Chapter 7

Treosulfan-based conditioning in pediatric hematopoietic stem cell transplantation: a prospective study on pharmacokinetics and early clinical outcomes

Marloes H. ten Brink

Robbert G.M. Bredius

Juliëtte Zwaveling

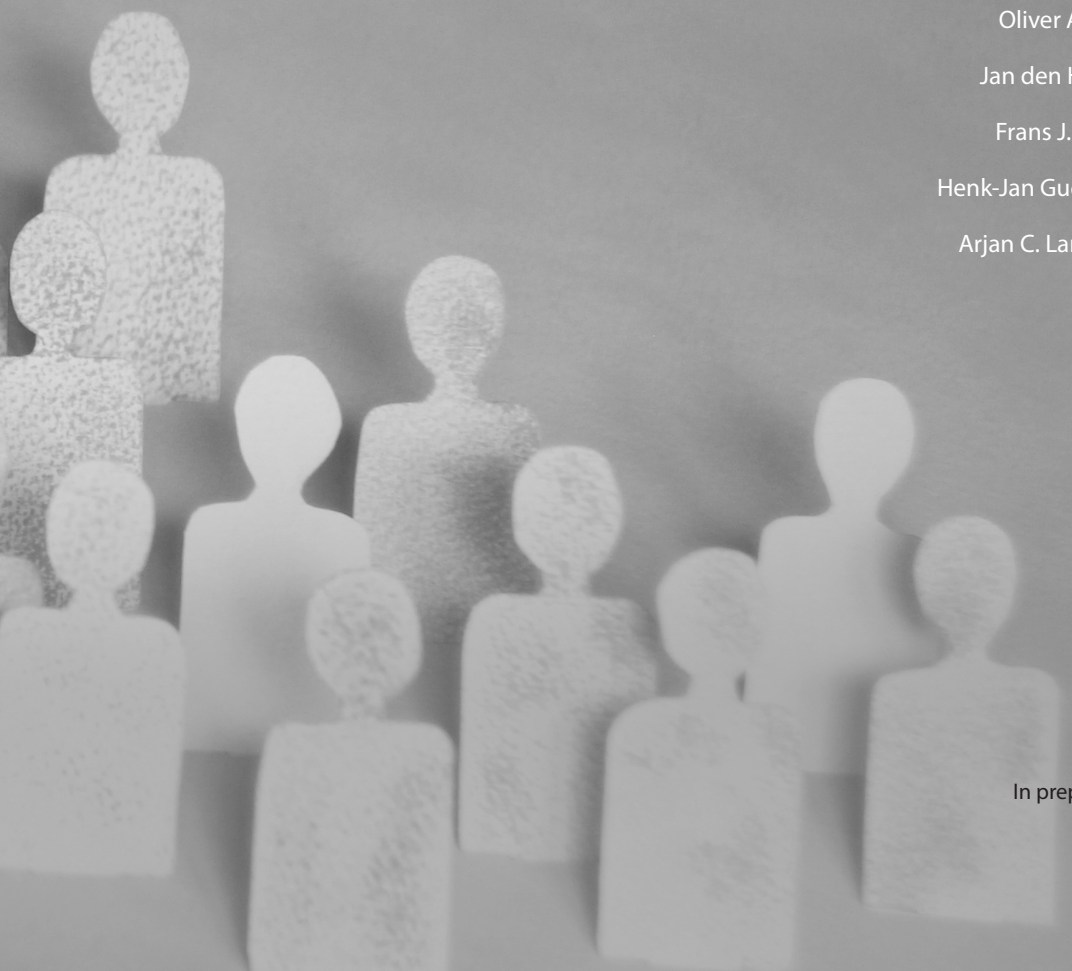
Oliver Ackaert

Jan den Hartigh

Frans J. Smiers

Henk-Jan Guchelaar

Arjan C. Lankester



In preparation

ABSTRACT

Treosulfan is an alkylating agent applied in regimens prior to allogeneic hematopoietic stem cell transplantation (allo-HSCT) in children. It has strong myeloablative and immunosuppressive activity and a relatively mild toxicity profile. In this article we describe the first results of an ongoing prospective study on the relation between the pharmacokinetic profile of treosulfan in pediatric patients undergoing HSCT and clinical outcome.

