Busulfan-fludarabine- or treosulfan-fludarabine-based myeloablative conditioning for children with thalassemia major

Luftinger, R.; Zubarovskaya, N.; Galimard, J.E.; Cseh, A.; Salzer, E.; Locatelli, F.; ... ; Corbacioglu, S.

Annals of Hematology (2022) 101:655–665
https://doi.org/10.1007/s00277-021-04732-4

ORIGINAL ARTICLE

Busulfan–fludarabine- or treosulfan–fludarabine-based myeloablative conditioning for children with thalassemia major

Roswitha Lüftinger[1] · Natalia Zubarovskaya[1] · Jacques-Emmanuel Galimard[2] · Annamaria Cseh[1] · Elisabeth Salzer[1] · Franco Locatelli[3,4] · Mattia Algeri[3] · Akif Yesilipek[5] · Josu de la Fuente[6] · Antonella Isgrò[7] · Amal Alseraihy[8] · Emanuele Angelucci[9] · Frans J. Smiers[10] · Giorgia La La Nasa[11] · Marco Zecca[12] · Tunc Fisgin[13] · Emel Unal[14] · Katharina Kleinschmidt[15] · Christina Peters[1] · Arjan Lankester[10] · Selim Corbacioglu[15] on behalf of on behalf of the EBMT Pediatric Diseases, Inborn Errors Working Parties

Received: 20 June 2021 / Accepted: 26 November 2021 / Published online: 9 January 2022

Abstract

Significant advances in supportive care for patients with transfusion-dependent thalassemia major (TDT) have improved patients' life expectancy. However, transfusion-associated iron overload remains a significant barrier to long-term survival with good quality of life. Today, allogeneic hematopoietic stem cell transplantation (HSCT) is the current curative standard of care. Alongside selection of the best available donor, an optimized conditioning regimen is crucial to maximize outcomes for patients with TDT undergoing HSCT. The aim of this retrospective analysis was to investigate the role of busulfan–fludarabine-based and treosulfan–fludarabine-based conditioning in TDT patients undergoing HSCT. We included 772 patients registered in the European Society for Blood and Marrow Transplantation (EBMT) database who underwent first HSCT between 2010 and 2018. Four hundred ten patients received busulfan–fludarabine-based conditioning (median age 8.6 years) and 362 patients received treosulfan–fludarabine-based conditioning (median age 5.7 years). Patient outcomes were retrospectively compared by conditioning regimen. Two-year overall survival was 92.7% (95% confidence interval: 89.3–95.1%) after busulfan–fludarabine-based conditioning and 94.7% (95% confidence interval: 91.7–96.6%) after treosulfan–fludarabine-based conditioning. There was a very low incidence of second HSCT overall. The main causes of death were infections, graft-versus-host disease, and rejection. In conclusion, use of busulfan or treosulfan as the backbone of myeloablative conditioning for patients with TDT undergoing HSCT resulted in comparably high cure rates. Long-term follow-up studies are warranted to address the important issues of organ toxicities and gonadal function.

Keywords Hematopoietic stem cell transplantation · Conditioning · Thalassemia major · Busulfan · Treosulfan

Introduction

Hemoglobinopathies such as transfusion-dependent thalassemia major (TDT) and sickle cell disease are the most common recessive monogenic disorder worldwide. TDT is characterized by ineffective erythropoiesis and hemolysis due to deficient β-globin chain production.

Consequently, patients suffer from transfusion-dependent anemia and primary as well as secondary iron overload, leading to severe organ dysfunction [1, 2]. Despite significant advancements in supportive care [3, 4], particularly monitoring and treatment of iron overload and its complications, organ dysfunction progresses over time, resulting in significant morbidity and mortality [5, 6].

The only consolidated and widely available curative option remains allogeneic hematopoietic stem cell transplantation (HSCT), which is the current standard of care [7–10]. Early clinical trials on gene therapy and gene editing as alternative curative options are promising [11–14].

Over the last decades, outcomes of HSCT in patients with TDT have constantly improved, which can be attributed to improvement in risk stratification [15], donor

Roswitha Lüftinger, Natalia Zubarovskaya, Christina Peters, Arjan Lankester, and Selim Corbacioglu contributed equally to the manuscript.

✉ Roswitha Lüftinger
roswitha.lueftinger@stanna.at

Extended author information available on the last page of the article

selection, supportive care, and modified conditioning regimens (including pharmacokinetically adjusted intravenous busulfan (BU) [16].

Despite optimal supportive management, blood transfusions and organ damage from iron overload prior to HSCT predict worse transplantation outcome. Applying the classic Pesaro criteria, treatment-related mortality was 3% versus 8% in patients of Pesaro class 1–2 versus class 3, respectively. Studies have reported a rate of graft rejection of 8 to 12% in pediatric patients with TDT undergoing HSCT [17–19]. Furthermore, hypogonadism with potential loss of fertility may significantly impact on future quality of life and has been reported in 56% of females and 14% of males in a one cohort [20].

Transplant-related acute and long-term complications are mainly due to the intensity of the conditioning regimen. BU, treosulfan (TREO), fludarabine (FLU), and thiotepa (THIO) are used as part of the conditioning regimen for HSCT for malignant and non-malignant disorders. BU is an alkylating agent that is mainly eliminated through the liver. The most common toxicity in patients undergoing HSCT after preparation based on BU is sinusoidal obstruction syndrome. In several studies, hepatotoxicity of BU has been recognized to be dose-dependent [21, 22]. TREO is the prodrug of L-epoxybutane, a water-soluble, bifunctional alkylating agent. Due to its myeloablative and immunosuppressive properties, TREO has been used as preparation for HSCT, showing a reduced risk of hepatic, pulmonary, and nervous system toxicity compared with a BU-based regimen [23]. Subsequently, the safety and efficacy of a reduced-toxicity regimen with a TREO/FLU/THIO backbone have been shown in a large cohort of patients [24–31].

The additive effect of preexisting iron overload and the toxic hepatic and cardiac effects of BU and cyclophosphamide (Cy) (which for many years represented the standard conditioning regimen for HSCT in TDT [32–34]) commonly led to severe acute and long-term organ damage. When reducing the dose of alkylating agents, a high incidence of graft failure resulted in mortality rates of up to 35% and rejection in nearly 30% of high-risk patients [35].

The first trials of myeloablative conditioning regimens with reduced toxicity were conducted in adults with hematological malignancies. TREO, in combination with either Cy or FLU, was first used for HSCT in adult patients with hematologic malignancies considered unfit to receive other myeloablative preparative regimens [36–38]. Good outcomes with respect to toxicity, achievement of complete donor chimerism, low rate of graft-versus-host disease (GVHD), low treatment-related mortality, and low relapse rates were shown [39–41]. Consequently, TREO has increasingly been used for pediatric patients undergoing HSCT for both malignant and non-malignant diseases [27, 42, 43].

