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Impact of Treosulfan Exposure on Early and Long-Term Clinical Outcomes in Pediatric Allogeneic Hematopoietic Stem Cell Transplantation Recipients: A Prospective Multicenter Study

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A B S T R A C T

Treosulfan-based conditioning has gained popularity in pediatric allogeneic hematopoietic stem cell transplantation (HSCT) because of its presumed favorable efficacy and toxicity profile. Treosulfan is used in standardized dosing regimens based on body surface area. The relationships between systemic treosulfan exposure and early and long-term clinical outcomes in pediatric patients undergoing allogeneic HSCT for nonmalignant diseases remain unclear. In this a multicenter, prospective observational study, we assessed the association between treosulfan exposure and early and, in particular, long-term clinical outcomes. Our study cohort comprised 110 pediatric patients with nonmalignant diseases who underwent HSCT between 2011 and 2019 in Leiden, The Netherlands and Rome, Italy. Blood samples were collected, and treosulfan area under the receiver operating characteristic curve ($AUC_{0-\infty}$) was estimated as a measure of exposure. Cox proportional hazard survival analyses were performed to assess the relationships between treosulfan exposure and overall survival (OS) and event-free survival (EFS). The predictive value of systemic treosulfan exposure for the occurrence of toxicity within 28 days was evaluated using a multivariable logistic regression analysis. In the overall cohort, OS and EFS at 2 years were 89.0% and 75.3%, respectively, with an excellent OS of 97% in children age <2 years. The occurrence of grade II-IV acute graft-versus-host disease, 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 = .02) and all-grade mucositis (OR, 4.43; 95% CI, 1.43-15.50; P = .02), but not grade ≥2 mucositis (OR, 1.51; 95% CI, 0.52 to 4.58; P = .46) was related to high treosulfan exposure (>1750 mg*h/L). Our study demonstrates that standardized treosulfan-based conditioning results in a favorable OS and EFS in infants and children with nonmalignant diseases, independent of interindividual variation in treosulfan exposure. These outcomes can be achieved without the need for therapeutic drug monitoring, 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 to minimize early and long-term toxicity without compromising efficacy.

INTRODUCTION

Over the past decade, treosulfan is being increasingly used as part of conditioning regimens in pediatric allogeneic hematopoietic stem cell transplantation (HSCT) for both malignant and nonmalignant diseases [1–4]. Treosulfan (Trecondi), a prodrug and water-soluble alkylating agent, is nonenzymatically,

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pH-dependently converted into a monoepoxide derivative and a diepoxide derivative, which are thought to cause DNA alkylation [5,6]. Treosulfan has gained popularity because of its myeloablative and immunoablative properties, along with an apparently favorable toxicity profile. This makes treosulfan an interesting choice as the backbone of conditioning regimens, particularly in patients with nonmalignant diseases.

In recent years, pharmacologic studies have provided evidence supporting therapeutic drug monitoring as an important tool to optimize the efficacy and limit the toxicity of chemotherapeutic agents, especially in pediatric patients. The wide interindividual variation in 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 outcomes for antithymocyte globulin, alemtuzumab, and fludarabine [9–12]. Treosulfan is used in standardized dosing regimens in both children and adults, based mainly on body surface area. A retrospective pediatric study found no correlation between total dose and clinical outcome [13]; however, various single-center and multicenter studies have reported a wide interindividual variability in treosulfan exposure in patients [14–18]. To date, only 3 studies, including a study from our group, have analyzed the relationships among treosulfan exposure, treatment-related toxicity, and clinical outcome [19–21]. These studies showed associations among treosulfan exposure, toxicity, and survival, although the results were inconsistent. We previously reported the pharmacokinetic behavior of treosulfan and its relationship with early toxicity in a cohort of pediatric patients who underwent HSCT for malignant and nonmalignant diseases. In the present study, the largest multicenter prospective observational study in pediatric patients with nonmalignant diseases reported to date, we assessed the association between treosulfan exposure and early, in particular long-term, clinical outcomes.