A total of 21 patients were included in the study with a median age of 5.2 (0.13–16.8) years and a median follow-up of 1 year (168 days – 1.9 years). Patients received intravenous treosulfan prior to their HSCT for various malignant and non-malignant indications combined with fludarabine and thiotepa. Nine of 21 patients received treosulfan as a reduced toxicity conditioning, due to preexisting comorbidities. A one-compartment model was used and clearance and volume of distribution were allometrically scaled using body weight. The mean AUC was 1534 mg*h/L and the interpatient variability was 14%.

The overall toxicity profile was relatively mild and consisted mainly of liver toxicity (48%), skin toxicity (28%) and mucositis (38%). Of 19 evaluable patients, 95% had a primary engraftment. In this study we did not find a relation between treosulfan exposure and HSCT outcome parameters engraftment, chimerism, toxicity, and survival. This is the first study report in which the treosulfan pharmacokinetic profile in pediatric patients is described and exposure is related to clinical outcome. Analysis of larger cohorts of pediatric HSCT recipients is warranted to determine the relation between treosulfan exposure and HSCT outcome in age- and disease-specific subgroups.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curative option for a variety of malignant and non-malignant hematologic diseases in pediatric patients. The conditioning regimen given prior to an allo-HSCT has two goals: suppression of the immune system of the host to prevent graft rejection and allow donor engraftment, and ablation or suppression of host hematopoiesis, including malignant cells. Based on differences in underlying diseases, various conditioning regimens have been developed which differ in immuno- and myeloablative potential. One of the strategies to improve HSCT outcome is reducing the toxicity caused by the conditioning regimen given prior to the HSCT. Treosulfan is an alkylating agent, which is increasingly applied in HSCT due to its beneficial toxicity profile in comparison to busulfan and total body irradiation.

Treosulfan (L-threitol 1,4-bismethanesulphonate, Ovastat®) is a prodrug and a water-soluble alkylating agent. It is non-enzymatically, pH-dependent converted into a monoepoxide and a diepoxide, which are responsible for DNA alkylation and DNA crosslinking.[1,2]

In vivo, treosulfan gives a rapid and sustained myeloablation, which is comparable to busulfan. Furthermore, the immunosuppressive profile of treosulfan was demonstrated to be stronger in comparison to busulfan and more durable than cyclophosphamide.[3] *In vitro* data suggest that treosulfan has a stronger cytotoxic effect against leukemic cells in pediatric patients than busulfan.[4]

In several studies treosulfan was applied in combination with other cytostatic and immunosuppressive drugs prior to allo-HSCT and demonstrated a safe and effective regimen.[5–7] Clinical outcome of allo-HSCT using busulfan-based condtioning is associated with the exposure of busulfan, and therapeutic drug monitoring is often applied to target exposure. Similarly, we assume that clinical outcome after allo-HSCT with a treosulfan based regimen might also be dependent on treosulfan exposure. To date, only two studies investigated the pharmacokinetic profile in pediatric patients. However, these studies focused on PK and did not report the correlation of exposure with clinical outcome.[8,9] In the first study, interpatient variability in exposure was large (CV = 70%). The second study was performed by our group and included 20 patients.[9] It comprised the development of a bioanalytical method to determine treosulfan in serum, a pharmacokinetic model and limited sampling strategy in order to determine treosulfan exposure in pediatric patients.

In the present prospective study, we describe the pharmacokinetics treosulfan in pediatric HSCT recipients and we studied whether the exposure could be related to clinical outcome.

