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Outcome of treosulfan-based reduced-toxicity conditioning regimens for HSCT in high-risk patients with primary immune deficiencies

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Abstract

Introduction: HSCT is the curative therapeutic option in PIDs. Due to the increase in survival rates, reduced-toxicity conditioning regimens with treosulfan have become another alternative. The purpose of this retrospective study was to analyze the outcome of treosulfan-based conditioning before HSCT for patients with PID.

Method: A total of 15 patients that received a treosulfan-based conditioning regimen for HSCT were recruited. Type of diagnosis, donor and stem cell source, pretransplant organ damage, infections, engraftment, chimerism, and transplant-related toxicities were analyzed.

Results: At a median follow-up time of 32 months, the overall survival was 86.7%. Following HSCT, 14 of 15 patients had engraftment, with 86.7% of the cohort having full-donor chimerism. The most common toxicity was seen on the skin (53.3%). Acute GVHD and chronic GVHD were documented in 53% and 20% of the study population, respectively. Although the cohort consisted of patients with pretransplant liver damage, SOS manifestations were documented in 20%.

Conclusion: Treosulfan-based conditioning regimens before HSCT are associated with lower toxicity compared to myeloablative regimens, are safe, and have high engraftment rates with full-donor chimerism in patients having PID, regardless of the specified genetic diagnosis and donor type.

KEYWORDS

hematopoietic stem cell transplantation, non-myeloablative conditioning, primary immune deficiencies, Treosulfan

1 | INTRODUCTION

HSCT has become the standard of care for certain PIDs. The aim of HSCT in PID was to produce stable donor engraftment after partial or full ablation of the host immunity. Conventional myeloablative regimens consist of busulfan, a myeloablative agent with unpredictable pharmacokinetic characteristics. Busulfan was reported to be associated with SOS and neurotoxicity. Moreover, it is responsible for long-term pulmonary toxicities such as pulmonary fibrosis and interstitial pneumonitis. Conditioning regimens with reduced toxicity have become an inevitable

Abbreviations: ATG, antithymocyte globulin; BM, bone marrow; CsA, cyclosporine A; Cy, cyclophosphamide; DOCK8, dedicator of cytokinesis 8; Flu, fludarabine; G-CSF, granulocyte colony-stimulating factor; GvHD, graft-vs-host disease; Haplo, haploidentical; HSCT, hematopoietic stem cell transplantation; ITK, inducible tyrosine kinase; LAD, leukocyte adhesion deficiency; MFD, matched family donor; MHC, major histocompatibility; MMF, mycophenolate mofetil; MMUD, mismatched unrelated donor; MSD, matched sibling donor; MTX, methotrexate; MUD, matched unrelated donor; PBSC, peripheral blood stem cell; PCR, polymerase chain reaction; PID, primary immune deficiency; SCID, severe combined immunodeficiency; SOS, sinusoidal obstruction syndrome; Treo, treosulfan.

strategy for patients with PID, particularly those with pretransplant infections and organ damage due to inflammatory burden.[1-4]

Treosulfan is a prodrug of an alkylating agent structurally related to busulfan and has similar myelosuppressive and immunosuppressive properties. Having a different mode of alkylation and not being activated by liver enzymes, the potential of treosulfan for causing liver toxicity as well as other tissue damage is reduced.[5-7] It has become an attractive candidate for use in patients with lymphoid and myeloid malignancies and been utilized for standard conditioning regimens before HSCT.[5-7] High engraftment rate and high overall survival were reported with minimal toxicities. Liver toxicity, especially SOS, pulmonary hypertension, interstitial pneumonitis, skin toxicity, mucositis, and seizures were lower compared with traditional combinations of busulfan and Cy. Also, low GvHD rates were observed in previous studies.[2,3,8-12] Treosulfan has increasingly been used for pediatric patients undergoing HSCT for both malignant and non-malignant diseases.[8-10,12]

The purpose of this single-center retrospective study was to analyze the outcome of treosulfan-based conditioning regimens before HSCT for patients with PID. Here, we reviewed the previous studies and reported the results of 15 patients with PID, who selectively underwent HSCT using treosulfan-based conditioning regimens instead of standard busulfan-based ones as a consequence of their pretransplant organ damage.