The three categories of conditioning regimens—myeloablative conditioning, reduced-intensity conditioning, and non-myeloablative conditioning—are defined based upon the agent, dose, and combination of drugs [32].

To compare outcomes in patients with TDT given either a BU–FLU-based or TREO–FLU-based conditioning regimen for HSCT, we performed a retrospective analysis on behalf of the European Society for Bone and Marrow Transplantation (EBMT) Paediatric Diseases and Inborn Errors Working Parties.

Materials and methods

Study design

This was a retrospective multicenter registry study according to the EBMT guidelines for retrospective studies, non-interventional prospective trials, and prospective clinical trials. EBMT is a society of more than 600 voluntary transplant centers, principally located in Europe. Each center has to report all conducted HSCTs and follow-up data. Informed consent for registration and data collection within the EBMT database was obtained from all patients, their parents, or legal guardians according to the ethical principles of the Declaration of Helsinki. The EBMT statistical office performed the analyses on behalf of the EBMT Paediatric Diseases and Inborn Errors Working Parties.

Participants

Patients included in this study were children aged < 18 years at time of HSCT, receiving a first allogeneic HSCT for TDT between January 2010 and December 2018. Patients who underwent myeloablative BU–FLU-based conditioning or TREO–FLU-based conditioning (regimen declared as myeloablative by the centers following EBMT guidelines: total BU dose > 8 mg/kg, total Treo dose ≥ 30 g/m^2) followed by first HSCT with bone marrow or peripheral blood stem cells (PBSC) from a matched sibling donor, other related donor, or unrelated donor were included. Unrelated donors were classified as matched (10/10 loci) or mismatched at 9/10 or 8/10 loci according to high-resolution HLA allele typing at loci A, B, C, DRB1, and DQ. As this was a retrospective study, the choice of conditioning regimen was at the discretion of treating physicians.

Endpoints

The primary endpoint was overall survival (OS), defined as the probability of survival irrespective of disease state at any point in time. Secondary endpoints were incidence of second HSCT and incidence of neutrophil engraftment.

Time to engraftment was defined as the time elapsing between date of HSCT and that of neutrophil engraftment (which was defined as the first of 3 days with neutrophil count $> 0.5 \times 10^9$/L). All patients without an event were censored at time of last follow-up.

Time to acute GVHD (aGVHD) was defined as first occurrence (date of diagnosis) of aGVHD after HSCT. Time to chronic GVHD (cGVHD) was defined as the first episode of cGVHD (date of diagnosis) after HSCT. aGVHD and cGVHD severity were graded according to the Glucksberg criteria [44].

Statistical analysis

Demographic and baseline characteristics were described by conditioning regimen and for the overall study population. Continuous data were summarized using median and range (minimum, maximum). Categorical data were summarized using the number and percentage. Baseline characteristics of the two conditioning groups were compared using the chi-squared or Fischer's exact test for categorical data, and the Wilcoxon test for continuous data. aGVHD was described as frequency due to missing dates of events. The median follow-up was calculated using the reverse Kaplan–Meier method. The probability of OS was calculated using the Kaplan–Meier method. Neutrophil engraftment, cGVHD, and incidence of second transplant were calculated using the cumulative incidence estimator to accommodate competing risks with death as a competing event. Univariate analyses were done using the log-rank test for OS and Gray's test for cumulative incidence. Multivariate analyses were performed using the Cox proportional-hazards model. Included variables were regimen group, age at transplant, donor type, and year of transplant. The age at transplant was studied as binary variable using the optimal threshold (9.7 years old at transplant). This was according to its impact on OS, which was obtained using the Hothorn and Zeileis method. Results were expressed as the hazard ratio (HR) with a 95% confidence interval (CI). For neutrophil engraftment, HRs were inversed to be coherent with the other outcomes. *P* values were two-sided. Statistical analyses were performed using R 4.0.2 (R Development Core Team, Vienna, Austria) software.

Results

Baseline characteristics

The study included 772 patients. Patient-related information was derived from 91 EBMT-registered transplantation centers located in 42 countries. Patient and donor characteristics, conditioning regimen, stem cell source, and GVHD prophylaxis are provided in Table 1. There were 410 patients in the BU–FLU-based conditioning group and 362 patients in the TREO–FLU-based conditioning group. Median age at HSCT in the BU–FLU-based conditioning group was 8.6 years (range: 0.5, 17.9 years) and in the TREO–FLU-based conditioning group was 5.7 years (range: 0.7, 17.7), with a significant difference ($P < 0.0001$). At transplantation, most patients were in good clinical condition with Karnofsky/Lansky scores > 90, with a higher proportion of scores ≥ 90 in the TREO–FLU-based conditioning group (95.4%) versus BU–FLU-based conditioning group (91.7%) ($P = 0.049$).

In the BU–FLU-based conditioning group, most patients received a combination of BU/Cy/FLU with THIO (122 patients) or without THIO (161 patients). In the TREO–FLU-based conditioning group, nearly all patients received TREO/FLU/THIO for conditioning (353 patients). In both groups, GVHD prophylaxis was based on calcineurin inhibitors with or without in vivo T-cell depletion (anti-thymocyte globulin or alemtuzumab). There was a significantly higher proportion of patients who did not undergo in vivo T-cell depletion in the BU–FLU-based versus TREO–FLU-based conditioning group ($P < 0.0001$). More patients in the BU–FLU-based group received post-transplant Cy as GvHD prophylaxis (69 patients versus 24 patients, respectively).

In the total study population, 26.1% of the male patients were transplanted from a female donor, with nearly the same distribution in each conditioning group ($P = 0.74$). Distribution of donor type was significantly different between groups ($P < 0.0001$). An HSCT was received from a matched sibling donor by 218 patients (53.2%) in the BU–FLU-based conditioning group versus 171 (47.2%) in the TREO–FLU-based conditioning group. For a full breakdown by donor type, see Table 1.

In the total study population, the stem cell source was bone marrow for 80.8% of patients and PBSC for 19.2% of patients. More patients received a PBSC HSCT in the BU–FLU-based versus TREO–FLU-based conditioning group (25.6% versus 11.9%, respectively), whereas more patients received a bone marrow HSCT in the TREO–FLU-based versus BU–FU-based conditioning group (88.1% versus 74.4%, respectively) ($P < 0.0001$).

