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ARTICLE

Allogenic peripheral stem cell transplantation from HLA-matched related donors for adult sickle cell disease: remarkable outcomes from a single-center trial

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Abstract

Adult patients with sickle cell disease (SCD) are highly susceptible to stem cell transplant complications, including drug toxicity, graft versus host disease (GVHD), and graft rejection due to SCD-related tissue damage, endothelial activation, and inflammation. The scarcity of compatible stem cells for transplantation further limits treatment options, with only 43 cases of adult allogeneic peripheral blood stem cell transplantation (allo-PSCT) from human leukocyte antigen (HLA)-identical sibling donors reported in the international registry for the period 1986–2013. Herein we report remarkable outcomes in a cohort of adult SCD patients who underwent allo-PSCT using a fludarabine (Flu), busulfan (Bu), and anti-T-cell lymphocyte globulin (ATG)-based conditioning regimen in combination with very low dose total body irradiation (TBI), followed by post-transplant cyclophosphamide (Cy) and sirolimus as GVHD prophylaxis. We performed a single-center, retrospective study consisting of 20 consecutive patients (mean age 33.4 years) who underwent allo-PSCT from HLA-matched related donors with a conditioning regimen of Flu 150/Bu 3.2/Cy 29/ATG 30 (Fresenius)/TBI 200 between September 2013 and September 2017. Data were validated by an independent data audit group of the affiliated JACIE-accredited transplantation center. All patients experienced a sustained donor cell engraftment. Full donor chimerism (total cell) occurred within 180 days in all patients. Mean duration of follow-up was 13.8 months (range: 0.3–50 months), with 12 (60%) patients completing 12 months. No non-relapse mortality or graft rejection occurred. Successful treatment was achieved without the presence of graft loss, grade III–IV acute GVHD, extensive chronic GVHD, or other major complications. Allo-PSCT in combination with Flu 150/Bu 3.2/Cy 29/ATG 30(Fresenius)/TBI 200- Cy/Sirolimus therapy yielded encouraging outcomes with no mortality and low incidence of GVHD. Further controlled studies will be necessary to compare transplant protocols and long-term outcomes.

Introduction

Sickle cell disease (SCD) is a single gene disease caused by abnormal hemoglobin S production, resulting in vaso-occlusive crises, chronic hemolysis, and organ damage [1]. Left untreated, SCD can result in impaired quality of life (QoL), with a high incidence of morbidity and early mortality [2–4]. The pathogenesis of the disease is quite complex, mediated by rigid erythrocytes arising in an anoxic environment that results in micro-vascular ischemia, endothelial activation, and inflammation through an interaction between blood cells and endothelial cells [5]. Management of SCD is often challenging due to the inflammatory, multi-system nature of the disease, driven by an underlying genetic condition. In many cases, it is classified as a malignant condition due to its poor clinical outcomes

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despite the availability of hydroxyurea and other supportive treatments in adults [4–6].

While some may argue that gene therapy is among the curative treatment modalities for SCD, given that it is still an experimental approach allogeneic hematopoietic stem cell transplantation (Allo-HSCT) is the only available curative treatment of SCD. Allo-HSCT performed using bone marrow and cord blood from a human leukocyte antigen (HLA)-matched, related donor with ablative conditioning regimens including busulfan (Bu) and cyclophosphamide (Cy) has yielded successful outcomes in pediatric patients, with curative treatment achieved in >90% of cases. This is also valid for non-myeloablative regimens in childhood [7, 8]. It is therefore recommended for all preschool-aged patients with an eligible donor [7–11]. In contrast, allo-HSCT is substantially more difficult in adult patients, with significantly greater risk of organ damage and other complications [12–14]. Furthermore, the availability of HLA-matched stem cell donors is very low, severely limiting the transplant options in adult patients. As of 2013, only 154 cases of allo-HSCT from adult matched related/sibling donors for SCD have been reported in the European Society for Blood and Marrow Transplantation (EBMT) and Center of International Blood and Marrow Transplantation Registry (CIBMTR) [9], with only 11 adult patients listed in the 2015 registry [14]. Among these cases, graft versus host disease (GVHD), graft loss, infections, endothelial activation, and inflammation continue to be a problem [13–17]. Therefore, assessment of indications for allogeneic peripheral blood stem cell transplantation (allo-PSCT) and risk analysis are required in adult patients.