2 | PATIENTS AND METHODS

We reviewed the results of 15 patients who received a treosulfan-based conditioning regimen for HSCT, between 2008 and 2016. From 1997 to 2016, 128 transplants with diagnosis of PID were performed in 117 patients. Seventy transplants were conducted without conditioning, and busulfan-based myeloablative regimens were preferred in 29. Treosulfan was available in our country after 2008; all patients receiving treosulfan between 2008 and 2016 were recruited in this study. Selection of treosulfan conditioning was based on clinical decision due to a high risk of developing transplant-related toxicity, particularly SOS and infections. As treosulfan was an expensive option and difficult to obtain in our country, patients with previous lung and liver damages were specifically selected for treosulfan conditioning due to high risk of busulfan toxicity in this group of patients. Informed consent was taken from all parents according to the local center and European Blood and Marrow Transplantation guidelines and the Declaration of Helsinki. Patients' demographics, diagnosis, donor match and stem cell source, pretransplant organ damages, infections and engraftment, chimerism, post-transplant organ toxicity, and final outcome are presented in Tables 1 and 2, respectively.

2.1 | Engraftment

Engraftment was defined as the absolute neutrophil count being higher than 0.5×10^9/L and platelet counts higher than 50×10^9/L without transfusion for at least three consecutive days.

2.2 | Chimerism analysis

Donor chimerism was measured using PCR-based amplification of short tandem repeat sequences in DNA of the cells following the separation from peripheral blood samples by Automated Magnetic Cell Sorting (Miltenyi Biotech, Bergisch Gladbach, Germany). Full-donor chimerism was defined if >95% of the cells were originated from the donor. Unsorted PBMC, T-cell, and myeloid cell chimerism analyses are routinely performed, and if needed, B-cell chimerism is also analyzed. Chimerisms were monitored on + first, second, third, sixth, ninth, twelfth, and eighteenth months post-transplant and then on an annual basis.

2.3 | Conditioning regimens and GvHD prophylaxis

Treosulfan was administered at a dose of 42 g/m^2 or 36 g/m^2 in three divided doses in three consecutive days according to the EBMT guidelines. The lower dose was given to eight patients (53%) who were under 1 year of age (median age: 6 months); 42 g/m^2 treosulfan was given to seven patients older than 1 year (median age: 5 years). Treosulfan was combined with either Flu (150 mg/m^2) or Cy (200 mg/kg) (13 and two cases, respectively). Eight patients received only CsA for GvHD prophylaxis. Four patients (three MUD and one Haplo donor) received CsA and MMF, and three patients undergoing transplants from MFD received CsA and MTX for GvHD prophylaxis. CsA was replaced with tacrolimus due to nephrotoxicity in two patients. Additional serotherapy as ATG (n = 2) and alemtuzumab (n = 1) was used in three patients. Alemtuzumab was conventionally not available in Turkey and obtained via compassionate use program. It was administered at a dose of 0.6 mg/kg in a T-B-NK+ SCID patient who underwent HSCT from MUD. One patient having ITK deficiency, who was also diagnosed with EBV-induced Hodgkin lymphoma and was under remission although she had persistent EBV viremia, received rituximab on day −10. All patients received ursodeoxycholic acid during and after the conditioning, and two patients had prophylactic defibrotide. Transplant-related complications (GvHD, infection, mucositis, and other toxicities) were graded according to standard criteria indicated in references.[13-16]

3 | RESULTS

3.1 | Patient characteristics

The median age at HSCT was 12 months (range: 3-180 months). The diagnoses leading to HSCT of the patients were as follows: three SCID, five DOCK8 deficiency, three MHC class 2 deficiency, one CD3ζ-chain defect, one interleukin-2-inducible T-cell kinase (ITK) deficiency, one CD40L deficiency, and one LAD type 3.

Patients were transplanted from six MFD, four MSD, three unrelated (two matched and one mismatch of 9/10), and two Haplo donors. CD34+ stem cell selection and CD3+/CD19+ depletion were

performed for the Haplo transplants. BM and G-CSF-mobilized PBSCs were utilized as stem cell source in 13 (86.7%) and 2 (13.3%) patients, respectively. The two Haplo transplants were performed from PBSC.