MATERIALS AND METHODS

Patients and donor characteristicsand conditioning regimen

Pediatric patients (≤ 18 years) receiving treosulfan based conditioning prior to their first allo-HSCT in the Leiden University Medical Center in The Netherlands were included. The institutional Ethics Committee approved the treosulfan PK study protocol. Written informed consent was obtained from all parents of the study patients and patients themselves when they were older than 12 years according to the Helsinski Declaration.

Patients received an allo-HSCT for various malignant and non-malignant indications. Donor matching was based on HLA typing. All patients received treosulfan in a dose of 42 g/m^2 or 30 g/m2 if BSA < 0.5 m^2, divided over 3 days. Treosulfan was combined with fludarabine and in some patient also thiotepa was added. Thiotepa (8 mg/kg) was administered at day -8, treosulfan was administered at day -7 to day -5 and fludarabine 30 mg/m^2/day on day -7 to day -3. Serotherapy consisted of anti-thymocyteglobuline (ATG) 2.5 mg/kg/day from day -5 till day -2 or alemtuzumab 0.2 mg/kg/day from day -6 till day -2. All drugs were intravenously administered.

Graft-versus-Host disease prophylaxes consisted of cyclosporine A (CsA:1.5 mg/kg i.v. twice daily, and following engraftment equivalent oral dose),with dose adjustments based on through levels, which were 100–150 µg/L in malignant disease and 150–200 µg/L in non-malignant disease. CsA was combined with methotrexate in T cell replete transplants (10 mg/m^2 on day +1, +3 and +6) or prednisolone in cord blood transplants (1 mg/kg/day). In case of suspected toxicity or adverse events CsA was switched to mycophenolate mofetil or tacrolimus. In 9/21 patients G-CSF (5 µg/kg/day) was given to support engraftment on individual indication, mostly because of co-morbidity.

All patients were cared for in high-efficiency, particle-free air (HEPA)-filtered positive-pressure isolation rooms with total gut decontamination using non-absorbable antimicrobials and antifungal prophylaxis using azoles according to institutional guidelines.

Treosulfan assay

Patients received 10 or 14 g/m^2 treosulfan in a 3 hour infusion on three consecutive days. On day one, patient blood samples were collected at 1.5, 3.5, 4, 5, 7 and 9 or at 4-5 and 6-7 hours after the start of a three hour treosulfan infusion. For determination of the inter-occasion variability (IOV) in a selection of patients (weight >10 kg) samples were also taken on day 3. Blood samples

were collected in serum tubes without gel through the lumen of the catheter that was not used for intravenous treosulfan administration. Samples were centrifuged as quickly as possible after collection, but at least within 5 hours. A reversed phase high pressure liquid chromatography (RP-HPLC) method using Ultraviolet (UV) detection was applied to determine treosulfan in serum, as previously described by our group.[9] Briefly, treosulfan and the internal standard busulfan were made detectable with derivatization with sodium diethyldithiocarbamate (DDTC). Treosulfan can be determined over a range of 10 to 500 mg/L with a limit of quantification of 6.8 mg/L. Accuracies for each of the three QC samples were within the 90–110% limit. The coefficient of variation of the within-day imprecision and between-day imprecision was less than 5% for all three concentration levels.

Pharmacokinetics of treosulfan

The individual pharmacokinetic profile of each patient was determined by a population pharmacokinetic model, using non-linear mixed-effects modelling as implemented in the NONMEM software package (version 7 level 2; Icon Development Solutions, Ellicott City, Maryland, USA). A one-compartment model was used and clearance and volume of distribution were allometrically scaled using body weight, as previously published.[9] The scaling exponent for clearance was fixed at 0.75 and for volume of distribution at 1.0. For a number of patients, 2 serum samples were collected and a limited sampling strategy (LSS) was applied to estimate individual pharmacokinetic parameters. Interpatient variability (IPV) was calculated by the CV% of the treosulfan exposure between individuals and IOV by the calculating the mean difference between the AUC on day 1 and day 3 of each individual. IOV calculations were based on measurements in 7 patients.

