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Treosulfan-based conditioning regimens for allogeneic HSCT in children with acute lymphoblastic leukaemia

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Abstract

Standard myeloablative conditioning regimens for children with acute lymphoblastic leukaemia are based on total body irradiation (TBI). However, TBI causes profound short-term and long-term side effects, provoking the necessity for alternative regimens. Treosulfan combines a potent immunosuppressive and antileukaemic effect with myeloablative activity and low toxicity profile. We retrospectively studied toxicity and outcome of 71 paediatric patients with ALL undergoing hematopoietic stem cell transplantation (HSCT) following treosulfan-based conditioning aiming to identify risk factors for treatment failure and dose depending outcome differences. Early regimen-related toxicity was low. No case of veno-occlusive disease was reported. There was no association of toxicity with age or number of HSCT. Event free survival (EFS) of infants was significantly better compared to older children. Overall survival (OS) at three years was 51% and not significantly influenced by number of HSCT (first HSCT 54%, $\geq$ second HSCT 44%, $p=0.71$). In multivariate analysis, OS and EFS were significantly worse for patients transplanted without complete remission ($p=0.04$ and 0.004). Treatment related mortality was low at 14%. We conclude that treosulfan based conditioning is a safe and efficacious approach for paediatric ALL.

Keywords: treosulfan, ALL, paediatrics, stem cell transplantation, toxicity

Introduction

As outcome with chemotherapy for children with acute lymphoblastic leukaemia (ALL) has remarkably improved, indications for allogeneic hematopoietic stem cell transplantation (HSCT) in first complete remission (CR1) are rare. (1-3) However, for high risk patients and after relapse, HSCT still represents an established therapy approach.(4) Fractionated total body irradiation (TBI) is the most common myeloablative conditioning procedure for children with high relapse risk ALL. (5, 6) In most transplant approaches, the current standard conditioning regimen for matched related or unrelated donors consists of TBI (12Gy) and cyclophosphamide or etoposide. (1, 5) TBI has been proven to have an effective immunosuppressive and antileukaemic potential, however acute and late toxic effects are the major drawbacks of a potentially curative therapy. (7-11) In young patients or patients already treated with high irradiation doses before HSCT, TBI is often replaced by busulfan and cyclophosphamide (BU/CY). (5) However, high-dose busulfan is associated with a substantial toxicity profile as well, in particular hepatic, pulmonary and neurotoxic side effects. (7, 12) To reduce these side effects, there is an urgent need for less toxic conditioning regimens with comparable antileukaemic, immunosuppressive and myeloablative potential. Although reduced-intensity conditioning (RIC) is an established concept for adult ALL, its efficacy has not been proven so far in childhood.(13, 14) TBI-free conditioning regimens based on chemotherapy alone have been explored for decades. However so far no combination of chemotherapeutic drugs has achieved a similar outcome. (12, 15, 16) Treosulfan is a prodrug of a bifunctional alkylating cytotoxic agent with low organ toxicity in paediatric patients, even for patients undergoing second HSCT. (17-19) Moreover, treosulfan targets hematopoietic stem cells effectively as well as having profound antileukaemic and immunosuppressive properties. (20, 21) A recent EBMT study demonstrated the efficacy and safety of treosulfan based regimens for children with haematological malignancies.(19) Here we report on a cohort of 71 children with ALL who underwent HSCT following a treosulfan based conditioning regimen.

Patients and methods

Patients

In this retrospective study we evaluated 71 children and adolescents with ALL who underwent allogeneic HSCT between January 2005 and July 2010 in 24 international paediatric transplant centres from 10 countries (Germany, Italy, United Kingdom, Poland, Russia, Netherlands, Switzerland, Finland, Lithuania, Israel) following a treosulfan based conditioning regimen. This study represents a subanalysis of a large retrospective EBMT study on treosulfan for conditioning in children.(22) The study was conducted in accordance with the EBMT Guidelines for retrospective studies.