METHODS

Study Design and Patients

This prospective observational multicenter study was conducted between June 2011 and January 2019. Pediatric patients who received conditioning with treosulfan before their first allogeneic HSCT for a nonmalignant disease in the Willem-Alexander Children's Hospital/Leiden University Medical Center, Leiden, The Netherlands (LUMC; n = 69) and the Children's Hospital Bambino Gesù (OPBG), Rome, Italy (n = 41) were included in this study. The LUMC Institutional Ethics Committee approved the study protocol (P12.267), which was subsequently approved at OPBG. Written informed consent for participation in the study was obtained from the parents or legal guardians, as well as consent from patients age ≥12 years according to the Declaration of Helsinki (last amended in 2013, Fortaleza, Brazil). The short-term outcomes of 61 patients in the present cohort have been reported previously in an earlier study of a more heterogenous cohort [20]. In the present study, 49 new patients were added, resulting in our large cohort comprised exclusively of patients with nonmalignant diseases.

Procedures

Patients underwent HSCT according to institutional protocols and in line with the European Society for Blood and Marrow Transplantation Inborn Errors Working Party recommendations. Patients age >1 year received treosulfan at a total dose of 42 g/m^2, administered over 3 consecutive days (14 g/m^2 per day). Children age <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 to 160 mg/m^2, day -6 to day -2), with or without thiotepa (total dose 8 to 10 mg/kg, day -6). Serotherapy consisted of anti-T lymphocyte globulin, antithymocyte globulin, or alemtuzumab. In patients with a mismatched related donor, peripheral blood stem cell grafts were processed by either CD34$^+$ selection or selective elimination of $\alpha\beta^+$ T and CD19$^+$ B cells [22]. Pharmacologic 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 with cord blood transplants from day +8 onward. Both transplantation units are Joint Accreditation Committee ISCT-Europe-accredited, and supportive care was provided according to institutional guidelines.

Pharmacokinetics of Treosulfan

Blood sample collection was done as described previously [17,20]. Because of the low intraindividual variability of treosulfan pharmacokinetics, blood samples were collected only on day 1 as a good representation of total exposure, as demonstrated previously [20,23].

Treosulfan concentrations were measured with 2 different assays. The first part was measured in serum with a high-performance liquid chromatography-ultraviolet spectroscopy method as described previously [17,20]. The second part was measured with a validated liquid chromatography with tandem mass spectrometry assay developed and validated according to European Medicines Agency 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 thus it was not necessary to reanalyze all samples with one method. Subsequently, the patients who were included after this validation were measured with the new liquid chromatography-tandem mass spectrometry method. Details of sample preparation, quantification, and cross-validation are provided in Supplementary File S1.

A previously developed treosulfan pharmacokinetic model was used to estimate treosulfan area under the receiver operating characteristic curve ($AUC_{0-\infty}$) as a measure of exposure using the post hoc 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. OS was defined as survival from HSCT to last follow-up with death considered as the sole event. Engraftment was defined as the first of 3 days with a neutrophil count $\geq 0.5 \times 10^9$/L. Primary graft failure was defined as alive on day +28 with a neutrophil count $< 0.5 \times 10^9$/L. Secondary graft failure was defined as loss of previously functioning graft resulting in cytopenia involving at least 2 lineages. For hemoglobinopathies, this is recurrence of transfusion dependency. aGVHD 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 post-HSCT were used in this analysis. When chimerism was determined in both granulocytes and mononuclear cells, the mean percentage was used in the final analysis. Mixed chimerism was defined as a donor chimerism <90%. Early toxicity endpoints evaluated until day +28 post-HSCT included mucosal, skin, hepatic, and neurologic toxicity assessed according to the Common Terminology Criteria for Adverse Events and the criteria of Bearman et al. [27]. The relationships 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 relationships between treosulfan exposure and OS and EFS. The predictive value of systemic treosulfan exposure for the occurrence of toxicity within 28 days was evaluated using a multivariable logistic regression analysis. $AUC_{0-\infty}$ was tested as discrete variable, considering 3 exposure groups based on tertiles: low (<1350 mg*h/L: first tertile), medium (1350 to 1750 mg*h/L: second tertile), and high (>1750 mg*h/L: third tertile). Age was tested as 2 groups (<2 years and ≥2 years). This age cutoff point was used because children age <2 years have immature renal and metabolic drug elimination pathways, which could influence the pharmacokinetics of treosulfan [28]. All statistical considerations are described in detail in Supplementary File S2. All P values were 2-tailed, and considered significant at $P < .05$. Statistical analyses were performed with R version 4.0.0 and R studio version 1.2.5042 with the cmprsk, survival, car, and rms packages (R Foundation for Statistical Computing, Vienna, Austria).