Evaluation of clinical data

Clinical endpoints of this study were stem cell engraftment, chimerism, survival, relapse, treatment related mortality and toxicity (i.e. mucositis, skin toxicity, hepatic toxicity, neurologic toxicity and metabolic acidosis) and GVHD.

Stem cell engraftment was defined as platelet count > 50 x 10^9/L, without platelet support for 3 consecutive days together with neutrophils > 0.5 x 10^9/L on 2 consecutive measurements separated by at least 3 days (72 hours) without granulocyte support. Donor–recipient white blood cell chimerism was determined by VNTR polymorphism. Occurrence of aGvHD (within 100 days) and chronic GvHD is diagnosed and graded according to the scale defined by Przepiorka *et al*.[10] Toxicities were scored according to Bearman *et al*., in which toxicities are scored which

were likely due to the preparative regimen.[11] For GvHD and toxicities, events were graded from 0 (no adverse event) to 4 (severe).

Statistical analysis

Normally distributed parameters are shown as mean ± standard deviation and all log-normally distributed parameters as median (minimum and maximum). IPV was determined by calculating the mean coefficient of variation of AUCs for the total cohort.

Study endpoints included stem cell engraftment, rejection, toxicity, and survival. The relationships between AUC and transplantation outcomes were characterized using linear regression analysis. When appropriate, an independent Student's T-test was used. All p-values were 2-tailed and considered significant when $p < 0.05$. All statistical analyses were performed with ISB SPSS statistics, version 20.0 (IBM Corp., Armonk, NY, USA).

RESULTS

Patients characteristics, conditioning regimen and GvHD prophylaxis

A total of 21 pediatric patients receiving their first HSCT were included in the study. The median age was 5.2 (0.13–16.8) years and 12 patients were male. HSCT indications included hemoglobinopathies (n = 12), acute myeloid leukemia (n = 3), primary immune deficiency (n = 4), and bone marrow failure (n = 2), see also Table 7.1. Patient received treosulfan based conditioning as first line choice according to institutional guidelines (n = 12) or as reduced toxicity conditioning because of individual patient comorbidity (n = 9).

Eight patients received a transplant from an HLA identical sibling, 13 patients received a transplant from a matched unrelated donor, i.e. 9/10 allelic matching (n = 6) and 10/10 allelic matching (n = 7). Seventeen of the 21 patients received a T cell replete bone marrow graft, one patient peripheral blood stem cells and 3 patients a cord blood transplant.

All patients except one received a treosulfan dose of 14 g/m^2 per day during 3 consecutive days, the youngest patient, a 1.5-month-old boy with a body surface area of 0.3 m^2, received a dose of 10 g/m^2 per day. In 16 patients treosulfan was combined with fludarabine and thiotepa and 5 patients received only treosulfan and fludarabine. Twenty patients received serotherapy; 19 received ATG and 1 alemtuzumab.

Table 7.1 Patient characteristics

	Total (n = 21)
Characteristic	
Age (years, median (range)	5.2 (0.13–16.8)
Weight (kg, mean (sd))	26.9 (18.3)
Sex (n: M/F)	12/11
Conditioning	
Treo-Flu-Thiotepa	16
Treo-Flu	5
Treosulfan dose	
14 g/m^2	20
10 g/m^2	1
Serotherapy	
Yes	20
ATG	19
Alemtuzumab	1
No	1
Diagnosis for HSCT	
Hemoglobinopathies	12
Hematologic malignancy	3
Primary Immune deficiency	4
Bone marrow failure	2
Treosulfan indication	
Reduced toxicity	9
According to protocol	12
Stem cell source	
BM	17
PBSC	1
CB	3
Donor matching	
HLA id sib	8
MUD (≥ 9/10)	13
GvHD prophylaxis	
CsA	4
CsA / MTX	16
CsA / Pred	1