Conditioning and transplantation

Table 1 shows detailed patients characteristics. The majority of children were transplanted in >CR1 (58%), for the first time (69%), from an unrelated donor (UD, 56%) and with bone marrow as stem cell source (54%). Reasons why a treosulfan based conditioning regimen was chosen instead of TBI based were second or third HSCT, pre-transplant organ dysfunction or advanced disease stage, respectively. Treosulfan was combined with different chemotherapeutic agents, most frequently with cyclophosphamide (32%) or fludarabine and thiotepea (28%). The most frequent dose range for treosulfan was between 3x13 and 3x15g/sqm (55%). Standard dosages were used for the other chemotherapeutic agents for the majority of patients (total dosage cyclophosphamide 120 mg/kg , fludarabine 120-180mg/sqm, thiotepea 8-10 mg/kg, melphalan 140 mg/sqm). Twenty-two patients underwent subsequent HSCT, median time from first to second HSCT was 1.5 years, from second to third HSCT 1.4 years. Regarding age, gender, donor, treosulfan dose and additional chemotherapy patients undergoing subsequent transplantation did not differ significantly from patients at first transplant. Significant differences between the two groups were stem cell source (first transplant: BM 53%, CB 20%, PB 27%; subsequent transplant: BM 55%, CB 0%, PB 45%, $p=0.042$) and disease status with higher proportion of patient in >CR1 at subsequent transplant (77% vs 49%, $p=0.044$) while percentage of patients without CR was similar (both 14%).

Most children received a CsA containing GvHD prophylaxis (83%). Acute and chronic GvHD were graded according to the Seattle criteria. (23) Early regimen related toxicity (RRT) until day +100 was defined and graded using the Short Name based on the Common Terminology Criteria for Adverse Events v3.0 (CTCAE), available online at: <http://ctep.cancer.gov/forms/>.

Endpoints and statistics

Outcome was measured as follows: myeloid engraftment (3 consecutive days with an absolute neutrophil count (ANC) $>0,5 \times 10^9/L$), graft failure, early RRT until day +100, cumulative incidence of acute (at day +100) and chronic GvHD (at 1 year), overall survival (OS), disease related mortality (DRM), treatment related mortality (TRM), event-free survival (EFS), relapse incidence, non-relapse mortality at 3 years. For all endpoints, the time to event interval started at the day of HSCT, and ended at the day of the respective first event for uncensored or at the day of last follow up for censored individuals.

Univariate statistical analysis was done in prospectively identified subgroups defined by remission status, conditioning regimen, number of HSCT and patient age. Kaplan-Meier estimates and log-rank test were used to evaluate OS and EFS.(24, 25) For OS, deaths from any cause were considered an event. Relapse, progression or death from any cause were considered an event for EFS. Cumulative incidences of events were calculated by the method of Fine and Gray for censored data subject to competing risks, and compared using the Gray test.(26, 27) The cumulative incidence of non-relapse mortality was estimated taking into account the competing risk of mortality after relapse, the cumulative incidence of relapse/progression taking into account the competing risks of death without relapse and graft failure, the cumulative incidence of ANC engraftment taking into account the competing risks of lost graft, death without engraftment and subsequent HSCT, and the cumulative incidence of TRM taking into account the deaths after relapse and subsequent SCT without treosulfan-based conditioning. Mantel-Haenszel Chi-Square test and Chi-Square test were used to compare categorical non time-to event variables: toxicities, acute GvHD and chronic GvHD. Multivariate analysis was performed to study the impact of confounding factors on the defined outcomes. Cox regression was used to model the time to relapse, TRM, EFS and OS, and logistic regression was used to model the rate of acute GvHD of grade 3/4, and the 1 year rate of chronic GvHD.(24, 25, 28) Because of the relatively small sample size and hence low GvHD numbers, treosulfan dose and patient age were included into the logistic regressions as continuous variables and only a possible linear relationship with the respective dependent variable was investigated.

The statistical analysis was done with SAS System V9.2 (2008, SAS Institute, Cary, NC). All p-values below 0.05 were considered significant.