Successful outcomes have recently been reported with chemotherapy or alemtuzumab-based non-myeloablative conditioning in adults [18, 19]. To date, only a limited number of cases have been reported; graft rejection continues to be a problem, along with difficulties when terminating immune-suppressive therapies [16, 17]. Here we report the outcomes of SCD patients who underwent allo-HSCT with peripheral stem cells (allo-PSCT), using total body irradiation (TBI) in combination with a fludarabine (Flu), Bu, and anti-T-cell lymphocyte globulin (ATG)-based conditioning regimen, followed by posttransplant Cy and sirolimus as GVHD prophylaxis.

Patients and methods

Study design

This single-center, retrospective study was conducted between September 2013 and October 2017. A total of 20 consecutive adult SCD patients [mean age 33.4 years; range: 20–45 years] who underwent allo-HSCT using peripheral stem cells (allo-PSCT) from HLA-matched relative donors were included. Patients were treated by adding TBI to a Flu, Bu, ATG-based non-myeloablative conditioning regimen, with the combination of post-transplant Cy and sirolimus used for GVHD prophylaxis.

Data were obtained from forms created to evaluate transplant patients; the forms met the Joint Accreditation Committee: International Society for Cellular Therapy and European Blood and Marrow Transplantation (JACIE) criteria for the Nucleus electronic data management system (ver. 9.3.39; Monad Software Co., Ankara, Turkey). All data were verified by an independent data audit group. Clinical data included patient age, gender, diagnosis, treatment with hydroxyurea, number of painful crises per year, transfusion requirement, history of red cell exchange, acute chest syndrome, renal damage, heart failure, pulmonary hypertension, pulmonary thromboembolism, deep venous thrombosis, cerebrovascular events, and vaccination scheme. Data also included treatment responses, disease status, and side effects, arranged in accordance with the Common Toxicity Criteria of the National Cancer Institute (v.4.0). Laboratory findings consisted of steady-state hemoglobin (Hgb) level, leukocyte and platelet counts, the ratio of abnormal Hgb (Hgb S, Hgb F, Hgb A2) to total Hgb, iron status, cardiac and hepatic iron concentration (analyzed by T2* magnetic resonance imaging, MRI), cranial perfusion MRI, pulmonary arterial pressure, and tricuspid jet regurgitation velocity on echocardiography.

The primary end points were successful engraftment and overall survival (OS) at 1 year. Secondary end points included graft rejection at 1 year, cumulative incidence of GVHD, non-relapse mortality (NRM) at 1 year, proportion of patients for whom immunosuppressive drugs could be discontinued by year 1, and QoL.

Patients reporting concurrent systemic inflammatory disease or organ failure, severe infections 1 month prior to commencement of the conditioning regimen, pregnancy, breast feeding, and those under 18 years of age were excluded from this study. Local ethics committee approval was obtained prior to initiation of the study (KA17/162). Written and verbal informed consent was obtained from all patients.