Outcomes

The median follow-up of all patients was 2.4 (95% CI: 2.1–2.7) years. Two-year OS was 92.7% (95% CI: 89.3–95.1%) in the BU–FLU-based conditioning group and 94.7% (95% CI: 91.7–96.6%) in the TREO–FLU-based conditioning group ($P = 0.22$; Table 2, Fig. 1). In the multivariate analysis, OS was not significantly different for TREO–FLU-based versus BU–FLU-based conditioning (HR: 0.87 [95% CI: 0.45–1.69]; $P = 0.68$; Table 3). Factors

Table 1 Patient and donor characteristics

Characteristic	All TDT patients ($N=772$)	BU–FLU-based conditioning ($n=410$)	TREO–FLU-based conditioning ($n=362$)	*P* value
Time to follow-up, median years (95% CI)	2.4 (2.1–2.7)	1.4 (1.1–1.9)	3 (2.7–3.2)	
Age at HSCT, median (range)	7 (0.5–17.9)	8.6 (0.5–17.9)	5.7 (0.7–17.7)	< 0.0001
Patient sex				
Female	341 (44.5%)	185 (45.7%)	156 (43.1%)	0.47
Male	426 (55.5%)	220 (54.3%)	206 (56.9%)	
Data missing	5	5	0	
TDT type				
β^+ TDT	109 (32.8%)	66 (40%)	43 (25.7%)	
β^0 TDT	202 (60.8%)	92 (55.8%)	110 (65.9%)	
HbE/β TDT	3 (0.9%)	3 (1.8%)	0 (0%)	
HbS/β TDT (sickle cell + TDT)	5 (1.5%)	2 (1.2%)	3 (1.8%)	
Other heterozygous states	13 (3.9%)	2 (1.2%)	11 (6.6%)	
Data missing	440	245	195	
Lansky/Karnofsky scale				
< 90	45 (6.6%)	31 (8.3%)	14 (4.6%)	0.049
≥ 90	634 (93.4%)	341 (91.7%)	293 (95.4%)	
Missing data	93	38	55	
Donor sex				
Female	365 (48%)	200 (49.6%)	165 (46.1%)	0.33
Male	396 (52%)	203 (50.4%)	193 (53.9%)	
Missing data	11	7	4	
Donor type				
Matched sibling donor	389 (50.4%)	218 (53.2%)	171 (47.2%)	< 0.0001
Matched other related donor	98 (12.7%)	61 (14.9%)	37 (10.2%)	
Mismatched related donor	79 (10.2%)	57 (13.9%)	22 (6.1%)	
Fully matched unrelated donor	143 (18.5%)	55 (13.4%)	88 (24.3%)	
Mismatched unrelated donor (8/10 and 9/10 loci)	63 (8.2%)	19 (4.6%)	44 (12.2%)	
Female donor for male recipient				
No	563 (73.9%)	295 (73.4%)	268 (74.4%)	0.74
Yes	199 (26.1%)	107 (26.6%)	92 (25.6%)	
Missing	10	8	2	
Conditioning regimen				
BU/FLU	28 (3.6%)	28 (6.8%)	-	
BU/FLU/Cy	161 (20.9%)	161 (39.3%)	-	
BU/FLU/Cy/THIO	122 (15.8%)	122 (29.8%)	-	
BU/FLU/THIO	99 (12.8%)	99 (24.1%)	-	
TREO/FLU	4 (0.5%)	-	4 (1.1%)	
TREO/FLU/Cy	5 (0.6%)	-	5 (1.4%)	
TREO/FLU/THIO	353 (45.7%)	-	353 (97.5%)	
In vivo T-cell depletion				
No	172 (22.5%)	119 (29.2%)	53 (14.8%)	< 0.0001
Yes	593 (77.5%)	288 (70.8%)	305 (85.2%)	
Missing data	7	3	4	
Ex vivo T-cell depletion				
No	696 (91.1%)	361 (88.7%)	335 (93.8%)	0.01
Yes	68 (8.9%)	46 (11.3%)	22 (6.2%)	
Missing data	8	3	5	
Stem cell source				
Bone marrow	624 (80.8%)	305 (74.4%)	319 (88.1%)	< 0.0001

Table 1 (continued)

Characteristic	All TDT patients ($N=772$)	BU–FLU-based conditioning ($n=410$)	TREO–FLU-based conditioning ($n=362$)	*P* value
Peripheral blood stem cells	148 (19.2%)	105 (25.6%)	43 (11.9%)	
GVHD prophylaxis (calcineurin inhibitor)				
Calcineurin inhibitor monotherapy	58 (8%)	29 (7%)	29 (8%)	
Calcineurin inhibitor plus 1 agent	540 (70%)	263 (65%)	277 (77%)	
Calcineurin inhibitor plus 2 agents	23 (3%)	11 (3%)	12 (3%)	
Calcineurin inhibitor plus 3 agents	2 (1)	-	2 (1%)	
Other	12 (2%)	4 (1%)	8 (2%)	
GVHD prophylaxis (post-transplant Cy)				
Post-transplant Cy monotherapy	4 (0.4%)	3 (0.7%)	1 (0.3%)	
Post-transplant Cy plus 1 agent	17 (2%)	7 (2%)	10 (3%)	
Post-transplant Cy plus 2 agents	71 (8%)	58 (14%)	13 (4%)	
Post-transplant Cy plus ≥ 3 agents	1 (0.3%)	1 (1%)	-	
Missing data	8	4	4	
Anti-thymocyte globulin monotherapy	35 (5%)	30 (7%)	5 (1%)	

BU busulfan; *CI* confidence interval; *Cy* cyclophosphamide; *FLU* fludarabine; *GVHD* graft-versus-host disease; *HSCT* hematopoietic stem cell transplantation; *TDT* transfusion-dependent thalassemia major; *THIO* thiotepa; *TREO* treosulfan

associated with an increased risk of mortality were HSCT performed at ≥ 9.7 years versus < 9.7 years (HR: 2.78 [95% CI: 1.49–5.21]; $P=0.001$) and HSCT from a mismatched related donor (HR: 2.83 [95% CI: 1.21–6.64]; $P=0.02$) or mismatched unrelated donor (HR: 2.99 [95% CI: 1.19–7.52]; $P=0.02$) versus a matched sibling donor. Overall, 43 of 772 patients died due to TDT-associated or HSCT-related complications. Causes of death by conditioning regimen are shown in Table 4.

The 2-year incidence of second HSCT was 4.6% (95% CI: 2.6–7.5%) in the BU–FLU-based conditioning group versus 9% (95% CI: 6.2–12.4%) in the TREO–FLU-based conditioning group ($P=0.051$). In the multivariate analysis, incidence of second HSCT was significantly different between the two groups (for TREO–FLU-based versus BU–FLU-based conditioning, the HR was 2.24 [95% CI: 1.21–4.13%]; $P=0.01$). Receiving an HSCT from a mismatched related donor versus matched sibling donor increased the risk of second HSCT (HR: 4.03 [95% CI: 1.97–8.23], $P=0.001$).