Results

Early regimen related toxicity

Early regimen related toxicity is shown in detail in table 2. Stomatitis, diarrhea, vomiting, respiratory toxicity, elevated bilirubin, elevated SGOT, CNS toxicity and peripheral neurological (PN) toxicity $\geq$ grade 3 occurred in 26% (18/70), 17% (12/71), 8% (6/71), 10% (7/70), 10% (7/69), 19% (13/69), 3% (2/70) and 3% (2/68) of patients, respectively. Grade 4 toxicity of any kind was observed in less than 10% of patients. No patient developed veno-occlusive disease (VOD).

Overall, severe neurotoxicity was low (CNS and/or PN grade 3 or 4: 3/71, 4%), in particular no central and peripheral neurotoxicity was seen when treosulfan was combined with fludarabine and thiotepa.

Toxicity was similar in all age groups (table 3). Infants did not show a significantly higher toxicity compared to older children except for vomiting grade 3/4 which tended to occur more frequently in this younger age group.

Early RRT of any category was not higher for patients undergoing second or third HSCT compared to patients at first HSCT (table 3). There was no significant association between higher treosulfan dose and grade 3 or 4 toxicity (table 3).

Engraftment

Myeloid engraftment was achieved in 68/70 (97%) of patients at a median of 18 days (range (8-84)). The remainder two patients died on day +15 and +38 without signs of engraftment.

For one patient, information on engraftment was not available in the database.

Acute and chronic GvHD

Acute GvHD occurred in 54% (37/69) of patients, GvHD $\geq$ grade 3 was observed in 16% (11/69). There was no significant association between acute GvHD $\geq$ grade 3 and treosulfan dose ($<3 \times 11$ g/sqm 13%, 3×11 - 3×13 g/sqm 14%, $>3 \times 13$ g/sqm 18%, $p=0.817$).

Twenty-one % of patients (13/61) showed signs of chronic GvHD (extensive GvHD in 4/61, 7%). There was a significant influence of treosulfan dose and chronic GvHD with lower chronic GvHD rates in patients who received higher treosulfan dose ($<3 \times 11$ g/sqm 63%, 3×11 - 3×13 g/sqm 15%, $>3 \times 13$ g/sqm 15%, $p=0.027$). No patient in the fludarabine/thiotepa group suffered from acute GvHD grade 4 or extensive chronic GvHD.

Acute GvHD grade 3 /4 and chronic GvHD occurred in only 4/37 (11%) and 5/34 (15%) patients who received bone marrow as stem cell source compared to 7/22 (32%, $p=0.081$) and 5/18 (28%, $p=0.287$) following HSCT with peripheral blood stem cells. Occurrence of acute GvHD grade 3/4 or chronic GvHD was not significantly influenced by number of HSCT (first HSCT 21 and 10%, $>$ first HSCT 5 and 0%, p 0.09 and 0.16, respectively).

In a multivariate setting, we could not identify statistically significant independent risk factors (impact of number of HSCT, remission status, age, treosulfan dose, conditioning regimen, donor type and stem cell source) on the incidence of acute GvHD of grade 3/4 and chronic GvHD.

Outcome

Overall and event-free survival at 3 years for all 71 patients were $51 \pm 6\%$ and $39 \pm 6\%$, respectively. There was no statistically significant difference between OS and EFS of patients at first HSCT and patients who underwent $\geq$ second HSCT (figure 1A,B). **OS and EFS**

correlated significantly with remission status, with a significantly better outcome for patients in CR1 compared to >CR1 and no CR (figure 2A,B).

Treatment related mortality at 2 years was low for the whole group ($14 \pm 4\%$) and particularly low in patients undergoing HSCT in CR (CR1 $10 \pm 7\%$, >CR1 $12 \pm 5\%$), while patients not in CR at HSCT showed a tendency for higher TRM ($30 \pm 14\%$, $p=0.28$, figure 2C). Main cause for death was disease related ($32 \pm 6\%$), relapse incidence at 3 years being $47 \pm 6\%$ ($n=34$).

DRM was high in patients who underwent HSCT without having reached CR (figure 2D).

Infants had a better OS compared to older children, regarding EFS this difference was statistically significant (figure 1C, D). No infant died of treatment related toxicity (0.5-1 year 0%, 1-12 years $17 \pm 6\%$, >12 years $14 \pm 7\%$, $p=0.45$).