Prior to HSCT, hepatic problems were documented in nine patients (Table 1). Cirrhosis was documented in two patients, one of whom had sclerosing cholangitis and the etiology was unknown in the other. One patient had chronic hepatitis B infection, and six patients had previous history for hepatotoxicity. Eleven patients had one or more episodes of viral infections. Six patients had bronchiectasis, and three patients had chronic diarrhea. The etiology of diarrhea was severe inflammatory bowel disease in two patients and cryptosporidium parvum infection in one patient. Nine patients had BCG vaccination before the diagnosis of PID. All patients received ursodeoxycholic acid before and after HSCT.

3.2 | Engraftment

Fourteen of 15 patients had engraftment after the initial HSCT. Median time of engraftment for neutrophils, thrombocytes, and lymphocytes was 13, 13, and 15 days, respectively. Patient P8 underwent Haplo HSCT with CD3+/CD19+ depletion, which was the initial experience of such graft manipulation in our institution performed in line with the study of Slatter et al.[17] He had graft failure on day 60 post-transplant after receiving antituberculosis drugs isoniazid and rifampicin for BCGitis. He had full engraftment following a stem cell boost. Engraftment could be accomplished only after second transplant from a MFD in patient P2, who underwent HSCT initially with CD34+ selection from Haplo donor. BM aspiration revealed hypocellular niche in both patients, and conditioning regimen was not given to patients before the second transplant.

Thirteen patients had full-donor chimerism, and two patients (P8 and P9) had mixed chimera.

3.3 | Survival

In our patient cohort, the median post-transplant follow-up time was 32 months. Two patients died following HSCT making the overall survival 86.7%. P7 who had chronic atelectasis and bronchiectasis caused by recurrent pneumonia prior to HSCT died at 13 months post-transplant due to multiorgan failure following an exacerbation of chronic pulmonary disease. P15 was a SCID patient with Rag1 deficiency. She had chronic renal failure caused by transplant-associated microangiopathy likely secondary to tacrolimus. She had no response to eculizumab and renal replacement therapies. She died following a multidrug-resistant Klebsiella sepsis in intensive care unit at 7 months post-transplant.

3.4 | Toxicity

We did not observe severe treosulfan toxicity in our patients. The most common toxicity was seen on the skin (53.3%). Severe skin irritation was observed in infants, rather than in older patients. Three patients (P7, P8, and P15) had severe perianal dermatitis and ulcers, four patients (P6, P10, P12, and P14) had mild dermatitis, and one patient (P4) had balanitis. Six patients (40%) had grade 1-2, and 2 patients (13.3%) had grade 3 oral mucositis. Seven patients did not have any skin reactions or mucositis. Transplantation-related complications are summarized in Table 3.

Five patients had nausea, and four patients had vomiting; eight patients had mild increase in bilirubin and liver enzyme levels all of which were resolved spontaneously. Five patients had BCG reactivation after HSCT; P6, P7, P12, P13 had BCGitis, while P8 had both BCGitis and extrapulmonary mycobacterial infection. Eleven patients had one or more viral infections before HSCT (Table 1). Seven patients had CMV, and one patient had EBV antigenemia during the engraftment period (Table 3). Two patients (P10 and P11) who had not received Cy for conditioning developed hemorrhagic cystitis following BK virus infection. Both of them were treated with intravesical hyaluronic acid (Table 3).

Acute GvHD grade I-III was documented in 8 patients (53%). Patient P11, who underwent HSCT from 9/10 mismatch unrelated donor, had grade 2 skin and grade 3 intestinal aGvHD. However, aGvHD resolved with tacrolimus, MMF, and mesenchymal stem cells; he then had mild chronic GvHD on skin. Rest of the patients had either grade 1 or grade 2 skin aGVHD. Mesenchymal stem cells were infused to four patients having steroid-resistant aGvHD, and all of them significantly benefited. Chronic GvHD was developed in three patients (20%), two of whom also had acute skin GvHD. The most severe cGvHD was documented in patient P10 with extensive nodular sclerosing cGvHD on skin, causing contractures in joints.