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 to 97 months). Clinical and demographic characteristics are detailed in Table 1. The cohort included 71 males and 39 females and had a median age at HSCT of 5.2 years (range, 0.2 to 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 (TFT).

Table 1
Patient Characteristics (N = 110)

Characteristic	Value
Age, yr, median (range)	5.2 (0.2-18.8)
Weight, kg, median (range)	18 (3.8-75.0)
Sex, male/female, n	71/39
Diagnosis for HSCT, n (%)	
Inborn errors of immunity	38 (35)
Hemoglobinopathies	55 (50)
Bone marrow failure	17 (15)
Donor type, n (%)	
MSD	32 (30)
MUD (≥9/10)	50 (45)
MMFD (haplo)	28 (25)
Stem cell source, n (%)	
Bone marrow	73 (66)
Peripheral blood	
T cell replete	5 (5)
TCR $\alpha\beta$/CD19 depletion	19 (17)
CD34 enrichment	3 (3)
Cord blood	10 (9)
Conditioning, n (%)	
TFT	77 (68)
TF	37 (32)
Treosulfan dose, n (%)	
14 g/m^2	92 (84)
10 g/m^2	18 (16)
Treosulfan pharmacokinetics, median (IQR)	
$AUC_{0-\infty}$, mg*h/L (10 g/m^2)	1776 (1129-1977)
$AUC_{0-\infty}$, mg*h/L (14 g/m^2)	1562 (1140-1860)
Serotherapy, n (%)	
Yes	
ATG	55 (50)
ATLG	35 (32)
Alemtuzumab	12 (11)
No	8 (7)
Pharmacologic GVHD prophylaxis, n (%)	
CsA	6 (6)
CsA/MTX	61 (55)
CsA/prednisolone	5 (4)
Other	4 (4)
None	22 (20)
PTCy/CsA/MMF	12 (11)

MSD, matched sibling donor; MMFD, mismatched family donor; MUD, matched unrelated donor; ATG (Thymoglobulin), antithymocyte globulin, ATLG (Grafalon), anti-T lymphocyte globulin; CsA, cyclosporine A; MTX, methotrexate; PTCy, post-transplantation cyclophosphamide; MMF, mycophenolate mofetil.

Treosulfan Pharmacokinetics

Eighteen patients age <1 year received a treosulfan dose of 10 g/m^2, and 92 patients age ≥1 year received a dose of 14 g/m^2 on 3 consecutive days. The median day 1 treosulfan $AUC_{0-\infty}$ was 1776 (IQR, 1129 to 1977) mg*h/L in patients receiving 10 g/m^2 and 1562 (IQR, 1140 to 1860) mg*h/L in patients receiving 14 g/m^2, with large interindividual differences. Treosulfan clearance was lower in younger patients (Figure 1). The median age at transplantation was significantly lower in the IEI group compared with the HBP and BMF groups (1.5 years versus 8.5 years versus 7.2 years; $P < .001$), and thus treosulfan clearance also was significantly lower in the IEI group ($P < .001$). The TF group was significantly younger than the TFT group (median, 3.6 years versus 7.6 years; $P = .011$), resulting in corresponding higher treosulfan $AUC_{0-\infty}$ in the TF group (median, 1800 mg*h/L versus 1443 mg*h/L; $P < .001$).

Treosulfan Exposure and Clinical Outcome

Engraftment and chimerism

The cumulative incidence of engraftment was 97.1% (95% confidence interval [CI], 93.5% to 100.0%), with a median time to neutrophil engraftment of 20 days (11 to 43 days) and of platelet engraftment of 24 days (8 to 94 days). Three patients died before engraftment (on days 0, +11, and +17), 7 patients experienced primary graft failure (3 in the HBP group, 2 in the IEI group, and 2 in the BMF group). The mean $AUC_{0-\infty}$ in patients with primary graft failure and patients with successful engraftment showed no significant difference (1310 mg*h/L versus 1586 mg*h/L; $P = .20$). Three of the patients with primary graft failure died subsequently from transplantation-related complications. Four patients underwent a second transplantation; 3 patients successfully engrafted, but 1 patient rejected again and required autologous reinfusion. Eight patients, all with hemoglobinopathy as the underlying disease (14.5% of the HBP group [n = 55]), experienced secondary graft failure, 6 within 6 months, 1 at 2 years, and 1 after 5 years. Four patients underwent a subsequent transplantation, of whom 2 successfully engrafted and 2 rejected again. The other 4 patients either did not undergo a second transplantation or were scheduled for a new transplantation. More detailed information is provided in Supplementary File S3. The mean $AUC_{0-\infty}$ was 1699 mg*h/L for the patients who experienced secondary graft failure versus 1558 mg*h/L for those without secondary graft failure ($P = .31$).