EFS tended to be worse in the low dose group of treosulfan, however this difference was not significant ($<3 \times 11$ g/sqm $25 \pm 15\%$, 3×11 - 3×13 g/sqm $47 \pm 11\%$, $>3 \times 13$ g/sqm $38 \pm 8\%$, $p=0.51$).

Regarding the different combinations of conditioning drugs, OS, EFS, TRM, DRM and relapse incidence were not significantly different between the various conditioning regimens.

Multivariate analysis

In a Cox regression analysis of EFS, OS, TRM and relapse, the following risk factors were included: number of HSCT, remission status, age, treosulfan dose, conditioning regimen, donor type and stem cell source (table 4).

Undergoing HSCT without having achieved CR was associated with worse OS ($p=0.041$) and EFS ($p=0.008$). Despite a very small group of 6 patients with other combinations of conditioning drugs associated with worse outcome, OS, EFS and the incidence of relapse were not significantly influenced by the other parameters. All statistical calculations in the multivariate setting have to be regarded with caution because of the relatively small number of patients and events.

Discussion

More than 30 years after the first successful allogeneic HSCT, TBI regimens are still the most common conditioning regimen for children with ALL despite the association with significant acute and late toxic side effects. (1, 5, 29) The only available myeloablative alternative for a long time period, busulfan containing conditioning, harbours a substantial risk of hepatotoxicity, veno-occlusive disease, neurotoxicity and pulmonary injury associated with possible high TRM. (12, 15, 30, 31) In contrast to adult ALL, the concept of reduced intensity conditioning could not prove similar efficacy so far. (32, 33) This situation leads to an urgent requirement for less toxic conditioning regimens. Although in non-malignant diseases, progress was reported with iv BU and therapeutic drug monitoring (TDM), it has not been proven so far, whether such procedures can ameliorate toxicity in children with ALL. (34, 35) *In vivo* human ALL mouse models showed a significant antileukaemic activity of treosulfan comparable to busulfan and cyclophosphamide, while *in vitro* studies even demonstrated a superior activity of treosulfan compared to busulfan. (20, 36) In contrast, treosulfan results in a lower nonhaematological toxicity with the additional advantage of linear pharmacokinetics without the necessity to measure blood levels as with busulfan. (17, 18) Treosulfan based conditioning has been applied in adult patients with haematological malignancies with promising results and notably low toxicity. (37-40) Few studies so far have investigated treosulfan as part of the conditioning regimen in paediatric patients. (19, 41-43) In a retrospective EBMT study, we now evaluated the toxicity profile and outcome of 71 ALL patients undergoing HSCT following treosulfan based conditioning. The primary aim in these patients was to reduce the risk of severe acute organ toxicity due to either pre-existing organ dysfunctions or second transplantation. To our knowledge, this is the largest study to date investigating treosulfan use in paediatric ALL patients. We observed a high engraftment rate of 97% comparable to earlier studies using treosulfan. (19, 43, 44) Early regimen related toxicity in the whole group was impressingly low. In particular severe toxicity ($\geq$ grade 3) occurred in $< 20\%$ and consisted mainly of stomatitis and elevated liver enzymes (table 2). There was no significant association between higher treosulfan dose and grade 3/4 toxicity. Direct comparison with studies using TBI and busulfan based conditioning is difficult, especially when different toxicity grading scales were used. (7) High grade mucosal toxicity was seen in up to 45% of paediatric patients following TBI or busulfan/cyclophosphamide (7) and 43% in a recent paediatric study using a combination of busulfan with fludarabine (45) – this percentage is higher than in our cohort following treosulfan (26%). A higher rate of high grade mucositis following standard myeloablative conditioning (either TBI or busulfan based)

compared to treosulfan has been reported in adult patients with haematological malignancies as well. (37, 46)

Hepatic toxicity in our study was low; in particular, we did not observe a single case of VOD confirming a low rate of VOD in earlier treosulfan studies. (42-44, 47) Recent myeloablative busulfan containing regimens were reported to be associated with higher VOD rates especially when melphalan was added as a third drug. (34, 35, 48, 49) VOD rates following a conditioning with busulfan and fludarabine combined with TDM of busulfan showed promising lower rates of VOD, however this regimen has not been evaluated in a large cohort of paediatric ALL patients. (45, 50) In addition, the pulmonary toxicity profile of treosulfan based regimens was low (grade 3/4: 4% and 10%) compared to TBI and busulfan based regimens with deaths due to pulmonary toxicity in up to 14% of patients. (11) This observation is impressive as high-risk ALL patients are prone to develop either toxic or infectious lung complications.