Of nine patients having liver comorbidities, SOS and mild transaminitis were developed in three and two patients respectively. In four patients who had liver damages prior to HSCT, no additional liver problems were documented during or after HSCT. SOS manifestations were observed in three patients (20%). All patients started receiving ursodeoxycholic acid before HSCT. In three patients with DOCK8 deficiency (P10 had chronic liver failure prior to HSCT, P11 had chronic hepatitis B and P12 had moderate transaminitis due to recurrent CMV infection and drug side effects), prophylactic iv defibrotide (25 mg/kg/d) was administered before HSCT; however, grade 4 SOS developed despite prophylaxis. Upon developing SOS, defibrotide dose was increased to 40 mg/kg/d, alongside other clinical interventions such as fluid restriction, close monitoring of electrolyte levels, and coagulation parameters. P10 who had idiopathic chronic liver failure before HSCT exhibited severe hyperbilirubinemia (total bilirubin: 30 mg/dL, direct bilirubin: 22 mg/dL), which was controlled with selective plasmapheresis. Twelve months after HSCT, she underwent liver transplantation from a deceased donor (Table 3).

Pulmonary complications were observed in two patients (13%) who had bronchiectasis before HSCT. P14, patient with ITK deficiency and persistent EBV viremia, had bronchiectasis and underwent segmentectomy due to persistent pulmonary nodules. During the post-transplant period, her symptoms relieved and the FEV/FVC and diffusion capacity improved. P7, patient with CD3 zeta chain

TABLE 1 Transplantation-related data and pretransplant characteristics of patients

Patient ID	Diagnosis	Age at transplant (y)	Pretransplant infections	Pretransplant organ damage	Conditioning regimen	GvHD prophylaxis	Donor/resource of stem cells
P1	CD40L deficiency	4.5	C. parvum infection[a]	Sclerosing cholangitis Cirrhosis	Treo/Cy	CsA	MSD/BM
P2	T-B-NK+ SCID	0.9	Parainfluenza Type 3 pneumonia[a] Rota virus gastroenteritis[a]	Pneumonia (parainfluenza type 3) Hypertransaminasemia	Treo/Flu	CsA	First: Haplo/PBSC[b] Second: MFD/BM
P3	DOCK8 deficiency	6	Cryptosporidium parvum[a]	Hepatic fibrosis Intestinal plasmacytoma Chronic diarrhea	Treo/Flu	CsA	MSD/BM
P4	DOCK 8 deficiency	0.3	Vancomycin resistant enterococcus diarrhea	Chronic diarrhea Hypertransaminasemia	Treo/Flu	CsA	MFD/BM
P5	MHC class II deficiency	0.6	CMV Pneumocystis jirovecii pneumonia	P. jirovecii pneumonia Hypertransaminasemia	Treo/Flu	CsA	MFD/BM
P6	MHC class II deficiency	1	Pseudomonas aeruginosa pneumonia	Necrotizing pneumonia Hepatitis	Treo/Flu	CsA/MTX	MFD/BM
P7	CD3 zeta chain deficiency	1.5	CMV and Parainfluenza Type 3 pneumonia[a]	Bronchiectasis	Treo/Flu/thiotepa	CsA	Haplo/PBSC[b]
P8	T-B-NK+ SCID	0.9	Human rhino virus	Hepatitis	Treo/Cy	CsA/MMF	First: Haplo/PBSC[c] Second: Haplo/PBSC[b]
P9	LAD type 3	0.9	CMV, RSV, Rhinovirus[a]	Viral pneumonia	Treo/Flu	CsA	MSD/BM
P10	DOCK 8 deficiency	6	RSV, HPV, CMV	Cirrhosis Bronchiectasis	Treo/Flu	CsA	MFD/BM
P11	DOCK8 deficiency	15	CMV	Chronic hepatitis B infection Bronchiectasis Celiac disease	Treo/Flu/ATG	CsA/MMF	MMUD/BM
P12	DOCK8 deficiency	5	CMV[a]	Bronchiectasis	Treo/Flu	CsA	MFD/BM
P13	MHC class II deficiency	0.6	Rhinovirus[a]	Bronchiectasis	Treo/Flu	CsA, MTX	MFD/BM
P14	ITK deficiency	6	EBV	Bronchiectasis Granulomatous lung disease Hodgkin lymphoma	Treo/Flu/rituximab/ ATG	CsA, MMF	MUD, BM
P15	T-B-NK+ SCID	0.5	None	None	Treo/Flu/alemtuzumab	CsA, MMF	MUD, BM

[a]Those infections were active at the time of the transplant, and patients received therapies consequently.
[b]Graft manipulation: CD34+ selection.
[c]Graft manipulation: CD3+/CD19+ depletion.