Eighty-nine patients (81%) were evaluable for 1-year chimerism. Fifty-nine patients (66%) achieved ≥90% donor chimerism, 14 (16%) achieved 50% to 90% chimerism, and 16 (18%) had <50% chimerism. Treosulfan $AUC_{0-\infty}$ was not correlated with either donor chimerism at 1 year in whole blood ($P = .87$) or granulocyte chimerism in a subgroup (n = 53) in which these data were available. In contrast, the use of TF conditioning (odds ratio [OR], 4.96; 95% CI, 1.50 to 18.18; $P = .01$) and age <2 years (OR, 7.69; 95% CI, 2.00 to 35.82; $P = .005$) were significantly correlated with mixed chimerism at 1 year.

GVHD

The cumulative incidence of grade II-IV aGVHD was 12.4% (95% CI, 7.4% to 20.7%) and that of grade III-IV aGVHD was 5.1% (95% CI, 2.2% to 12.0%). Eight patients had grade II aGVHD (7%), 4 patients had grade III (3.6%), and 1 patient had grade IV (0.9%). The cumulative incidence of grade II-IV aGVHD was 8.8% (95% CI, 2.9% to 26.5%) in the TF group and 14.0% (95% CI, 7.8% to 25.0%) in the TFT group ($P = .36$). No relationship was found between treosulfan $AUC_{0-\infty}$ and the occurrence of aGVHD ($P = .42$). cGVHD was reported in 6 patients (cumulative incidence, 5.5%; 95% CI, 2.5% to 11.9%), of whom 3 had extensive cGVHD, including 2 with bronchiolitis obliterans. Treosulfan $AUC_{0-\infty}$ was not a significant risk factor for cGVHD ($P = .32$).

EFS and OS

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

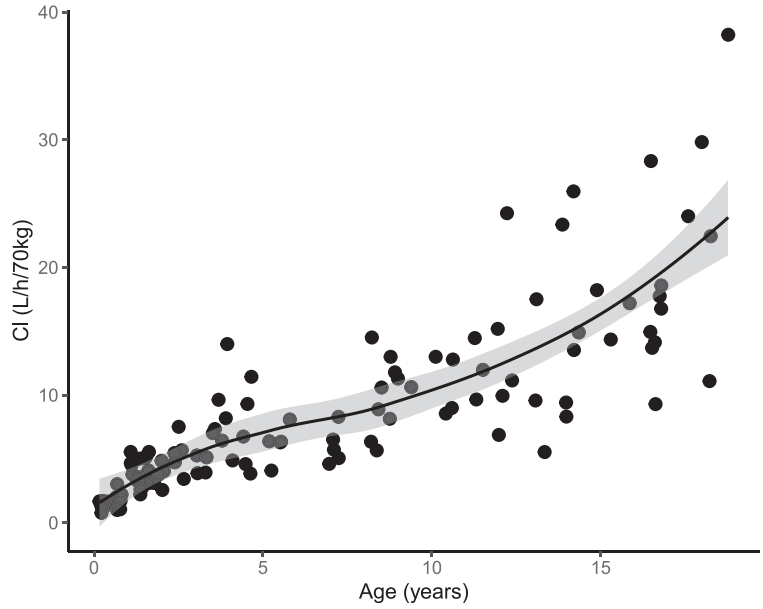

Figure 1. Treosulfan clearance versus age. Each dot represents the clearance of treosulfan (L/h/70 kg) of a patient plotted against age.

Multivariable Cox regression analysis demonstrated that treosulfan exposure was not correlated with 2 year OS (HR, 1.09; 95% CI, 0.22 to 5.46; P = .92 for treosulfan exposure >1750 mg*h/L) (Table 2; Figure 2). Underlying disease was a significant predictor for OS with the most favorable outcome for HBP (HR, 0.13; 95% CI, 0.03 to 0.64; P = .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).