Treatment related mortality at 2 years in our cohort was 14% compared to up to 35% for paediatric patients following TBI-based and between 17 and 23% for busulfan based myeloablative conditioning. (11, 12, 48) TDM might contribute to lower TRM after busulfan exposure. However, a recent study on paediatric patients reported TRM of up to 27% with TDM. (35) As our cohort contains a high number of patients with > first HSCT (31%) and considerable number of patients with pre-existing organ dysfunctions, our observed low TRM rate is remarkable. (51)

Infant patients undergoing HSCT are especially vulnerable for short and long term toxic side effects of the conditioning regimen, in particular TBI. (29, 52) (42) Hence, most groups currently employ chemotherapy- only conditioning regimens for children under 2-3 years, most frequently a backbone of busulfan and cyclophosphamide combined with melphalan (BuCyMel) (1, 49) However, this combination has substantial long-term and short-term toxic side effects in infant patients and relapse is still an unsolved problem. (52-54) Due to high treatment related mortality, the Interfant-06 study recently replaced BuCyMel with either busulfan or treosulfan combined with fludarabine and thiotepe. Treosulfan based reduced intensity conditioning has been shown to be well tolerated in infants with immunodeficiency disorders and seems to provide sufficient antineoplastic activity in infant ALL. (41, 42, 55) Our data also show low short-term toxicity in infants receiving treosulfan based myeloablative conditioning without a single case of treatment related death. Toxicity did not significantly differ from older children (table 3). In addition, outcome in infants was at least comparable to older age groups. Regarding EFS infants even showed significantly better

survival, although statistical calculations have to be regarded with caution as the group of infants was small. Prospective studies are needed to confirm lower long term side effects in infants receiving treosulfan compared to TBI or busulfan.

Our study has the limitations of being a registry-based retrospective study, hence we did not have sufficient data on the phenotypes of leukaemia, pre-transplant performance scores and information on the condition regimen of the first transplantation. Nevertheless, our cohort in general and specifically patients undergoing subsequent HSCT exhibited low toxicity. In our multivariate analysis, subsequent HSCT in comparison to first HSCT did not significantly influence EFS, TRM or relapse incidence. Hence, treosulfan based conditioning seems to be an adequate and promising option for patients undergoing subsequent HSCT. In addition, toxicity and outcome in an unselected ALL cohort at first HSCT is likely to be even lower than our reported results. Hilgendorf et al. reported before that adult patients undergoing HSCT for myelodysplastic syndrome with increased risk for side effects due to comorbidities showed lower TRM and better survival following treosulfan compared to TBI. (37)

Severe acute GvHD grade 3 or 4 was observed in 16% of patients comparable to earlier paediatric treosulfan studies, as well as TBI or busulfan based regimens. (7, 19, 43)

Outcome for the whole group was acceptable with OS and EFS of 51 and 39%, respectively. As expected, outcome correlated with disease status at transplant with high significance as previous studies have shown for HSCT following TBI or busulfan as well. Regarding our cohort, patients in CR1 had significantly better OS and EFS compared to patients in $\geq$ CR2 (70% vs. 52% and 70% vs 41%). This difference was also seen in paediatric patients following TBI conditioning: EFS for patients in CR1 was 83% compared to 47% in $\geq$ CR2.(15) Not surprisingly, patients undergoing HSCT without having reached CR had an extremely poor outcome with high DRM, high TRM and low EFS (40 and 30% at two years, EFS $10 \pm 9\%$, figure 2) comparable to earlier reports following treosulfan. (43, 56). It seems that outcome is more dependent on disease state than on number of transplant: Disease-free survival was reported as 17% for pediatric ALL patients who underwent first HSCT after TBI without having reached CR while according to another study overall survival was 15.3% for patients in Non-CR undergoing second transplant following TBI or busulfan-based myeloablative conditioning.(48, 57) This outcome is comparable to our patient cohort at first HSCT (EFS $14 \pm 13\%$). Our data and that of previous studies underlie the need to reduce leukaemia burden before HSCT.