TABLE 2 Post-transplant toxicities, outcome, and follow-up

Patient ID	Diagnosis	Viral reactivation	GvHD/Grade	SOS	Post-transplant organ damage	Chimerism	Outcome/duration of follow-up (mo)
P1	CD40L Deficiency	-	aGvHD/grade 2 skin cGvHD/ mild liver	None	Occasional hypertransaminasemia	97% unsorted cell	Alive, 98 mo
P2	T-B-NK+ SCID	CMV	aGvHD/grade 2 skin and liver	None	Occasional hypertransaminasemia	100% unsorted cell 100% T cell	Alive, 56 mo[a]
P3	DOCK8 Deficiency	-	None	None	None	100% unsorted cell 100% T cell	Alive, 44 mo
P4	DOCK 8 Deficiency	-	None	None	None	97% unsorted cell 97% T cell	Alive, 42 mo
P5	MHC Class II Deficiency	CMV	None	None	None	100% unsorted cell 99% T cell	Alive, 34 mo
P6	MHC Class II Deficiency		aGvHD/grade 1 skin	None	Bronchiectasis	94% T cell	Alive, 34 mo
P7	CD3 Zeta Chain Defect	CMV	aGvHD/grade 2 skin	None	Bronchiectasis, chronic atelectasis Pulmonary hypertension	99% T cell	Died of chronic lung disease and pulmonary hypertension at 13 mo post-transplant
P8	T-B-NK+ SCID	-	None	None	BCGitis, BCG osteomyelitis	64% unsorted cell 96% T cell	Alive, 27 mo[b]
P9	LAD Type 3	CMV	None	None	None	98% unsorted cell 99% T cell	Alive, 22 mo
P10	DOCK 8 Deficiency	CMV, BK	cGvHD/severe intestinal and skin (nodular sclerosing GvHD)	Grade 4 Prophylactic defibrotide	Liver failure Liver transplantation	98% unsorted cell 98% T cell	Alive, 21 mo
P11	DOCK8 Deficiency	CMV, BK	aGvHD/grade 2 skin, grade 3 intestinal cGvHD/mild skin	Grade 4	None	96% unsorted cell 99% T cell	Alive, 16 mo
P12	DOCK8 Deficiency	CMV	aGvHD/grade 2 skin	Grade 4	None	98% unsorted cell 98% T cell	Alive, 12 mo
P13	MHC Class II Deficiency	-	None	None	None	98% unsorted cell 99% T cell	Alive, well 9 mo
P14	ITK Deficiency	EBV	aGvHD/grade 2 skin	None	Bronchiectasis	96% unsorted cell 97% T cell	Alive, well 8 mo
P15	T-B-NK+ SCID	-	aGvHD/grade 2 skin and liver	None	Tacrolimus-associated thrombotic microangi-opathy End-stage renal disease	Unsorted cell 99% 99% T cell	Died of chronic renal failure and multidrug-resistant Klebsiella sepsis at 7 mo post-transplant

[a]P2 first underwent Haplo HSCT with CD34+ selection but did not have engraftment. He then had second transplant from MFD, by which engraftment was achieved.
[b]P8 underwent Haplo HSCT initially with CD3+/CD19+ depletion and had a graft failure on day 60 post-transplant. He had full engraftment following a stem cell boost.

deficiency, had severe lung damage and bronchiectasis due to persistent CMV before HSCT. She developed pulmonary hypertension and had respiratory failure 13 months after HSCT.