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 the IEI group, 18% ((n = 3) for the BMF group, and 35% (n = 19) for the HBP group. In multivariable analysis, high treosulfan exposure (>1750 mg*h/L) (OR, 4.43; 95% CI 1.43 to 15.50; P = .01) and age >2 years (OR, 5.69; 95% CI, 1.90 to 19.44; P = .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 to 0.57; P = .01) than IEI and HBP. However, mucositis grade ≥2, which is clinically more relevant, was not significantly correlated with high treosulfan exposure (OR, 1.51; 95% CI, 0.52 to 4.58; P = .46) (Table 3).

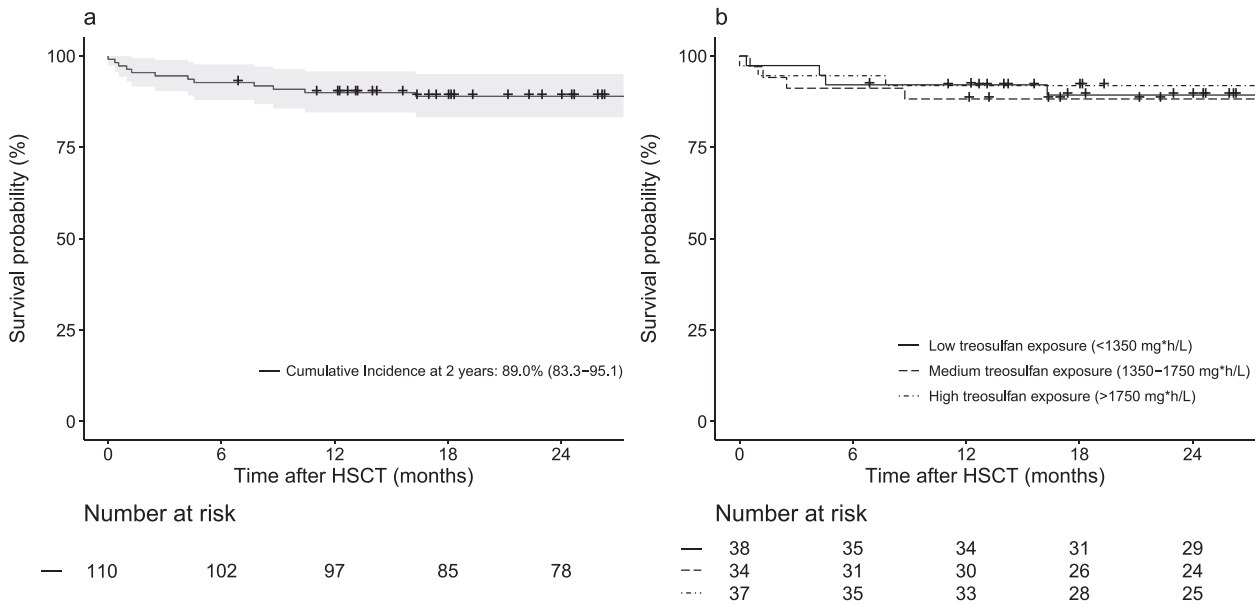

Figure 2. Kaplan-Meier plots of OS (A) and OS stratified by treosulfan exposure: low (<1350 mg*h/L; solid line), medium (1350-1750 mg*h/L; dashed line) (P = .36), or high (>1750 mg*h/L; dotted-dashed line) (P = .92) (B).

Table 2
Multivariable Analysis of EFS and OS

	EFS at 2 yr		OS at 2 yr	
Covariate	HR (95% CI)	*P* Value	HR (95% CI)	*P* Value
Treosulfan $AUC_{0-\infty}$, mg*h/L				
Low (<1350)	1.00 (ref)		1.00 (ref)	
Medium (1350-1750)	1.67 (0.65-4.31)	.29	2.08 (0.44-9.96)	.36
High (>1750)	1.03 (0.37-2.89)	.95	1.09 (0.22-5.46)	.92
Age				
<2 yr	1.00 (ref)		1.00 (ref)	
≥2 yr	0.83 (0.32-2.18)	.71	4.81 (0.92-25.28)	.06
Conditioning regimen				
TF	1.00 (ref)		1.00 (ref)	
TFT	1.17 (0.46-2.97)	.74	1.77 (0.38-8.30)	.47
Donor type				
MSD	1.00 (ref)		1.00 (ref)	
MUD (≥ 9/10)	1.58 (0.59-4.26)	.36	1.51 (0.27-8.54)	.64
MMFD (haploidentical)	2.65 (0.92-7.69)	.07	3.24 (0.61-17.34)	.17
Underlying disease				
Inborn errors of immunity	1.00 (ref)		1.00 (ref)	
Bone marrow failure	1.09 (0.28-4.33)	.90	0.22 (0.04-1.29)	.09
Hemoglobinopathies	1.36 (0.44-4.21)	.60	0.13 (0.03-0.64)	.01

Adjusted multivariable analyses were done using a Cox proportional hazards model.

