekte (GvHD) te voorkomen. Na de stamceltransplantatie migreren de toegediende stamcellen naar het beenmerg van de patiënt om van daaruit te prolifereren en te differentiëren, wat zal resulteren in een gezond hematopoëtisch systeem.

Aanvankelijk waren de conditioningsschema's veelal erg intensief, om zoveel mogelijk maligne cellen en aangedane stamcellen te elimineren. Het nadeel hiervan was dat er dientengevolge regelmatig ernstige bijwerkingen en orgaanschade optraden vlak na het geven van de conditionering, maar ook op de lange termijn, zoals het verminderen of verdwijnen van de vruchtbaarheid. Ook bestond er een aanzienlijke kans om ten gevolge van de ernstige bijwerkingen aan de behandeling te overlijden. Deze conditioneringsschema's bestonden veelal uit een combinatie van bijvoorbeeld een hoge dosis radiotherapie en chemotherapie (bij maligniteiten), dan wel een combinatie alkylerende chemotherapeutica (een middel dat de celdeling remt, waardoor de cellen sterven), zoals busulfan en cyclofosfamide. Er wordt veel onderzoek gedaan naar het veiliger en effectiever maken van deze conditioneringsschema's. Van busulfan weten we nu bijvoorbeeld dat een gepersonaliseerde dosering met behulp van het meten en monitoren van bloedspiegels, therapeutic drug monitoring (TDM), het risico op acute

toxiciteit, afstoting van het transplantaat en terugkeer van de ziekte kan verminderen en daarmee de kans op een goede uitkomst vergroot.

In de afgelopen jaren is het middel treosulfan in toenemende mate gebruikt bij allogene stamceltransplantatie bij kinderen (en volwassenen). Dit alkylerende middel heeft aan populariteit gewonnen omdat het de eigenschap heeft om de stamcellen van de donor te elimineren, immuun onderdrukkend is, maar ook over een relatief mild toxiciteitsprofiel beschikt. Treosulfan lijkt qua chemische structuur veel op busulfan, maar wordt in het lichaam op een andere manier omgezet. Hoewel er talloze studies zijn die de farmacokinetiek (wat het lichaam doet met het geneesmiddel) van busulfan bestuderen, zijn er slechts een paar studies uitgevoerd die de farmacokinetiek van treosulfan in kinderen onderzoeken. Bovendien waren er bij het begin van dit onderzoeksproject geen studies uitgevoerd die de relatie tussen blootstelling aan treosulfan en klinische uitkomsten, zoals toxiciteit, rejectie en (ziektevrije) overleving onderzoeken. Omdat treosulfan nog maar relatief kort gebruikt wordt bij allogene stamceltransplantaties op de kinderleeftijd is de kennis van acute en late bijwerkingen in deze setting met het huidige aanbevolen dosisbereik (30-42 g/m^2) beperkt.

Doel van dit proefschrift

Dit proefschrift heeft als doel vragen te beantwoorden met betrekking tot de farmacokinetiek en farmacodynamiek (wat de gevolgen zijn van het geneesmiddel op de patiënt en zijn/haar ziekte) van treosulfan bij allogene stamceltransplantaties bij kinderen.

Specifiek zijn deze drie doelen opgesteld:

1. Het onderzoeken van het farmacokinetisch (PK) gedrag van treosulfan in kinderen en de ontwikkeling van een populatie PK model. Populatie PK modellen beschrijven de concentraties van het geneesmiddel in een bepaalde populatie en verklaren verschillen tussen individuele concentraties.
2. Het onderzoeken van de relatie tussen de blootstelling van treosulfan in het lichaam, vroege toxiciteit en klinische uitkomsten.
3. Kennis opdoen over de acute en lange termijn effecten van treosulfan.