Five patients (33%) required admission to the intensive care unit during their pre-/post-transplant hospital course. Before HSCT, two patients (P13 and P5) with severe pneumonia and one patient with severe necrotizing pneumonia (P6) needed admittance to intensive care unit. All three patients responded to supportive therapies in the ICU and thereafter HSCT ensued. During the post-transplant period, other two patients (P7 and P15) required intensive care support due to respiratory failure and renal insufficiency, both died of multiorgan failure as described above.

4 | DISCUSSION

HSCT has become the lifesaving therapeutic option in many PIDs for more than 30 years. As a result of the improvement in prognosis and increase in survival rates, toxicities of HSCT are of greater concern. Reduced toxicity regimens are being preferred for lower rates of acute and long-term complications. In previous studies, treosulfan-based conditioning protocols used in children with PID were shown to have high engraftment rates and high overall survival around 80% without the complications associated with traditional myeloablative regimens.[8-10,12,18,19] In our study group, at 32 months of follow-up time, overall survival is 86.7%. Following HSCT, 14 of 15 patients had engraftment, with 86.7% of the cohort having full-donor chimerism.

In this retrospective study, we evaluated the transplant-related toxicity profiles and outcomes of 15 patients with PID who received treosulfan-based conditioning regimens before HSCT. Our study was conducted in a patient cohort having a variety of specific defects leading to PIDs. The selection of conditioning was based on clinical decision due to a high risk of developing transplant-related toxicity, predominantly SOS and infections. Patients with previous organ damages, particularly liver and lung, were specifically recruited for treosulfan use. Although our study group was composed of high-risk patients having various diagnoses of PID, the overall survival documented is similar to former studies.[8-10,12,19]

Major toxicities were not reported in patients receiving treosulfan-based regimens. Similarly, toxicities were low in our study; however, minor skin complications were more frequent in our patients compared to other groups. Skin toxicity was also the most common adverse effect attributed to treosulfan, reported as high as 49% of the patients in the previous studies.[8,9,19] Skin rashes with perianal ulcers and exfoliative diaper dermatitis were the common presentations of dermatological toxicity. In our group, skin toxicity was documented in 53.3% of the patients and severe perianal ulceration was seen in three patients. It is known that the perianal dermatitis is likely due to the urinary excretion of active treosulfan metabolites, but resolves with local treatment, barrier creams, and pain relief. Although we used intensive skin care and frequent bathing (four times a day), skin reactions were more frequent in our patients, especially in the younger babies under 1 year of age. We could not specify a reason for this observation, but it may be because of genetic variation in our patient cohort causing susceptibility to skin reactions with treosulfan metabolites.

TABLE 3 Transplantation-related complications

	n (%)
Second transplantation	2 (13.3)
Secondary engraftment failure	1 (6.7)
Primary engraftment failure	1 (6.7)
Deaths	2 (13.3)
Survival	13 (86.7)
Skin reactions	8 (53.3)
Mucositis	8 (53.3)
Pulmonary complications	2 (13.3)
Seizures/peripheral neuropathy	None
Acute GvHD	8 (53.3)
Chronic GvHD	3 (20.0)
Vomiting/diarrhea < grade 3	9 (60.0)
SOS	3 (20)
Hemorrhagic cystitis	2 (13.3)
BCGitis	5 (33.3)
Viral reactivations after HSCT	8 (53.3)
CMV PCR	7 (46.6)
EBV PCR	1 (6.7)
BK virus PCR	2 (13.3)[a]

[a]Patients with BK virus were also positive for CMV PCR.

In line with the other studies, we did not observe any neurotoxicity or cardiotoxicity documented during the other myeloablative regimens.

Rates of GvHD were generally reported low in several preceding studies. Slatter et al[8] observed aGvHD in 26% of patients. The incidence of grade 1-2 aGvHD was 19% and that of grade 3-4 was 6% in the study of Greystoke et al[9] Four of their patients had chronic GvHD. In the study of Dinur-Schejter et al,[10] which recruited 44 patients with non-malignant diseases, 27 patients had PID and severe pretransplant toxicity. GVHD was observed in 44% of total patient cohort. Burroughs et al[12] reported that grade 2 to 4 aGVHD occurred in 62%.