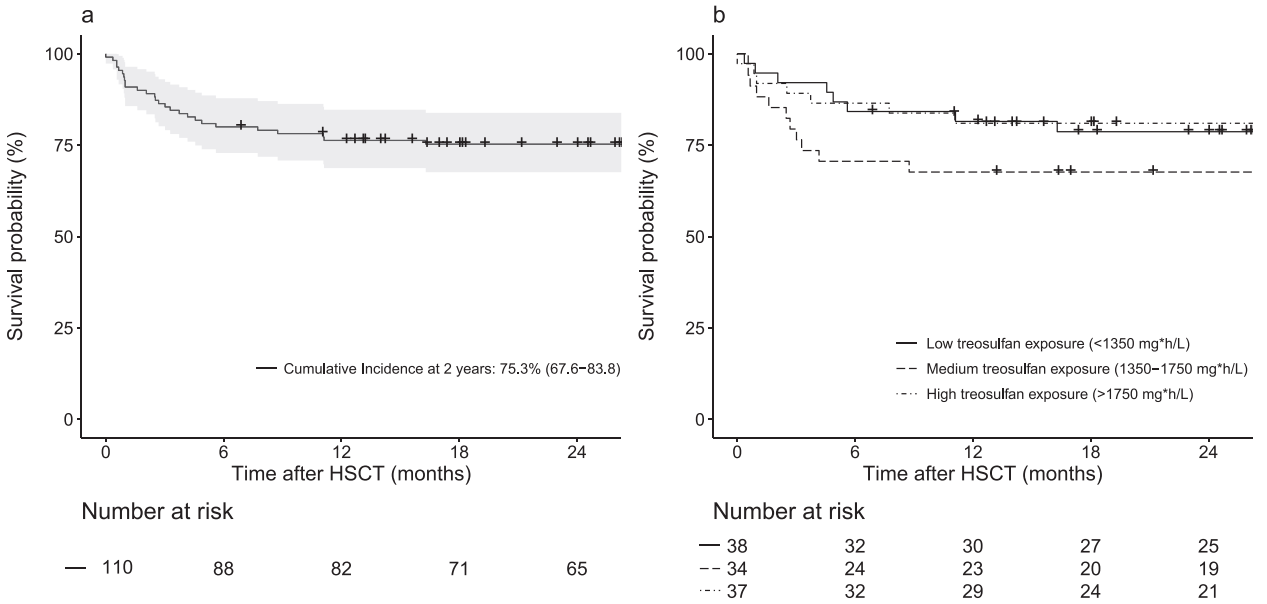

Figure 3. Kaplan-Meier plots of EFS (A) and EFS stratified by treosulfan exposure: low (<1350 mg*h/L; solid line), medium (1350-1750 mg*h/L; dashed line) (*P* = .29), or high (>1750 mg*h/L; dotted-dashed line) (*P* = .95) (B).

Moderate to severe skin toxicity (grade ≥2) occurred in 31% of patients, with high treosulfan exposure (>1750 mg*h/L) as a risk factor (OR, 3.97; 95% CI, 1.26 to 13.68; *P* = .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 to 6.06; *P* = .29). Grade ≥2 hepatic and neurologic toxicity occurred in 33% and 6% of patients, respectively, and was not correlated with treosulfan exposure (*P* = .67 and .60, respectively), age, or conditioning regimen.