In **hoofdstuk 2** hebben we een populatie PK model van treosulfan in kinderen ontwikkeld en is onderzocht welke patiënt- en transplantatie karakteristieken voorspellend zijn voor de farmacokinetiek. De klaring uit het lichaam van treosulfan werd significant beïnvloed door lichaamsgewicht en leeftijd, maar andere factoren zoals onderliggende ziekte of nierfunctie deden dat niet. Ook hebben we een strategie ontwikkeld waarbij we bij kinderen met bloedafnames op slechts 3 tijdsstippen nauwkeurig de blootstelling kunnen berekenen.

In **hoofdstuk 3** is de relatie tussen blootstelling aan treosulfan en acute toxiciteit beschreven in een cohort van 77 patiënten uit twee verschillende centra. We zagen een hoge variabiliteit tussen patiënten in blootstelling aan treosulfan terwijl zij een uniforme dosis hadden gekregen. Het risico op matige tot ernstige mucositis en huidtoxiciteit was hoger bij patiënten met een hoge blootstelling aan treosulfan, vergeleken met patiënten met een lage blootstelling. Deze studie levert het eerste bewijs dat er een relatie tussen blootstelling en toxiciteit op de korte termijn bestaat. In **hoofdstuk 4** hebben we ons gericht op het onderzoeken van de relatie tussen blootstelling aan treosulfan en de lange termijn klinische uitkomsten in een cohort van 110 patiënten, getransplanteerd voor een niet-maligne aandoening. Er werden geen associaties gevonden met uitkomsten zoals afstoting van het transplantaat en overleving na 2 jaar. Er werden ook geen relaties gevonden met andere uitkomstmaten, zoals de mate waarin de donorcellen zich hebben genesteld in het beenmerg van de patiënt (donor chimerisme) en GvHD. Net als in hoofdstuk 3, vonden we een relatie met matige tot ernstige huidtoxiciteit, maar de relatie met matige tot ernstige mucositis was minder sterk. Samen wijzen deze resultaten op een matige relatie tussen blootstelling en vroeg optredende toxiciteit, maar een relatie met 'event-free survival' en algehele overleving kon niet worden gevonden. Hieruit concluderen we dat de toegevoegde waarde van TDM zou kunnen liggen in de preventie van huidtoxiciteit, hoewel de implementatie van richtlijnen voor preventieve huidverzorging tijdens de behandeling mogelijk ook de incidentie van cutane complicaties zou kunnen verminderen. Op dit moment vinden we dat de huidige resultaten de toepassing van TDM in de routine patiëntenzorg niet ondersteunen, maar dat het nuttig kan zijn in specifieke gevallen en subgroepen. Voor de definitie van deze subgroepen is verder onderzoek noodzakelijk.

Hoewel treosulfan een relatief oud geneesmiddel is, oorspronkelijk geregistreerd voor de palliatieve behandeling van ovariumcarcinoom in het midden van de jaren '90, werd het pas onlangs geregistreerd als onderdeel van de conditionering voorafgaand aan allogene stamceltransplantatie bij volwassen en pediatrische patiënten. Treosulfan wordt in 3 opeenvolgende doses toegediend met een totale dosis tot 42 g/m2, een ander doseringsschema dan bij ovariumcarcinoom. In het pediatrische stamceltransplantatie programma van het Willem Alexander Kinderziekenhuis in Leiden ervoeren sommige patiënten spier- en gewrichtspijn na conditionering met treosulfan, bijwerkingen die niet worden genoemd in de oorspronkelijke wetenschappelijke bijsluiter.