In the current study, we observed acute GvHD in 53.3% (8/15) of the patients, mostly grade 1-2. Chronic GvHD developed in three patients (20%). Although P10 did not have acute GvHD, she had chronic skin and intestinal GvHD. She had an idiopathic chronic hepatic failure before the HSCT, which led her to liver transplantation following HSCT. She is currently functional under tacrolimus treatment. P11 had aGvHD (skin and intestinal) and limited chronic skin GVHD. This may be explained with unrelated mismatched (9/10) donor transplantation. Our acute GvHD rates are higher than other studies, but our cohort consisted of heterogeneous group of patients with relatively high pretransplant organ damages, which may

cause an inflammatory burden leading to aGvHD. None of our patients died of GVHD.

We selected the conditioning regimen based on clinical decision, and pretransplant liver damage was a factor to prefer treosulfan- rather than busulfan-based regimens. Busulfan has a variable metabolism, and its inactivation requires hepatic conversion of metabolites. The drug clearance is also age-dependent, which results in unpredictable toxicities. The stable pharmacokinetics of treosulfan and the low rate of liver toxicity were important factors in the selection of conditioning. Our study group consisted of patients with higher risk of chronic liver impairment following HSCT. One of our patients even underwent liver transplantation following HSCT. Consequently, there is a selection bias in our patient cohort regarding the liver impairment. Nine of our patients (56%) had to some extent liver damage. Four patients had severe liver damages, and SOS was observed in three of them. Three of the patients with SOS received defibrotide, and all of them responded to therapy. In the two previous studies, not reporting any SOS cases, they did not include patients with severe liver damage.[11,12] In a retrospective analysis of HSCTs for non-malignant diseases registered in the EBMT database, SOS was observed in 5% of the patients receiving treosulfan-based conditioning regimen.[18] In another retrospective study consisting of 109 HSCTs for both malignant and non-malignant disorders, hepatic SOS was documented in only three patients (2.8%).[19] Concisely, treosulfan is a good therapeutic option for conditioning in patients with high risk of liver damage.

The viral reactivation rate was 53.3% in our study similar to the other studies.[8,10,12]

Intensive care unit admissions were required during pre- and post-transplant periods in three and two patients, respectively. P7 had preexisting respiratory impairment due to recurrent CMV and parainfluenza type 3 infections, and BCGitis during the pretransplant period. She died in the +13th month due to respiratory impairment and heart failure, although she had full-donor chimerism. P15 was a SCID patient with Rag1 deficiency. She encountered with transplant-associated thrombotic microangiopathy due to tacrolimus toxicity. She had full-donor chimerism, but succumbed to gram-negative bacterial sepsis.

Here, we reported our single-center experience regarding the treosulfan-based conditioning regimens before HSCT in our patients with PID, having a variety of diagnoses. From 1997 to 2016, of 128 transplants, 29 received busulfan-based myeloablative regimens whereby the overall survival rate was recorded as 75.8%, which is significantly lower than reduced toxicity treosulfan regimen (Table S1). Compared to myeloablative regimens, we did not observe conditioning-associated morbidity frequently although the patients receiving treosulfan had a high prevalence of pretransplant morbidity, particularly liver problems and serious infections. As a study from single center, we have limited number of patients from a heterogeneous background compared to previous multicentered data. However, as a result of our experience we suggest that treosulfan-based conditioning regimens before HSCT are associated with lower toxicity compared to myeloablative regimens, are safer, and have high engraftment rate with full-donor chimerism in patients having PID, regardless of the specified genetic diagnosis and donor type. Long-term follow-up is required for determining late effects of chemotherapy.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest and source of funding regarding the publication of this article.

AUTHORS' CONTRIBUTION

ŞH, EFD, and Aİ: Designed the study; ŞH, SKB, Cİ, and TK: Collected the data; ŞH and SKB: Wrote the manuscript; and DA, TK, EFD, and Aİ: Revised the manuscript.