Treo: treosulfan, Flu: fludarabine, Thio: thiotepa, ATG: Anti-thymocyteglobuline, BM: bone marrow, PBSC: peripheral blood stem cells, CB: cord blood, HLA id sib: Human leukocyte antigen identical sibling, MMFD: mismatched family donor, MUD: matched unrelated donor, GvHD: Graft-versus-Host Disease, CsA: Ciclosporin A, MTX: methotrexate, Pred: prednisolone.

Treosulfan pharmacokinetics

In total 28 AUCs were determined in 21 patients; in 7 patients also on day 3 treosulfan samples were taken for determination of the IOV. After development and validation of a limited sampling strategy, it was possible to calculate AUCs with LSS. In 17 patients, AUCs were calculated based on rich data curves. A total of 7 AUCs, in 4 patients, were analyzed based on the LSS. The median clearance and volume of distribution were 5.7 L/h (1.2–20.5 L/h) and 10.8 L (3.0–42.9 L), respectively. The mean AUC of treosulfan was 1534 mg*h/L and the interpatient variability (IPV) was 14%. The IOV was approximately 5%, based on the day 1 and day 3 results of 7 patients. One patient received a lower dose because of his young age and small body surface area i.e. 10 g/m^2. The AUC of this patient was 1578 mg*h/L, which is similar to the mean AUC seen in the population receiving 14 g/m^2. There was a linear relationship between age and treosulfan exposure (p = 0.026, R2 = 23%), see Figure 7.1. Exposure was not different between the various disease groups, i.e. hemoglobinopathies, malignancies, immune deficiencies, and bone marrow failure (data not shown).

Clinical outcome and treosulfan PK

Two patients of the total cohort died on day +11 and +18 due to toxicity and infections prior to engraftment, respectively. Resulting in an overall survival of 90%, primary engraftment

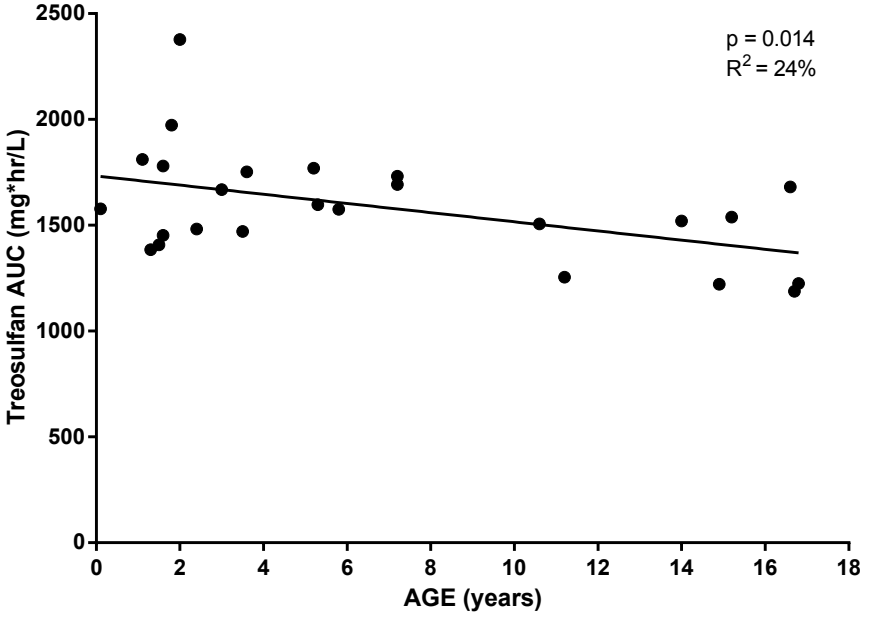


Figure 7.1 Treosulfan exposure versus age of the patient.