In patients with thalassemia, the combination of treosulfan with fludarabin and thiotepa was shown to be safe and efficacious.(44) In our cohort, this combination was associated with a good outcome (OS 50%), low toxicity, low TRM (10%) and low rate of severe acute GvHD (15%). As thiotepa crosses the blood-brain barrier, this combination should have beneficial effects in preventing leukemic CNS relapse following HSCT as well. (58)

This retrospective analysis serves as a basis for prospective study questions. The international ALL SCTped 2012 FORUM protocol (EudraCT number 2012-003032-22) compares outcome and toxicity of treosulfan- or busulfan-containing regimen versus TBI based conditioning in a prospective randomized study and has recently recruited the first patients.

Summary:

Treosulfan based conditioning is a safe approach for paediatric ALL patients undergoing HSCT allowing sufficient and early engraftment with efficacy in high relapse risk and advanced ALL. Treosulfan is associated with low toxicity, even in high risk patients as infants or patients undergoing subsequent HSCT.

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Figure legends

Figure 1. Overall (A) and event-free survival (B) were not significantly different between patients undergoing first or subsequent stem cell transplantation. Infant patients tended to have a better overall survival (C) while event-free survival was significantly better for this age group (D).

Figure 2. Remission status at transplant had a significant influence on outcome: Overall (A) and event-free survival (B) were significantly better for patients in first complete remission. Patients who underwent transplant without having reached remission showed a higher tendency for treatment- (C) and disease-related mortality (D).

Table 1 Patient and HSCT procedure characteristics

Number of patients	71
Age	Median 9.1 years (0.8-17.9); <1 year, n= 7 (10%)
Sex	Female, n= 30 (42%); male, = 41 (58%)
Disease state	CR1, n= 20 (28%); >CR1, n= 41 (58%); no CR, n= 10 (14%)
Number of HSCT	1st, n= 49 (69%); >1st, n= 22 (31%)
Donor type	MFD, n= 24 (34%); MMFD, n= 7 (10%); UD, n= 40 (56%)
Stem cell source	BM, n= 38 (54%); PB, n= 23 (32%); CB, n= 10 (14%)
<i>Preparative regimen according to treosulfan dose (g/sqm)</i>	
Treosulfan <3x9	n=3 (4%); +CY, 2; +FLU, 1
Treosulfan 3x9-<3x11	n= 5 (7%); +CY, 1; +FLU, 3; +other, 1
Treosulfan 3x11-<3x13	n=21 (30%); +CY, 13; +FLU, 2; +FLU/MEL, 3; +FLU/Thio, 2; +other, 1
Treosulfan 3x13-3x15	n=39 (55%); +CY, 7; +FLU, 8; +FLU/MEL, 2; +FLU/Thio, 18; +other, 4
Treosulfan >3x15	n=3 (4%); +FLU, 3
GvHD prophylaxis	CsA w/o MTX/MMF ± other, n=28 (40%)
	CsA + MTX w/o MMF ± other, n=30 (42%)
	CsA + MMF w/o MTX ± other, n=1 (1%)
	other combinations, n=12 (17%)

Abbreviations: HSCT=hematopoietic stem cell transplantation; CR= complete remission;
MFD= matched family donor; MMFD= mismatched family donor; UD= unrelated donor
BM= bone marrow; PB= peripheral blood; CB= cord blood; CY=cyclophosphamide;
FLU= fludarabine; MEL= melphalan; Thio= thiotepea; CsA= ciclosporin A; MTX= methotrexate;
MMF= mycophenolate mofetil