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REFERENCES

1. Chiesa R, Veys P. Reduced-intensity conditioning for allogeneic stem cell transplant in primary immune deficiencies. *Exp Rev Clin Immunol*. 2012;8(3):255-266; quiz 67.
2. Barrett AJ, Savani BN. Stem cell transplantation with reduced-intensity conditioning regimens: a review of ten years experience with new transplant concepts and new therapeutic agents. *Leukemia*. 2006;20(10):1661-1672.
3. Baronciani D, Depau C, Targhetta C, et al. Treosulfan-fludarabine-thiotepa conditioning before allogeneic haemopoietic stem cell transplantation for patients with advanced lympho-proliferative disease. A single centre study. *Hematol Oncol*. 2016;34(1):17-21.
4. Alousi A, de Lima M. Reduced-intensity conditioning allogeneic hematopoietic stem cell transplantation. *Clin Adv Hematol Oncol*. 2007;5(7):560-570.
5. ten Brink MH, Zwaveling J, Swen JJ, Bredius RG, Lankester AC, Guchelaar HJ. Personalized busulfan and treosulfan conditioning for pediatric stem cell transplantation: the role of pharmacogenetics and pharmacokinetics. *Drug Discov Today*. 2014;19(10):1572-1586.
6. Ten Brink MH, Ackaert O, Zwaveling J, et al. Pharmacokinetics of treosulfan in pediatric patients undergoing hematopoietic stem cell transplantation. *Ther Drug Monit*. 2014;36(4):465-472.
7. Sjoo F, Hassan Z, Abedi-Valugerdi M, et al. Myeloablative and immunosuppressive properties of treosulfan in mice. *Exp Hematol*. 2006;34(1):115-121.
8. Slatter MA, Rao K, Amrolia P, et al. Treosulfan-based conditioning regimens for hematopoietic stem cell transplantation in children with primary immunodeficiency: United Kingdom experience. *Blood*. 2011;117(16):4367-4375.
9. Greystoke B, Bonanomi S, Carr TF, et al. Treosulfan-containing regimens achieve high rates of engraftment associated with low transplant morbidity and mortality in children with non-malignant disease and significant co-morbidities. *Br J Haematol*. 2008;142(2):257-262.
10. Dinur-Schejter Y, Krauss AC, Erlich O, et al. Bone marrow transplantation for non-malignant diseases using treosulfan-based conditioning. *Pediatr Blood Cancer*. 2015;62(2):299-304.
11. Casper J, Knauf W, Kiefer T, et al. Treosulfan and fludarabine: a new toxicity-reduced conditioning regimen for allogeneic hematopoietic stem cell transplantation. *Blood*. 2004;103(2):725-731.

12. Burroughs LM, Nemecek ER, Torgerson TR, et al. Treosulfan-based conditioning and hematopoietic cell transplantation for nonmalignant diseases: a prospective multicenter trial. *Biol Blood Marrow Transplant*. 2014;20(12):1996-2003.
13. Bensinger W, Schubert M, Ang KK, et al. NCCN Task Force Report prevention and management of mucositis in cancer care. *J Natl Compr Canc Netw*. 2008;6(Suppl. 1):S1-S21; quiz S2-4.
14. Harris AC, Young R, Devine S, et al. International, multicenter standardization of acute graft-versus-host disease clinical data collection: a report from the Mount Sinai acute GVHD International Consortium. *Biol Blood Marrow Transplant*. 2016;22(1):4-10.
15. Lee SJ. Classification systems for chronic graft-versus-host disease. *Blood*. 2017;129(1):30-37.
16. Mohty M, Malard F, Abecassis M, et al. Revised diagnosis and severity criteria for sinusoidal obstruction syndrome/veno-occlusive disease in adult patients: a new classification from the European Society for Blood and Marrow Transplantation. *Bone Marrow Transplant*. 2016;51(7):906-912.
17. Slatter M, Nademi Z, Patel S, et al. Haploidentical hematopoietic stem cell transplantation can lead to viral clearance in severe combined immunodeficiency. *J Allergy Clin Immunol*. 2013;131(6):1705-1708.
18. Slatter MA, Boztug H, Potschger U, et al. Treosulfan-based conditioning regimens for allogeneic haematopoietic stem cell transplantation in children with non-malignant diseases. *Bone Marrow Transplant*. 2015;50(12):1536-1541.
19. Beier R, Schulz A, Honig M, et al. Long-term follow-up of children conditioned with Treosulfan: German and Austrian experience. *Bone Marrow Transplant*. 2013;48(4):491-501.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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