Day-60 neutrophil engraftment was 94.6% (95% CI: 91.9–96.5%) in the BU–FLU-based versus 97.1% (95% CI: 94.6–98.5%) in the TREO–FLU-based conditioning group ($P=0.01$). In the beginning, a faster neutrophil engraftment was observed in the BU–FLU-based conditioning group (data not shown), but at the end of day-60 neutrophil engraftment, the incidence is slightly higher in the TREO–FLU-based group. Multivariate analysis did not show a lower incidence of day-60 neutrophil engraftment in the TREO–FLU-based conditioning group, but after adjustment, we saw a higher risk of non-neutrophil engraftment in the TREO–FLU-based versus BU–FLU-based group (HR: 1.37 [95% CI: 1.17–1.61], $P=0.001$).

Factors associated with a reduced probability of day-60 neutrophil engraftment were HSCT ≥ 9.7 years versus < 9.7 years (HR: 1.22 [95% CI: 1.04–1.43]; $P=0.02$) and an HSCT from mismatched related donor (HR: 1.74 [95% CI: 1.32–2.30]; $P=0.001$) or mismatched unrelated donor (HR: 1.57 (95% CI: 1.18–2.08); $P=0.002$) versus a matched sibling donor. Factors associated with a higher probability of day-60 neutrophil engraftment were more recent year of transplant (HR for 5-year increment: 0.79 [95% CI: 0.68–0.92]; $P=0.003$) and a matched sibling donor versus other matched related donor (HR: 0.71 [95% CI: 0.57–0.89], $P=0.003$).

The overall incidence of grade II aGVHD was 7.7%, grade III aGVHD 5.6% and grade IV aGVHD 2.1% (Table 5). The 2-year incidence of cGVHD was 13.1% (95% CI: 10.3–16.2%, based on 619 patients), and the 2-year incidence of extensive cGVHD was 5.2% (95% CI: 3.5–7.4%, based on 614 patients). Due to missing data, the impact of baseline variables on incidences of cGVHD and extensive cGVHD was not assessed.

Further results of univariate and multivariate analyses are shown in Tables 2 and 3.

Discussion

The only established and widely available curative standard of care for patients with TDT remains allogeneic HSCT. The best outcomes—with survival rates over 90%—can be obtained when HSCT is performed in patients before complications related to frequent blood transfusions such as iron overload, alloimmunization, and adverse effects of chelation

Table 2 Univariate analysis

Variable	Groups	2-year OS, median (95% CI)	2-year incidence of second HSCT, median (95% CI)	60-day incidence of neutrophil engraftment, median (95% CI)
Age at HSCT, years	9.7–18	88.6 (83.4–92.2)	6 (3.3–9.8)	97.8 (95.9.1–98.8)
	<9.7	96 (93.7–97.5)	7.7 (5.3–10.7)	92 (87.9–94.8)
	P value	0.0002	0.71	0.008
Patient sex	Male	94.3 (91.4–96.2)	7 (4.6–10)	94.6 (91.9–96.4)
	Female	92.7 (88.9–95.2)	7.7 (4.8–11.6)	97.3 (94.6–98.6)
	P value	0.48	0.82	0.11
Patient CMV status	Negative	97.5 (94.2–99)	6.3 (3.3–10.6)	97.5 (93.7–99)
	Positive	91.9 (88.9–94.1)	7.9 (5.5–10.8)	95.5 (93.3–96.9)
	P value	0.01	0.67	0.43
Donor sex	Male	94.3 (91.2–96.3)	7.2 (4.7–10.5)	95 (92.2–96.8)
	Female	93.3 (89.8–95.6)	7.4 (4.6–11)	96.9 (94.3–98.3)
	P value	0.64	0.65	0.99
Donor type	Matched sibling donor	95.7 (92.9–97.4)	5.3 (3.1–8.4)	97.6 (95.3–98.8)
	Matched other related donor	92.6 (83.9–96.7)	4.7 (1.2–12.3)	96.8 (93.3–100)
	Mismatched related donor	87.7 (77.5–93.4)	15.6 (8.5–24.7)	80.9 (69.6–88.3)
	Fully matched unrelated donor	94.9 (89–97.7)	6.4 (2.8–12.1)	100
	Mismatched unrelated donor (8/10 and 9/10 loci)	88.2 (76.6–94.2)	12.5 (5.4–22.8)	90.3 (78.9–95.7)
	P value	0.04	0.01	<0.0001
Female donor for male recipient	No	93.8 (91.3–95.7)	7.8 (5.5–10.7)	95.7 (93.6–97.2)
	Yes	93.2 (88.4–96.1)	5.9 (3–10.3)	96.4 (92.3–98.3)
	P value	0.8	0.79	0.92
Conditioning regimen	BU–FLU-based	92.7 (89.3–95.1)	4.6 (2.6–7.5)	94.6 (91.9–96.5)
	TREO–FLU-based	94.7 (91.7–96.6)	9 (6.2–12.4)	97.1 (94.6–98.5)
	P value	0.216	0.051	0.01
In vivo T-cell depletion	No	95.6 (91–97.9)	4.5 (1.8–9.1)	96.9 (94.3–99.6)
	Yes	93 (90.4–94.9)	7.7 (5.5–10.4)	95.6 (93.6–97)
	P value	0.38	0.29	0.04
Ex vivo T-cell depletion	No	93.5 (91.2–95.2)	6.8 (4.8–9.2)	97 (95.4–98.1)
	Yes	93.9 (84.5–97.7)	12.2 (5.6–21.5)	83.6 (71.9–90.7)
	P value	0.93	0.13	0.03
Stem cell source	Bone marrow	94 (91.6–95.7)	6.7 (4.7–9.2)	96.8 (95–98)
	PBSC	92 (85.6–95.7)	9.2 (4.9–15.1)	91.6 (85.5–95.2)
	P value	0.46	0.22	0.01

BU busulfan; *CI* confidence interval; *CMV* cytomegalovirus; *FLU* fludarabine; *HSCT* hematopoietic stem cell transplantation; *OS* overall survival; *PBSC* peripheral blood stem cells; *TREO* treosulfan

therapy emerge [45]. Our data corroborate these findings. In our cohort, patients had a 2-year OS of 93.6%, with a significantly higher 2-year OS observed in patients who were transplanted before the age of 9.7 years. This outcome was independent of the conditioning regimen, with 2-year OS of 92.7% and 94.7% observed in the BU–FLU-based and TREO–FLU-based conditioning groups, respectively. High survival rates reflect the low number of deaths and treatment-related mortality (6% in the BU–FLU-based versus 5% in the TREO–FLU-based conditioning group). This observation is in line with another recently reported cohort [46].