Patient/donor selection

All patients were of Eti-Turk origin and had been diagnosed with homozygous Hgb S or heterozygous combinations of Hgb S and thalassemia (Hgb S-β thalassemia, Hgb S-α thalassemia) by Hgb electrophoresis or genetic screening. Patients were evaluated for transplant indications based on the SCD high-risk group criteria of the bone marrow transplantation list of the Social Security Institution of Turkey (https://organ.saglik.gov.tr/). Indications for

transplant included two or more of the following criteria: frequent painful crises despite hydroxyurea treatment (>3/year), pulmonary hypertension, acute neurological attack, acute chest syndrome while on hydroxyurea, silent cranial ischemia, widespread osteo-necrosis, and allo-immunization. Donors were selected in accordance with the standard operating procedure for donor selection and assessment (SOP: KIT-KU-003). Donors included both HLA 10/10-matched Hgb S trait and non-Hgb S trait relatives. Once a donor was identified, a second round of HLA confirmatory testing of the recipient was performed. Major, minor, or bidirectional ABO mismatches were permitted as necessary. Donor follow-up was performed in accordance with SOPs.

Definitions

Patients who had not required medication to treat painful conditions for the previous 4 weeks were considered to have steady-state disease. A painful crisis was defined as the need for hospital admission due to pain not related to any cause other than SCD or for the use of parenteral non-steroidal anti-inflammatory drugs, metamizol, or narcotics for SCD-related pain treatment. The crisis frequency was defined as rare if the patient experienced fewer than three painful crises a year and frequent if the number of yearly painful crises was three or more. Microalbuminuria was defined as 30–300 mg microalbumin per day in a spot urine test in an afebrile patient with episodes of pain. Nephropathy was defined as the presence of at least one indicator of renal dysfunction, such as microalbuminuria and proteinuria, hyperechogenicity and/or thinning of the renal cortex on ultrasonography, and low creatinine clearance. Pulmonary hypertension was defined as a mean resting pulmonary artery pressure of >30 mm Hg following exercise and tricuspid regurgitant jet velocity >2.5 m/s at least 3 weeks after a vaso-occlusive crisis. Hydroxyurea use was defined as the regular use of minimum 15 mg hydroxyurea/kg/day for at least 1 month. Successful engraftment was defined as normalization of cell counts in the peripheral blood, absence of Hgb S in patients who received stem cells from a non-Hgb S trait relative donor, <40% Hgb S (relative to total Hgb) in patients who received product from an Hgb S trait donor, and full total peripheral blood cell chimerism within 100 days.

Preparation for transplant

Patients and donors were vaccinated against seasonal influenza; patients also completed pneumococcal, meningococcal, and *Haemophilus influenzae* vaccinations before transplant. Once a decision for transplantation was made, patients were scheduled to undergo an exchange transfusion to decrease Hgb S concentrations to <30% of total Hgb before commencement of conditioning regimen. Patients who could not undergo exchange transfusion due to allo-immunization and who underwent plasma exchange started conditioning regimen with >90% Hgb S of total hemoglobin. Iron chelation was stopped ≥48 h before initiating the conditioning regimen. Hydroxyurea was discontinued 1 day before the conditioning regimen.

Stem cell collection

All donors were given 10 µg/kg/day filgrastim (Neupogen; Amgen-Roche, Thousand Oaks, CA, USA) in two equal doses for 5 days prior to peripheral stem cell collection, with the aim of mobilizing stem cells from the bone marrow into the peripheral blood. An apheresis device employing a continuous flow technique (Cobe Spectra V. 7.0; Terumo BCT, Lakewood, CO, USA) was used to collect stem cells.

Transplantation procedure

Patients received a non-myeloablative preparative of Cy (Endoxan) at a dose of 14.5 mg/kg on days −8 and −7, Flu (Fludara) 30 mg/kg on days −7 to −3, Bu (Bisulfex) 0.8 mg/kg BID on days −4 and −3, and rabbit anti-T lymphocyte globin (Grafalon, Fresenius, Germany) 5 mg/kg on days −7 to −5, followed by 200 cGy TBI given as a single dose on day −1. Sirolimus (Rapamune) was started on day −2 at a dose of 12-mg oral loading dose, followed by 4 mg per day orally in a single morning dose with the goal of maintaining drug levels between 10 and 15 ng/mL. The peripheral blood stem cell graft was targeted to harvested $\geq 8 \times 10^6$ CD34+ cells/kg. All patients received post-transplant Cy of 33 mg/kg/day intravenously on days +3 and +4.