In **hoofdstuk 5** onderzochten we de incidentie, duur, locatie en ernst van spierpijn na toediening van treosulfan met behulp van een natural language processing (NLP) en text mining tool om elektronische patiëntendossiers te doorzoeken en vergeleken de resultaten met een cohort van patiënten die met busulfan zijn behandeld. In een cohort van 114 patiënten die met treosulfan waren geconditioneerd, trad spierpijn op bij 30% van de patiënten. Van deze groep had 44% sterke opiaten en adjuvante geneesmiddelen zoals pregabaline, gabapentine of ketamine nodig om de bijwerking te behandelen. Patiënten die voor sikkelcelziekte of bèta-thalassemie werden getransplanteerd, hadden een hogere incidentie van spierpijn dan patiënten die voor andere onderliggende ziekten werden getransplanteerd. Het is niet bekend waarom deze specifieke ziektegroepen een hoger risico op deze bijwerking hebben. Dit kan in toekomstig onderzoek worden onderzocht. Deze studie heeft belangrijke nieuwe kennis opgeleverd over bijwerkingen van treosulfan en laat de grote mogelijkheden zien van NLP en text mining tools in toepassingen voor de gezondheidszorg.

Nu meer pediatrische patiënten na stamceltransplantatie overleven en volwassen worden, zullen de late effecten van de transplantatieprocedure steeds belangrijker worden. Endocriene complicaties, zoals gonadale dysfunctie (dysfunctie van de geslachtsorganen), kunnen een grote impact hebben op de kwaliteit van leven. Het is niet bekend of de mate van blootstelling aan geneesmiddelen tijdens de transplantatieprocedure invloed heeft op de prevalentie van gonadale dysfunctie. In

hoofdstuk 6 evalueerden wij de blootstelling aan busulfan en treosulfan in relatie tot gonadale dysfunctie bij pediatrische patiënten die getransplanteerd waren voor een niet-maligne aandoening in een retrospectieve studie. In het busulfan cohort trad gonadale dysfunctie op bij 63% van de patiënten waarbij relatief lage blootstelling aan busulfan (zogenaamde "reduced intensity" conditionering) niet geassocieerd was met een verminderd risico op gonadale dysfunctie. In de treosulfan groep kwam gonadale dysfunctie veel minder vaak voor (28%) en vonden we evenmin een verband met blootstelling.

In **hoofdstuk 7** geven we een overzicht van het beschikbare bewijs voor de relatie tussen farmacokinetische parameters en klinische uitkomsten of toxiciteit van de meest gebruikte geneesmiddelen in de conditionering en serotherapie in pediatrische allogene stamceltransplantaties en bespreken we per middel op basis van de beschikbare literatuur of TDM van toegevoegde waarde is om klinische uitkomsten te optimaliseren.

In **hoofdstuk 8** worden alle studieresultaten besproken met perspectieven voor toekomstig onderzoek. Dit proefschrift heeft belangrijke nieuwe inzichten in de farmacokinetiek en dynamiek van treosulfan heeft opgeleverd, maar toekomstig onderzoek is nodig om de mogelijke toegevoegde waarde van treosulfan TDM in specifieke ziektecategorieën of patiëntengroepen verder te onderzoeken. Ook kan de integratie van PK-gegevens van andere conditionerings- en serotherapie middelen de klinische uitkomsten en immuun reconstitutie mogelijk verder optimaliseren. Ook moet onderzoek worden verricht naar de late complicaties van treosulfan, zoals endocriene, tandheelkundige, neurocognitieve, haar-, oog- en longproblemen, aangezien dit aspect steeds belangrijker wordt naarmate meer (zeer jonge) patiënten een stamceltransplantatie ondergaan met treosulfan in het conditioneringsschema.

LIST OF PUBLICATIONS

This thesis

van der Stoep MYEC, Bertaina A, Ten Brink MH, Bredius RG, Smiers FJ, Wanders DCM, Moes DJAR, Locatelli F, Guchelaar HJ, Zwaveling J, Lankester AC. High interpatient variability of treosulfan exposure is associated with early toxicity in paediatric HSCT: a prospective multicentre study. *Br J Haematol. 2017;179(5):772-80.*