In both conditioning groups, we observed a 60-day incidence of neutrophil engraftment of about 94% and a low incidence of graft failure necessitating second transplantation. Nevertheless, in the TREO–FLU-based conditioning group, significantly more patients required a second HSCT than in the BU–FLU-based conditioning group. Information on indications for second HSCT were not available in the

Fig. 1 Overall survival

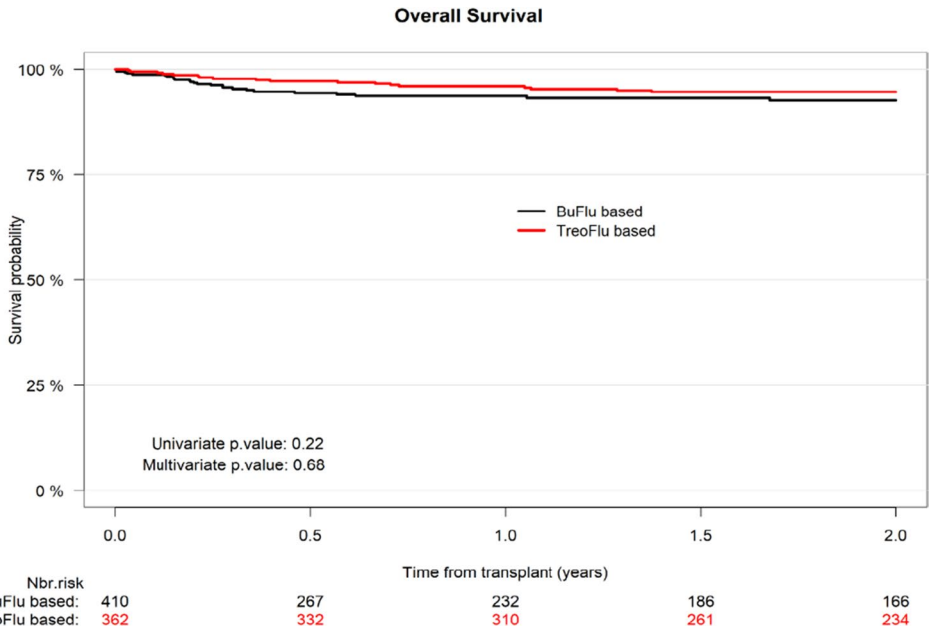

Table 3 Multivariate analysis

Variable	Groups	OS		Incidence of second HSCT		Neutrophil engraftment	
		HR (95% CI)	*P* value	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Conditioning regimen	BU–FLU-based	1		1		1	
	TREO–FLU-based	0.87 (0.45–1.69)	0.68	2.24 (1.21–4.13)	0.01	1.37 (1.17–1.61)	0.001
Age at HSCT, years	< 9.7	1		1		1	
	9.7–18	2.78 (1.49–5.21)	0.001	0.99 (0.55–1.80)	0.98	1.22 (1.04–1.43)	0.02
Donor type	Matched sibling donor	1		1		1	
	Matched related donor	1.90 (0.74–4.90)	0.19	0.67 (0.20–2.24)	0.52	0.71 (0.57–0.89)	0.003
	Mismatched related donor	2.83 (1.21–6.64)	0.02	4.03 (1.97–8.23)	0.001	1.74 (1.32–2.30)	0.001
	Fully matched unrelated donor	1.28 (0.49–3.34)	0.62	0.82 (0.36–1.84)	0.63	1.04 (0.85–1.28)	0.67
	Mismatched unrelated donor (8/10 and 9/10 loci)	2.99 (1.19–7.52)	0.02	1.71 (0.76–3.86)	0.2	1.57 (1.18–2.08)	0.002
Year at HSCT (5-year increment effect)		0.82 (0.44–1.52)	0.52	0.69 (0.39–1.22)	0.20	0.79 (0.68–0.92)	0.003

BU busulfan; *CI* confidence interval; *FLU* fludarabine; HR hazard ratio; *HSCT* hematopoietic stem cell transplantation; *OS* overall survival; *TREO* treosulfan

Table 4 Causes of death

Cause of death	BU–FLU-based conditioning ($n=410$)	TREO–FLU-based conditioning ($n=362$)
Any cause	24	18
Rejection	4	3
Bleeding	4	1
GVHD	5	6
Infection	7	7
Toxicity	2	1
Other	2	0

BU busulfan; *FLU* fludarabine; *GVHD* graft-versus-host disease; *TREO* treosulfan

database, but we assume that the most common reason was primary or secondary graft failure or graft loss. But we are unable to explore the reasoning for a higher rate of second HSCT with TREO–FLU-based conditioning.

Grade II aGVHD occurred in 7.2% of patients in the BU–FLU-based conditioning group and 8.3% in the TREO–FLU-based conditioning group. Severe aGVHD was observed in a low number of patients overall, without a significant difference between conditioning groups (grade III aGVHD occurred in 5.1% of patients in the BU–FLU-based versus 6.2% in the TREO–FLU-based conditioning group, and grade IV aGVHD occurred in 1.6% of patients in the BU–FLU-based versus 2.7% in

Table 5 Grade of acute GVHD

	All TDT ($n=772$)	BU–FLU-based conditioning ($n=410$)	TREO–FLU-based conditioning ($n=362$)
No aGvHD	523 (73.2%)	286 (76.1%)	237 (70.1%)
Grade I	78 (10.9%)	38 (10.1%)	40 (11.8%)
Grade II	55 (7.7%)	27 (7.2%)	28 (8.3%)
Grade III	40 (5.6%)	19 (5.1%)	21 (6.2%)
Grade IV	15 (2.1%)	6 (1.6%)	9 (2.7%)
Grade unknown	3 (0.4%)	0 (0%)	3 (0.9%)
Missing data	58	34	24

BU busulfan; *FLU* fludarabine; *GVHD* graft-versus-host disease; *TDT* transfusion-dependent thalassemia major; *TREO* treosulfan

the TREO–FLU-based conditioning group). Two-year extensive cGVHD occurred in 4.3% of patients (95% CI: 2.2–7.6%) in the BU–FLU-based conditioning group versus 5.8% (95% CI: 3.4–9.0) in the TREO–FLU-based group. Interpretation of these data has to be done carefully as there were missing data.

GVHD has no benefit after HSCT in non-malignant disorders because no graft-versus-leukemia effect is needed. Severe cGVHD is a devastating disease with substantial mortality and high negative impact on health-related quality of life; indeed, the risk of GVHD is one of the most important reasons that some patients or their parents opt out of HSCT in non-malignant disorders [47].