Table 2 Early regimen related toxicity

	Grade 0		Grade 1		Grade 2		Grade3		Grade 4	
	All	+ FLU/Thio	All	+ FLU/Thio	All	+ FLU/Thio	All	+ FLU/Thio	All	+ FLU/Thio
Stomatitis (%)	22/70 (31)	5/20 (25)	22/70 (31)	8/20 (40)	8/70 (11)	3/20 (15)	13/70 (19)	3/20 (15)	5/70 (7)	1/20 (5)
Diarrhea (%)	17/71 (24)	6/20 (30)	29/71 (41)	7/20 (35)	13/71 (18)	4/20 (20)	6/71 (8)	2/20 (10)	6/71 (8)	1/20 (5)
Vomiting (%)	23/71 (32)	9/20 (45)	14/71 (20)	4/20 (20)	28/71 (39)	7/20 (35)	2/71 (3)	0/20 (0)	4/71 (6)	0/20 (0)
Respiratory (%)	51/70 (72)	14/20 (70)	2/70 (3)	0/20 (0)	10/70 (14)	2/20 (19)	2/70 (3)	1/20 (5)	5/70 (7)	3/20 (15)
Bilirubin (%)	40/69 (58)	10/18 (56)	13/69 (20)	4/18 (22)	9/69 (13)	4/18 (22)	5/69 (7)	0/18 (0)	2/69 (3)	0/18 (0)
SGOT (%)	16/69 (23)	6/18 (33)	28/69 (41)	8/18 (44)	12/69 (17)	2/18 (11)	12/69 (17)	2/18 (11)	1/69 (1)	0/18 (0)
VOD (%)	68/68 (100)	19/19 (100)								
CNS (%)	65/70 (93)	19/19 (100)	1/70 (1)	0/19 (0)	2/70 (3)	0/19 (0)	0/70 (0)	0/19 (0)	2/70 (3)	0/19 (0)
PN (%)	65/68 (96)	19/19 (100)	1/68 (1)	0/19 (0)	0/68 (0)	0/19 (0)	1/68 (1)	0/19 (0)	1/68 (1)	0/19 (0)

Abbreviations: VOD= veno-occlusive disease; CNS= central nervous system; PN= peripheral neuropathy

Table 3 Early regimen related toxicity in correlation with treosulfan dose, age and number of HSCT

Toxicity ≥ grade 3 (%)	Treo sulfan dose (g/sqm)				number of HSCT			age at HSCT (years)			
	<3*11	3*11-3*13	>3*13	p	first	> first	p	0-1	1-12	>12	p
Stomatitis	0/7 (0)	9/21 (43)	9/42 (21)	1.00	12/48 (25)	6/22 (27)	0.84	0/6 (0)	10/42 (24)	8/22 (36)	0.18
Diarrhea	2/8 (25)	2/21 (10)	8/42 (19)	1.00	9/49 (18)	3/22 (14)	0.62	2/7 (29)	7/42 (17)	3/22 (14)	0.66
Vomiting	1/8 (13)	4/21 (19)	1/42 (2)	1.00	5/49 (10)	1/22 (5)	0.43	2/7 (29)	3/42 (7)	1/22 (5)	0.12
Max. gastrointestinal toxicity	2/8 (25)	10/21 (48)	14/42 (33)	1.00	19/49 (39)	7/22 (32)	0.57	3/7 (43)	14/42 (33)	9/22 (41)	0.78
Respiratory	0/8 (0)	2/20 (10)	5/42 (12)	0.42	5/48 (10)	2/22 (9)	0.86	0/7 (0)	4/41 (10)	3/22 (14)	0.58
Bilirubin	1/8 (13)	1/21 (5)	5/40 (13)	0.79	5/48 (10)	2/21 (10)	0.91	0/7 (0)	3/40 (8)	4/22 (18)	0.27
SGOT	1/8 (13)	4/21 (19)	8/40 (20)	0.83	10/48 (21)	3/21 (14)	0.52	1/7 (14)	6/42 (15)	6/22 (27)	0.47
VOD	0/8 (0)	0/21 (0)	0/39 (0)								
Max. liver toxicity	1/8 (13)	5/21 (24)	11/40 (28)	0.44	13/48 (27)	4/21 (19)	0.48	1/7 (14)	8/40 (20)	8/22 (36)	0.29
CNS	0/8 (0)	1/21 (5)	1/41 (2)	1.00	2/49 (4)	0/21 (0)	0.35	0/7 (0)	1/41 (2)	1/22 (5)	0.80
PN	0/8 (0)	1/19 (5)	1/41 (2)	1.00	1/47 (2)	1/21 (5)	0.55	0/7 (0)	0/39 (0)	2/22 (9)	0.12
Max. neurological toxicity	0/8 (0)	1/21 (5)	2/41 (5)	0.72	2/49 (4)	1/21 (5)	0.90	0/7 (0)	1/41 (2)	2/22 (9)	0.39