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van der Stoep MYEC, Bertaina A, Moes D, Algeri M, Bredius RGM, Smiers FJW, Berghuis D, Buddingh EP, Mohseny AB, Guchelaar HJ, Locatelli F, Zwaveling J, Lankester AC. Impact of treosulfan exposure on early and long-term clinical outcome in pediatric allogeneic HSCT recipients: a prospective multicenter study. *Transplant Cell Ther. 2022; 28(2): p.99.e1-99.e7.*

van der Stoep MYEC, Berghuis D, Bredius RGM, Buddingh EP, Mohseny AB, Smiers FJW, Guchelaar HJ, Lankester AC, Zwaveling J. Treosulfan-induced myalgia in pediatric hematopoietic stem cell transplantation identified by an electronic health record text mining tool. *Sci Rep. 2021;11(1):19084.*

van der Stoep MYEC, Oostenbrink LVE, Bredius RGM, Moes DJAR, Guchelaar HJ, Zwaveling J, Lankester AC. Therapeutic Drug Monitoring of Conditioning Agents in Pediatric Allogeneic Stem Cell Transplantation; Where do We Stand? *Front Pharmacol. 2022; 13: 826004.*

van der Stoep MYEC, Bense JE, de Kloet LC, von Asmuth EGJ, de Pagter APJ, Hannema SE, Guchelaar HJ, Zwaveling J, Lankester AC. Effect of busulfan and treosulfan on gonadal function after allogeneic stem cell transplantation in children and adolescents with nonmalignant diseases is not exposure-dependent. *Submitted.*

Other publications

Jacobse J, Mensink H, **van der Stoep-Yap MYEC**, Kollen WJW, Bresters D, Bredius RGM. Long-term aprepitant for nausea and vomiting associated with gastroparesis in hematopoietic stem cell transplantation. *Bone Marrow Transplant. 2018; 53(10):1372-1374*

De Kloet LC, Bense JE, **van der Stoep MYEC**, Louwerens M, von Asmuth EGJ, Lankester AC, de Pagter APJ, Hannema SE. Late endocrine effects after hematopoietic stem cell transplantation in children with nonmalignant diseases. *Bone Marrow Transplant. 2022 Jul 15. doi: 10.1038/s41409-022-01755-x. Online ahead of print*

PORTFOLIO

Presentations			
2016	Pharmacokinetics of treosulfan in children undergoing hematopoietic stem cell transplantation	Oral presentation	EBMT Inborn Errors Working Party Meeting (Ghent, Belgium)
2017	Treosulfan-based conditioning in pediatric hematopoietic stem cell transplantation: a prospective study on treosulfan exposure and clinical outcome	Oral presentation	EBMT Annual Meeting (Marseille, France)
2018	High treosulfan exposure is associated with early toxicity in pediatric hematopoietic stem cell transplantation: a prospective multicenter study	Oral presentation	BMT Tandem Meetings (Salt Lake City, USA)
2019	Population pharmacokinetics of treosulfan in pediatric patients undergoing hematopoietic stem cell transplantation	Poster presentation	EBMT Annual Meeting (Frankfurt, Germany)
2020	Treosulfan exposure in paediatric hematopoietic stem cell transplantation is associated with early toxicity but not with event free survival	Oral presentation (Presidential Symposium)	EBMT Virtual Meeting
2020	Treosulfan-induced myalgia in pediatric hematopoietic stem cell transplantation	Poster presentation	EBMT Virtual Meeting
2021	Identification of treosulfan-induced myalgia in pediatric hematopoietic stem cell transplantation using an electronic health record text mining tool	Poster Presentation	ASCPT Annual Meeting 2021
2021	Identification of treosulfan-induced myalgia in pediatric hematopoietic stem cell transplantation using an electronic health record text mining tool	Poster presentation	TCT Annual Meeting
2021	Individualized treatment in (pediatric) SCT: Pharmacogenetics and therapeutic drug monitoring	Oral presentation	EBMT Virtual Meeting
2022	Effect of busulfan and treosulfan exposure on gonadal function after allogeneic stem cell transplantation in children and adolescents with non-malignant diseases	Oral presentation	EBMT Inborn Errors Working Party Meeting (Paris, France)