It has been reported that HSCT in TDT protects patients against, potentially reverses, disease-related organ damage [48] and improves health-related quality of life [49]. However, considering that TDT is not an immediately life-threatening disorder, the risks and benefits of HSCT have to be carefully balanced. The reduction of toxicity (especially in the long-term) and other HSCT-related complications (such as aGVHD, cGVHD, and graft rejection) poses a challenge in the choice of the appropriate conditioning regimen. Based on these considerations, there has been an increase in the use of TREO-based conditioning over the years. We hypothesize that the lower hepatotoxicity profile of TREO in comparison to BU [50] and the known high incidence of sinusoidal obstruction syndrome in patients who received the conventional BU-Cy regimen might led treating physicians to favor TREO-based preparative regimens.

In our TREO–FLU-based conditioning group, nearly all patients (97.5%) were treated with TREO/FLU/THIO. Our results indicate that a TREO–FLU-based myeloablative conditioning regimen that includes THIO is effective and well-tolerated for HSCT in patients with TDT. Based on the overall low incidence of a second transplantation in our cohort in contrast to historical data, we speculate that the addition of THIO as well as the choice of post-transplant immunosuppression impact significantly on relevant outcome parameter such as rejection and GVHD.

Another finding of our analysis is that more patients in the TREO–FLU group had a matched unrelated donor than did patients in BU–FLU group and yet 2-year OS and 60-day neutrophil engraftment remained excellent in both conditioning groups. This finding confirms previous reports in smaller (single center) cohorts, concluding that matched unrelated donors should be considered for HSCT in TDT, broadening the donor pool.

The optimal timing for an HSCT should be determined on a case-by-case basis. However, our data confirm that a significantly higher 2-year survival is achieved if patients are transplanted early, in our series < 9.7 years. Based on this finding, we recommend that treating physicians should consider HSCT in patients with TDT early as results with alternative as well as haploidentical donors are steadily improving [51]. Therefore HSCT should be considered also in younger patients lacking a matched donor.

Two strengths of our retrospective registry study are the high number of transplanted TDT patients and the balanced distribution between the two conditioning regimens, which allowed for a well-founded comparison. A limitation of the study is the retrospective character associated with heterogeneity within particular groups and in the lack of detailed data both on dosing and scheduling of chemotherapies and on short-term and long-term toxicity. Data on cGVHD, fertility, and health-related quality of life would have been of special interest because these are among the most important adverse effects of HSCT in non-malignant disorders. Future prospective randomized trials assessing short and long-term toxicity are warranted [52].

In conclusion, our retrospective analysis of a large and international cohort of TDT patients confirms the efficacy and safety of both BU–FLU-based and TREO–FLU-based myeloablative conditioning regimens. TREO, FLU, and THIO are increasingly becoming the standard of care in non-malignant HSCT in Europe and seem to be appropriate for minimizing the risk of short-term life-threatening complications of HSCT in patients with TDT. Moreover we speculate that there is a potential advantage for TREO–FLU-based

conditioning regimen, based on the excellent outcome related to 2-year OS and 60-day neutrophil engraftment and with no significant difference related to incidence of aGVHD and extensive cGVHD between both groups, although more patients in the TREO–FLU group had a matched unrelated donor than did patients in the BU–FLU group.

Early data indicate also a potential advantage for TREO-based conditioning regimens with regard to fertility preservation [53], but longer follow-up is required to evaluate the difference in late complications and fertility. These data could also be interesting in the field of gene therapy, where the intensity and tolerability of the conditioning regimen are important.