Abbreviations: max= maximal, SGOT=serum glutamyl oxaloacetic transaminase, VOD= venoocclusive disease, CNS= central nervous system, PN= peripheral neurological

Table 4 Multivariate analysis

	OS			EFS			TRM			Relapse		
	p-value	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI
Number of HSCT												
> 1st HSCT vs. 1st HSCT	0.87	1.1	0.5 - 2.4	0.85	0.9	0.5 - 1.9	0.82	0.8	0.2 - 4.1	0.97	1.0	0.5 - 2.3
Status (vs. CR1)	0.04			0.008			0.16			0.06		
> CR1	0.59	1.4	0.4 - 4.1	0.19	2.0	0.7 - 5.3	0.83	1.2	0.2 - 8.2	0.34	1.8	0.5 - 5.9
no CR	0.04	4.4	1.1 - 18.3	0.004	6.5	1.8 - 23.5	0.13	7.0	0.6 - 88.7	0.03	5.3	1.2 - 23.9
Age (vs. 1-12 years)	0.12			0.13			0.87			0.18		
0.5-1 year	0.26	0.3	0.0 - 2.7	0.23	0.2	0.0 - 2.4	0.997	NA	NA	0.20	0.2	0.0 - 2.3
> 12 years	0.05	0.4	0.1 - 1.0	0.07	0.5	0.2 - 1.1	0.60	0.6	0.1 - 3.4	0.12	0.4	0.2 - 1.2
Treosulfan-Dose (vs. > 3*13 g/sqm)	0.62			0.67			0.70			0.21		
3*11-3*13 g/sqm	0.38	0.7	0.3 - 1.7	0.63	0.8	0.4 - 1.9	0.40	0.5	0.1 - 2.7	0.77	0.9	0.3 - 2.4
<3*11 g/sqm	0.82	1.1	0.4 - 3.6	0.53	1.4	0.5 - 4.3	0.996	0.0	0.0	0.12	2.7	0.8 - 9.4
Conditioning: (vs. + Fludarabine+Thiotepa)	0.03			0.15			0.36			0.68		
Cyclophosphamide±	0.41	1.7	0.5 - 5.9	0.39	1.6	0.5 - 4.8	0.26	4.1	0.4 - 45.7	0.85	1.1	0.3 - 4-0
Fludarabine	0.94	1.0	0.4 - 2.9	0.73	1.2	0.5 - 2.8	0.55	1.9	0.2 - 15.8	0.81	1.1	0.4 - 3.1
other	0.007	4.6	1.5 - 14.1	0.03	2.9	1.1 - 7.9	0.08	5.7	0.8 - 38.5	0.26	2.1	0.6 - 7.4
Donor (vs. other)												
MSD	0.998	1.0	0.4 - 2.6	0.91	1.0	0.5 - 2.4	0.25	0.3	0.0 - 2.2	0.53	1.4	0.5 - 3.6
Stem cell source (vs. pB)	0.43			0.44			0.986			0.23		
BM	0.41	0.7	0.3 - 1.7	0.48	0.7	0.3 - 1.7	0.87	0.9	0.2 - 3.7	0.73	0.8	0.3 - 2.5
CB	0.23	0.4	0.1 - 1.9	0.21	0.4	0.1 - 1.7	0.996	NA	NA	0.66	0.7	0.1 - 3.4

Abbreviations: HR= hazard ratio, CI= confidence interval, HSCT= hematopoietic stem cell transplantation, CR= complete remission, vs= versus, MSD= sibling donor, pB= peripheral blood, BM= bone marrow, CB= cord blood

A

B

C

D