Courses and lectures	
2016	Basiscursus regelgeving en Organisatie Klinisch onderzoek (BROK), renewed in 2020
2016	Boerhaave Cursus Klinische Onderwijskunde
2017	PhD Introductory course
2017	Teach The Teachers-Plus, Feedback en coachen op competenties
2017	EBMT Statistics Course 5th edition
2018	Basic Methods and Reasoning in Biostatistics
2020	IEWP-EBMT Midterm Focus Meeting on Non-Malignant Diseases
2021	Teach the Teachers plus: de leerzame overdracht

Symposia and congresses (attendance)		
2016	42nd EBMT Annual Meeting	Valencia, Spain
2016	EBMT Inborn Errors Working Party Meeting	Ghent, Belgium
2017	43rd EBMT Annual Meeting	Marseille, France
2018	BMT Tandem Meetings (ASBMT/CIBMTR)	Salt Lake City, USA
2019	45th EBMT Annual Meeting	Frankfurt, Germany
2020	46th EBMT Annual Meeting	Virtual
2021	47th EBMT Annual Meeting	Virtual
2021	ASCPT Annual Meeting	Virtual
2021	TCT Annual Meeting (ASBMT/CIBMTR)	Virtual
2022	48th EBMT Annual Meeting	Virtual
2022	EBMT Inborn Errors Working Party Meeting	Paris, France

Teaching activities		
2015-2018	Practical lecture dermatology	BSc Medicine
2015-2018	Lecture Practical Pharmacotherapy	BSc Medicine
2015-2018	Practical lecture Pharmacokinetics	BSC Medicine
2015-2022	Lectures on a clinical topic	Hospital Pharmacists
2016	Practical tutor pharmaceutical compounding	BSc Pharmacy
2016 -2017	Supervision MSc Thesis	MSc Pharmacy
2017	Practical lectures ADME pregnancy and lactation / premature neonates to adolescents	MSc Pharmacy
2017 -2018	Supervision pharmacy interns	MSc Pharmacy
2021	Practical lecture Supportive Care	MSc Pharmacy

CURRICULUM VITAE

Eileen van der Stoep, *née* Yap, was born on April 12th, 1987 in Dordrecht, the Netherlands. After she finished secondary school at the Johan de Witt Gymnasium in Dordrecht in 2005, she studied Pharmacy at Utrecht University. In 2011, she obtained her Master's degree and started working as a Medical Information Officer at Roche Pharmaceuticals in Woerden. In 2012, she made a switch to the department of Clinical Pharmacy and Toxicology of Leiden University Medical Center (LUMC). There she worked as a pharmacist under the supervision of dr. I.M. Teepe-Twiss and dr. K.J.M. Schimmel. In January 2014, she started her four-year hospital pharmacy residency at the LUMC in collaboration with Apotheek Haagse Ziekenhuizen in The Hague (supervisors dr. K.J.M. Schimmel and dr. L.E. Visser). During her residency, she became interested in pediatric stem cell transplantation and in October 2015, she started her PhD project studying the pharmacokinetics and dynamics of treosulfan in pediatric allogeneic stem cell transplantation. This PhD project was a collaboration between the department of Pediatrics (supervisor prof. dr. A.C. Lankester) and the department of Clinical Pharmacology and Toxicology (supervisors prof. dr. H.J. Guchelaar and dr. J. Zwaveling). In 2018, she finished her residency and started working as a hospital pharmacist in the LUMC. As of 2021, she works both at the department of Clinical Pharmacy and Toxicology and the Center for Cell and Gene therapy. She specializes in the development and manufacturing of cell and gene therapies, chemical synthesized products and radiopharmaceuticals.

DANKWOORD

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