Table 7.2 Main toxicities and adverse events in pediatric patients receiving treosulfan based conditioning

Adverse event		N	Mean AUC ± sd		N	Mean AUC ± sd	p-value
Mucositis	Grade 0–1	13	1517 ± 187	Grade 2–3	8	1560 ± 262	0.70
SOS	No	20	1528 ± 217	Yes	1	1668	NA
Hepatic toxicity	No–mild	11	1465 ± 197	Moderate/severe	10	1610 ± 214	0.21
Skin toxicity	No	15	1535 ± 234	Grade 1–2	6	1533 ± 169	0.98
GI-bleeding	No	18	1558 ± 213	Grade 3–4	3	1392 ± 182	0.25
Neurologic toxicity	No	20	1534 ± 219	Yes	1[a]	1538	NA
Viral reactivation	No	15	1539 ± 210	Yes	6	1523 ± 243	0.89
aGvHD	Grade 0–1	18	1517 ± 190	Grade 2–3	3	1636 ± 361	0.63
cGvHD	No	20	1527 ± 216	Limited	1	1681	NA

Occurrence or grading of event, number of patients (N) and mean AUC ± standard deviation. [a] Hallucinations and disorientation in end stage disease. SOS: sinusoidal obstruction syndrome, aGvHD and cGvHD: acute and chronic graft-versus-host disease, NA: not applicable.

was seen in 95% of the 19 evaluable patients. Of the 18 patients that engrafted the median neutrophil and platelet engraftment occurred on day 23 and 35, respectively. After 100 days 11 patients had full donor chimerism and 7 patients had mixed chimerism which persisted until latest follow-up. The median follow-up time was 1 year (168 days – 1.9 years).

Of the 19 survivors, 74% are disease free (n = 14),four patients had a recurrence of their initial disease, and one patient had a primary rejection followed by rapid autologous reconstitution.

The toxicities observed were mucositis, skin- and hepatic toxicity, as shown in Table 7.2. In 38% of the patients mucositis grade 2 or 3 was observed. Twenty eight percent of the patients had any grade skin toxicity and 38% had a moderate transient hepatic toxicity within 100 days after treosulfan administration. Two patients had severe hepatic toxicity of which one patient developed sinusoidal obstruction syndrome (SOS). Both patients received treosulfan as a reduced toxicity conditioning (RTC), one patient because of pre-existent liver damage due to *Cryptosporidium parvum* infection and the second patient due to heavily pre-treatment for secondary AML after initial osteosarcoma treatment.

Gastro-intestinal bleeding occurred in 3 patients (14%) and in one patient neurological toxicity was observed. Only three patients (14%) were diagnosed with aGVHD (grade 2 (n = 1) and grade 3 (n = 2)) and one patient was diagnosed with a limited cGHVD. There is a trend towards higher toxicities in patients receiving treosulfan as a RTC in comparison to patients receiving treosulfan according to protocol, see Table 7.3. Furthermore, there was no significant difference

Table 7.3 Incidence of toxicities based on treosulfan indication

Adverse event	According to protocol (n = 12)	Reduced toxicity conditioning (n = 9)	p-value
Mucositis (grade 2–3)	25%	56%	0.33
SOS	0%	22%	0.88
Hepatic toxicity (moderate/severe)	33%	67%	0.28
Skin toxicity (grade 1–2)	17%	44%	0.36
GI-bleeding (grade 3–4)	8%	22%	0.79
Neurologic toxicity	0%	22%	0.88
Viral reactivation	25%	33%	1.0
aGvHD (grade 2–3)	8%	22%	0.79
cGvHD (limited)	8%	0%	1.0

SOS: sinusoidal obstruction syndrome, aGvHD and cGvHD: acute and chronic graft-versus-host disease.

in treosulfan exposure in these two groups. In this limited cohort, no relation was found between treosulfan exposure and HSCT outcome parameters, e.g. engraftment, chimerism, toxicity and survival.