DISCUSSION

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

In our cohort, 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 an increase in body weight. 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, because age also

Table 3
Early Regimen-Related Toxicity

	Mucositis, All Grades		Mucositis Grade ≥2		Skin Toxicity Grade ≥2	
Covariate	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value	OR (95% CI)	*P* Value
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)	.38	0.62 (0.20-1.88)	.40	1.61 (0.53-4.99)	.40
High (>1750)	4.43 (1.43-15.50)	.02	1.51 (0.52-4.58)	.46	3.97 (1.26-13.68)	.02
Age						
<2 yr	1.00 (ref)		1.00 (ref)		1.00 (ref)	
≥ 2 yr	5.69 (1.90-19.45)	.003	4.02 (1.30-14.16)	.02	2.71 (0.86-9.55)	.10
Conditioning regimen						
TF	1.00 (ref)		1.00 (ref)		1.00 (ref)	
TFT	2.13 (0.74-6.46)	.17	1.30 (0.44-4.00)	.64	1.85 (0.61-6.06)	.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)	.01	0.22 (0.04-0.99)	.06	1.17 (0.26-5.20)	.84
Hemoglobinopathies	0.66 (0.20-2.05)	.48	0.59 (0.18-1.85)	.37	1.66 (0.50-5.93)	.41

Adjusted multivariable analyses were done using logistic regression.

differed significantly between these groups (the TF group being younger), this is the most probable explanation for the observed difference in $AUC_{0-\infty}$.

An important observation is the lack of correlation between treosulfan exposure and the level of donor chimerism at 1 year but a positive correlation between conditioning regimen (i.e., TF versus TFT) and age. Chiesa et al. [19] reported a trend toward an association between low-level (≤20%) myeloid chimerism and low treosulfan $AUC_{0-\infty}$ in IEI patients treated with TF, but only in univariable analysis. We found a greater risk of mixed donor chimerism (<90%) in the TF group compared with the TFT group; however, the risk was independent of treosulfan exposure. This information could be of value when deciding between these 2 regimens in diseases in which 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, although we note that the impact of adding thiotepa to TF on long-term toxicity, especially fertility, is currently unresolved.

We have demonstrated 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 also may 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 likely made only a minor contribution to 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, confirming our observation. Although skin toxicity occurs frequently, preventive measures can help reduce the incidence of cutaneous complications. Preventive care guidelines for thiotepa-induced skin toxicity, such as those suggested by Van Schandevyl and Bauters [29], also could be implemented for treosulfan.

Interestingly, although we previously observed a relationship between high treosulfan exposure and the risk of grade ≥2 mucositis in a smaller and mixed cohort [20], in the present study of patients with nonmalignant diseases exclusively, this correlation was observed only for all-grade mucositis, which is clinically less relevant. This difference is likely due to the lack of patients with malignant diseases, of whom 50% experienced grade ≥2 mucositis. Our findings are in accordance with those of Chiesa et al. [19], 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.

In our cohort, the 2-year OS was 89.0%, similar to that in other reports of patients with nonmalignant diseases treated with treosulfan-based conditioning [1,4,13,19,21]. Remarkably, OS of infants age <2 years was 97%, emphasizing the excellent efficacy and safety profile of treosulfan-based regimens in this vulnerable category of patients. In both the TF and TFT groups, 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 to a daily exposure >2000 mg*h/L) was associated with higher transplantation-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 and 4882 mg*h/L, and Mohanan et al. [21] reported $AUC_{0-\infty}$ values of 129 to 4267 mg*h/L. Although our patients were treated with similar dosing regimens, their $AUC_{0-\infty}$ values ranged between 366 and 3368 mg*h/L and 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 (eg, comorbidities) that could be cofactors explaining the unfavorable outcome.

The EFS rate in our study was very favorable, with 75.3% at 2 years post-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 patients with thalassemia treated with the same TFT regimen. Our

combined results of >200 patients with nonmalignant diseases demonstrate that with current dosage 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, infertility) in children treated with treosulfan-based conditioning has yet to be established.

In recent years, clear relationships between busulfan exposure and clinical outcome and toxicity have become evident, leading to the establishment of 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 given the relatively mild toxicity profile of treosulfan, the added value and clinical relevance of introducing individualized dosing will be limited. Our findings may raise the question of whether a lower treosulfan $AUC_{0-\infty}$ can be sufficient to achieve effectiveness. In addition, lower treosulfan exposure could be beneficial in limiting late effects of conditioning, especially gonadal insufficiency. These questions 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 nonmalignant diseases translates into very favorable clinical outcomes. Our data demonstrate that standardized dosage regimens can be applied in the vast majority of patients to achieve favorable OS and EFS.

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SUPPLEMENTARY MATERIALS

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