References

1. Betts M, Flight PA, Paramore LC et al (2020) Systematic literature review of the burden of disease and treatment for transfusion-dependent beta-thalassemia. Clin Ther 42(322–337):e322. https://doi.org/10.1016/j.clinthera.2019.12.003
2. Borgna-Pignatti C, Cappellini MD, De Stefano P et al (2005) Survival and complications in thalassemia. Ann N Y Acad Sci 1054:40–47. https://doi.org/10.1196/annals.1345.006
3. Angelucci E, Pilo F (2016) Management of iron overload before, during, and after hematopoietic stem cell transplantation for thalassemia major. Ann N Y Acad Sci 1368:115–121. https://doi.org/10.1111/nyas.13027
4. Choudhry VP (2017) Thalassemia minor and major: current management. Indian J Pediatr 84:607–611. https://doi.org/10.1007/s12098-017-2325-1
5. Higgs DR, Engel JD, Stamatoyannopoulos G (2012) Thalassaemia. Lancet 379:373–383. https://doi.org/10.1016/s0140-6736(11)60283-3
6. Pinto VM, Poggi M, Russo R et al (2019) Management of the aging beta-thalassemia transfusion-dependent population - the Italian experience. Blood Rev 38:100594. https://doi.org/10.1016/j.blre.2019.100594
7. Angelucci E, Matthes-Martin S, Baronciani D et al (2014) Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. Haematologica 99:811–820. https://doi.org/10.3324/haematol.2013.099747
8. Anurathapan U, Hongeng S, Pakakasama S et al (2016) Hematopoietic stem cell transplantation for homozygous beta-thalassemia and beta-thalassemia/hemoglobin E patients from haploidentical donors. Bone Marrow Transplant 51:813–818. https://doi.org/10.1038/bmt.2016.7
9. Lucarelli G, Galimberti M, Polchi P et al (1990) Bone marrow transplantation in patients with thalassemia. N Engl J Med 322:417–421. https://doi.org/10.1056/nejm199002153220701
10. Mathews V, Srivastava A, Chandy M (2014) Allogeneic stem cell transplantation for thalassemia major. Hematol Oncol Clin North Am 28:1187–1200. https://doi.org/10.1016/j.hoc.2014.08.009
11. Srivastava A, Shaji RV (2017) Cure for thalassemia major - from allogeneic hematopoietic stem cell transplantation to gene therapy. Haematologica 102:214–223. https://doi.org/10.3324/haematol.2015.141200
12. Frangoul H, Altshuler D, Cappellini MD et al (2021) CRISPR-Cas9 gene editing for sickle cell disease and beta-thalassemia. N Engl J Med 384:252–260. https://doi.org/10.1056/nejmoa2031054
13. Thompson AA, Walters MC, Kwiatkowski J et al (2018) Gene therapy in patients with transfusion-dependent beta-thalassemia. N Engl J Med 378:1479–1493. https://doi.org/10.1056/NEJMoa1705342
14. Marktel S, Scaramuzza S, Cicalese MP et al (2019) Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent ss-thalassemia. Nat Med 25:234–241. https://doi.org/10.1038/s41591-018-0301-6
15. Angelucci E, Pilo F, Coates TD (2017) Transplantation in thalassemia: revisiting the Pesaro risk factors 25 years later. Am J Hematol 92:411–413. https://doi.org/10.1002/ajh.24674
16. Gaziev J, Nguyen L, Puozzo C et al (2010) Novel pharmacokinetic behavior of intravenous busulfan in children with thalassemia undergoing hematopoietic stem cell transplantation: a prospective evaluation of pharmacokinetic and pharmacodynamic profile with therapeutic drug monitoring. Blood 115:4597–4604. https://doi.org/10.1182/blood-2010-01-265405
17. Anurathapan U, Pakakasama S, Mekjaruskul P et al (2014) Outcomes of thalassemia patients undergoing hematopoietic stem cell transplantation by using a standard myeloablative versus a novel reduced-toxicity conditioning regimen according to a new risk stratification. Biol Blood Marrow Transplant 20:2066–2071. https://doi.org/10.1016/j.bbmt.2014.07.016
18. Lucarelli G, Clift RA, Galimberti M et al (1996) Marrow transplantation for patients with thalassemia: results in class 3 patients. Blood 87:2082–2088
19. Mathews V, George B, Deotare U et al (2007) A new stratification strategy that identifies a subset of class III patients with an adverse prognosis among children with beta thalassemia major undergoing a matched related allogeneic stem cell transplantation. Biol Blood Marrow Transplant 13:889–894. https://doi.org/10.1016/j.bbmt.2007.05.004
20. Rahal I, Galambrun C, Bertrand Y et al (2018) Late effects after hematopoietic stem cell transplantation for beta-thalassemia major: the French national experience. Haematologica 103:1143–1149. https://doi.org/10.3324/haematol.2017.183467
21. Slattery JT, Clift RA, Buckner CD et al. Marrow transplantation for chronic myeloid leukemia: the influence of plasma busulfan levels on the outcome of transplantation. Blood 1997; 89: 3055–3060. https://doi-rg.ez.srv.meduniwien.ac.at/10.1182/blood.V89.8.3055
22. Slattery JT, Sanders JE, Buckner CD et al (1995) Graft-rejection and toxicity following bone marrow transplantation in relation to busulfan pharmacokinetics. Bone Marrow Transplant 16:31–42
23. Romanski M, Wachowiak J, Glowka FK (2018) Treosulfan pharmacokinetics and its variability in pediatric and adult patients undergoing conditioning prior to hematopoietic stem cell transplantation: current state of the art, in-depth analysis, and perspectives. Clin Pharmacokinet 57:1255–1265. https://doi.org/10.1007/s40262-018-0647-4
24. Bernardo ME, Piras E, Vacca A et al (2012) Allogeneic hematopoietic stem cell transplantation in thalassemia major: results of a reduced-toxicity conditioning regimen based on the use of treosulfan. Blood 120:473–476. https://doi.org/10.1182/blood-2012-04-423822
25. Bernardo ME, Zecca M, Piras E et al (2008) Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in patients with thalassaemia major. Br J Haematol 143:548–551. https://doi.org/10.1111/j.1365-2141.2008.07385.x
26. Choudhary D, Sharma SK, Gupta N et al (2013) Treosulfan-thiotepa-fludarabine-based conditioning regimen for allogeneic transplantation in patients with thalassemia major: a single-center experience from north India. Biol Blood Marrow Transplant 19:492–495. https://doi.org/10.1016/j.bbmt.2012.11.007
27. Slatter MA, Boztug H, Potschger U et al (2015) Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell

transplantation in children with non-malignant diseases. Bone Marrow Transplant 50:1536–1541. https://doi.org/10.1038/bmt.2015.171
28. Slatter MA, Rao K, Amrolia P et al (2011) Treosulfan-based conditioning regimens for hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience. Blood 117:4367–4375. https://doi.org/10.1182/blood-2010-10-312082
29. Strocchio L, Zecca M, Comoli P et al (2015) Treosulfan-based conditioning regimen for allogeneic haematopoietic stem cell transplantation in children with sickle cell disease. Br J Haematol 169:726–736. https://doi.org/10.1111/bjh.13352
30. Sureda A, Bader P, Cesaro S et al (2015) Indications for allo- and auto-SCT for haematological diseases, solid tumours and immune disorders: current practice in Europe, 2015. Bone Marrow Transplant 50:1037–1056. https://doi.org/10.1038/bmt.2015.6
31. Yerushalmi R, Shem-Tov N, Danylesko I et al (2015) Fludarabine and treosulfan compared with other reduced-intensity conditioning regimens for allogeneic stem cell transplantation in patients with lymphoid malignancies. Bone Marrow Transplant 50:1526–1535. https://doi.org/10.1038/bmt.2015.174
32. Bacigalupo A, Ballen K, Rizzo D et al (2009) Defining the intensity of conditioning regimens: working definitions. Biol Blood Marrow Transplant 15:1628–1633. https://doi.org/10.1016/j.bbmt.2009.07.004
33. Lucarelli G, Isgro A, Sodani P, Gaziev J (2012) Hematopoietic stem cell transplantation in thalassemia and sickle cell anemia. Cold Spring Harb Perspect Med 2:a011825. https://doi.org/10.1101/cshperspect.a011825
34. Lucarelli G, Polchi P, Galimberti M et al (1985) Marrow transplantation for thalassaemia following busulphan and cyclophosphamide. Lancet 1:1355–1357. https://doi.org/10.1016/s0140-6736(85)91784-2
35. Iannone R, Casella JF, Fuchs EJ et al (2003) Results of minimally toxic nonmyeloablative transplantation in patients with sickle cell anemia and beta-thalassemia. Biol Blood Marrow Transplant 9:519–528. https://doi.org/10.1016/s1083-8791(03)00192-7
36. Scheulen ME, Hilger RA, Oberhoff C et al (2000) Clinical phase I dose escalation and pharmacokinetic study of high-dose chemotherapy with treosulfan and autologous peripheral blood stem cell transplantation in patients with advanced malignancies. Clin Cancer Res 6:4209–4216
37. Baronciani D, Depau C, Targhetta C et al (2016) Treosulfan-fludarabine-thiotepa conditioning before allogeneic haemopoietic stem cell transplantation for patients with advanced lympho-proliferative disease. A single centre study. Hematol Oncol 34:17–21. https://doi.org/10.1002/hon.2187
38. Baronciani D, Rambaldi A, Iori AP et al (2008) Treosulfan/fludarabine as an allogeneic hematopoietic stem cell transplant conditioning regimen for high-risk patients. Am J Hematol 83:717–720. https://doi.org/10.1002/ajh.21240
39. Wandt H, Schaefer-Eckart K, Wilhelm M (2005) Two allogeneic hematopoietic stem cell transplantations without the use of blood-product support. Haematologica 90:1292–1294
40. Beelen DW, Trenschel R, Casper J et al (2005) Dose-escalated treosulphan in combination with cyclophosphamide as a new preparative regimen for allogeneic haematopoietic stem cell transplantation in patients with an increased risk for regimen-related complications. Bone Marrow Transplant 35:233–241. https://doi.org/10.1038/sj.bmt.1704784
41. Klaudel-Dreszler MA, Kalwak K, Kurenko-Deptuch M et al (2009) Treosulfan-based conditioning regimen in a second matched unrelated peripheral blood stem cell transplantation for a pediatric patient with CGD and invasive aspergillosis, who experienced initial graft failure after RIC. Int J Hematol 90:571–575. https://doi.org/10.1007/s12185-009-0433-z
42. Wachowiak J, Sykora KW, Cornish J et al (2011) Treosulfan-based preparative regimens for allo-HSCT in childhood hematological malignancies: a retrospective study on behalf of the EBMT pediatric diseases working party. Bone Marrow Transplant 46:1510–1518. https://doi.org/10.1038/bmt.2010.343
43. Boztug H, Sykora KW, Slatter M et al (2016) European Society for Blood and Marrow Transplantation analysis of treosulfan conditioning before hematopoietic stem cell transplantation in children and adolescents with hematological malignancies. Pediatr Blood Cancer 63:139–148. https://doi.org/10.1002/pbc.25764
44. Glucksberg H, Storb R, Fefer A et al (1974) Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 18:295–304. https://doi.org/10.1097/00007890-197410000-00001
45. Peters C (2018) Allogeneic hematopoietic stem cell transplantation to cure transfusion-dependent thalassemia: timing matters! Biol Blood Marrow Transplant 24:1107–1108. https://doi.org/10.1016/j.bbmt.2018.04.023c
46. Li C, Mathews V, Kim S et al (2019) Related and unrelated donor transplantation for beta-thalassemia major: results of an international survey. Blood Adv 3:2562–2570. https://doi.org/10.1182/bloodadvances.2019000291
47. Lertkovit O, Anurathapan U, Hongeng S et al (2021) Chronic graft-versus-host disease in children and adolescents with thalassemia after hematopoietic stem cell transplantation. Int J Hematol 113:556–565. https://doi.org/10.1007/s12185-020-03055-w
48. Muretto P, Angelucci E, Lucarelli G (2002) Reversibility of cirrhosis in patients cured of thalassemia by bone marrow transplantation. Ann Intern Med 136:667–672. https://doi.org/10.7326/0003-4819-136-9-200205070-00009
49. Shenoy S, Angelucci E, Arnold SD et al (2017) Current results and future research priorities in late effects after hematopoietic stem cell transplantation for children with sickle cell disease and thalassemia: a consensus statement from the second pediatric blood and marrow transplant consortium international conference on late effects after pediatric hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 23:552–561. https://doi.org/10.1016/j.bbmt.2017.01.009
50. Danylesko I, Shimoni A, Nagler A (2012) Treosulfan-based conditioning before hematopoietic SCT: more than a BU look-alike. Bone Marrow Transplant 47:5–14. https://doi.org/10.1038/bmt.2011.88
51. Anurathapan U, Hongeng S, Pakakasama S et al (2020) Hematopoietic stem cell transplantation for severe thalassemia patients from haploidentical donors using a novel conditioning regimen. Biol Blood Marrow Transplant 26:1106–1112. https://doi.org/10.1016/j.bbmt.2020.01.002
52. Chaudhury S, Ayas M, Rosen C et al (2017) A multicenter retrospective analysis stressing the importance of long-term follow-up after hematopoietic cell transplantation for beta-thalassemia. Biol Blood Marrow Transplant 23:1695–1700. https://doi.org/10.1016/j.bbmt.2017.06.004
53. Faraci M, Diesch T, Labopin M et al (2019) Gonadal function after busulfan compared with treosulfan in children and adolescents undergoing allogeneic hematopoietic stem cell transplant. Biol Blood Marrow Transplant 25:1786–1791. https://doi.org/10.1016/j.bbmt.2019.05.005

Authors and Affiliations

Roswitha Lüftinger[1] · Natalia Zubarovskaya[1] · Jacques-Emmanuel Galimard[2] · Annamaria Cseh[1] · Elisabeth Salzer[1] · Franco Locatelli[3,4] · Mattia Algeri[3] · Akif Yesilipek[5] · Josu de la Fuente[6] · Antonella Isgrò[7] · Amal Alseraihy[8] · Emanuele Angelucci[9] · Frans J. Smiers[10] · Giorgia La La Nasa[11] · Marco Zecca[12] · Tunc Fisgin[13] · Emel Unal[14] · Katharina Kleinschmidt[15] · Christina Peters[1] · Arjan Lankester[10] · Selim Corbacioglu[15] on behalf of on behalf of the EBMT Pediatric Diseases, Inborn Errors Working Parties

1 Department of Stem Cell Transplantation, St. Anna Children's Hospital, Medical University of Vienna, Kinderspitalgasse 6, 1090 Vienna, Austria

2 EBMT Statistical Unit, Pediatric Diseases Working Party, Paris, France

3 Department of Pediatric Hematology and Oncology, IRCCS Ospedale Pediatrico Bambino Gesù, Rome, Italy

4 Department of Pediatric Sciences, University of Pavia, Pavia, Italy

5 Department of Pediatric Hematology and Stem Cell Transplantation Unit, Medical Park Antalya Hastanesi, Antalya, Turkey

6 Department of Pediatrics, St Mary's Hospital, Imperial College Healthcare NHS Trust, London, UK

7 Fondiazione IME Policlinico Tor Vergata Rome, Rome, Italy

8 Department of Pediatric Hematology Oncology, King Faisal Specialist Hospital and Research Centre, Riyadh, Saudi Arabia

9 Hematology and Transplant Center, IRCCS Ospedale Policlinico San Martino, Genoa, Italy

10 Department of Pediatrics, Division of Immuno-Hematology and Stem Cell Transplantation, Willem-Alexander Children's Hospital, Leiden University Medical Center, Leiden, The Netherlands

11 Centro Trapianti, Unico Di CSE Adulti E Pediatrico A. O Brotzu, Cagliari, Italy

12 Fondazione IRCCS Policlinico San Matteo Pediatric Hematology-Oncology, Pavia, Italy

13 Altinbaş University, Faculty of Medicine, Bahçelievler Medical Park Hospital, Pediatric Bone Marrow Transplantation Unit, Istanbul, Turkey

14 Ankara University Pediatric BMT Unit, Ankara, Turkey

15 Department of Pediatric Hematology, Oncology and Stem Cell Transplantation, University of Regensburg, Regensburg, Germany