DISCUSSION

This is the first report in which treosulfan exposure was analyzed in a comprehensive cohort of pediatric HSCT recipients and related to toxicity and clinical outcome parameters.

We found a relative small interpatient variability in PK of 14% only, which was much smaller than observed in an earlier study.[8] In this study of Glowka *et al*. three different doses were applied (10, 12, and 14 g/m^2/day) and they found a linear relationship between dose and exposure.[11] Interpatient variability based on treosulfan exposure measurements in 5 patients receiving 12 g/m^2 was 70%. The exposure in the patient receiving the highest dose of 14 g/m^2/day was relative high (e.g. 1960 mg/L*h).

In our patient population all, except one patient, received 14 g/m^2/day and the mean AUC is lower (i.e. 1534 mg/L*h) in comparison to Glowka *et al*. The inter-occasion variability was approximately 5%, which is much smaller than inter-individual variability. However, IOV is based on measurements on day 1 and 3 in only 7 patients. Notably, we found that treosulfan exposure decreases with age. This was not reported previously, but shows similarities with

the reported data on busulfan exposure and its relation with age.[12] In general, age and body weight are strongly correlated, and in the PK-model treosulfan clearance is allometrically scaled using body weight with a fixed scaling exponent of 0.75 to correct for the influence of growth.[9] The age effect observed in this study could be due to dosing based on body surface area instead of allometrically scaled body weight, or that the appropriate scaling component in the patient population diverge from 0.75. PK-data in a larger cohort is required to confirm this age effect on treosulfan exposure. One very young child received a lower dose of 10 g/m^2/day, but demonstrated an exposure similar to the older children having received 14 g/m^2/day. From these results, it may be concluded that younger patients (< 1 year) require a lower treosulfan dose to obtain an adequate exposure. Furthermore, in older patients dose-escalation could be possible to further increase efficacy of treosulfan-based conditioning. Studies in a larger cohort of children including a broad spectrum of ages, and particularly infants, will provide more insight in the pharmacokinetics of treosulfan.

Particularly in pediatric patients, the relation between treosulfan exposure and clinical outcome parameters has so far been unresolved. In this first report of an ongoing prospective pediatric study, the OS rate was 90% and primary engraftment was 95% in the 19 evaluable patients. OS and primary engraftment are similar or even better in comparison to other studies applying treosulfan-based conditioning in pediatric populations with malignant and non-malignant diseases.[13–15]

The toxicity profile in this study group was relatively mild, with mild to moderate mucositis and hepatic toxicity being most often reported. In these children, the toxicity parameters could not be related to treosulfan exposure. The high incidence of hepatic toxicity could be due to the preexistent hepatic risk profile in affected patients, which precluded the use of busulfan-based conditioning. This reflects the fact that treosulfan-based conditioning was used as reduced toxicity conditioning (RTC) in almost half of the patients because of significant preexisting co-morbidities. The incidence of acute and chronic aGvHD is low in comparison to other studies applying treosulfan based conditioning.[13,14,16] Furthermore, no relationship with any clinical outcome and treosulfan exposure was observed. However, the cohort was limited in size as to observe subtle effects of exposure on clinical outcome.

In conclusion, the optimal treosulfan exposure in pediatric allo-HSCT recipients, in relation to age and primary diseases remains to be defined. In this study we demonstrated that treosulfan conditioning is effective and well tolerated. However, primary engraftment and (long term) chimerismcould be improved, and probably a higher exposure of treosulfan may be beneficial to achieve this goal. This is not only of relevance to prevent disease recurrence in patients

with hematologic malignancies, but also to achieve adequate and long term graft function in various non-malignant diseases. Therefore, prospective treosulfan PK studies in larger cohorts of pediatric patients are warranted with the aim to define the most effective treosulfan exposure and dose while keeping its beneficial toxicity profile.

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