Supportive care

All patients were followed-up in rooms with a laminar airflow; irradiated, leukocyte-free blood products were used when transfusions were needed. Hemoglobin values were tried to be kept around 9–10 g/L during the transplantation process except alloimmunization. The threshold platelet level was $<50 \times 10^9$ cells/L for administration of concentrated platelets. Chemotherapy and anti-microbial drug use were conducted in accordance with SOPs, as set forth by JACIE guidelines (SOP: BMT-CU 032). Patients received acyclovir for herpes prophylaxis, sulphometoxazole for *Pneumocystis jirovecii* prophylaxis, and empirical sefalosporin-clavulonate and carbapenem until microbiological data were obtained if febrile neutropenia was evident; glycopeptides were given when Gram-positive bacterial infection was suspected. Viral and antifungal

prophylaxis was delivered in accordance with S, a JACIE-accredited SOP (BMT-TU 002). Pre-emptive gancyclovir was given to patients exhibiting cytomegalovirus (CMV) reactivation.

Posttransplant follow-up and GVHD assessment

Time to neutrophil engraftment was defined as the first of 3 consecutive days on which the absolute neutrophil count exceeded 0.5×10^9 cells/L. Time to platelet engraftment was defined as the first of 2 days with 50×10^9 cells/L without the need for platelet transfusion during a 5-day period. Sirolimus was prescribed for a minimum of 1 year for GVHD prophylaxis. The total cell chimerism was assessed by PCR analysis of the peripheral blood on days 30, 100, and 180 and at 1 year. Sirolimus was discontinued once patients exhibited full donor chimerism without GVHD. Women with childbearing potential were informed of the potential risks of pregnancy. Sunlight and ultraviolet light protection were advised while patients were taking sirolimus. Acute GVHD (aGVHD) was evaluated according to standard criteria [20, 21]. The diagnosis of chronic GVHD (cGVHD) was made on the basis of both clinical and histology criteria of the skin and other affected sites, as previously described [22, 23]. cGVHD was defined as any GVHD present after day 100. cGVHD was further defined as progressive onset if it manifested as a prolongation of any type of aGVHD, quiescent-onset if it developed after the resolution of aGVHD, and de novo onset if it was not preceded by an episode of aGVHD. Patients with grade II–IV aGVHD or cGVHD were primarily treated with sirolimus and a corticosteroid-based regimen (2 mg/kg/day). Second-line immunosuppressive therapy was defined as the initiation of secondary systemic immunosuppressive treatment replacing, or in addition to, primary first-line systemic therapy due to refractory or progressive GVHD. Grade II–IV patients also received extracorporeal photopheresis as a second-line therapy, in accordance with guidelines set forth by the Centers for Disease Control and Prevention (USA). Patients were offered seasonal influenza, hepatitis B, pneumococcus, diphtheria, and tetanus vaccinations once they had reached at least 6 months posttransplant. Endocrine and cardiology consultation was completed as required.

Health-related QoL (HRQoL)

HRQoL was measured using the short-form SF-36 health survey (ver. 1.0) normalized to the values of the Turkish population at two time points: before HSCT and 1 year after HSCT [23]. Based on the patients' responses to the 36 items, scores were calculated for each of the eight domains, and according to the two physical dimension components and the mental component, in accordance with a scoring algorithm [24, 25].

Table 1 Baseline patient-, disease-, donor-, and transplantation-related characteristics among 20 SCD patients

Characteristics	No. (%) of patients
Gender	
Female	7 (35)
Male	13 (65)
Age, years	
≤20	2 (10)
20–30	9 (45)
31–40	6 (30)
≥41	3 (15)
Disease genotype	
Homozygous Hgb S	17(85)
Hgb S-β thalassemia	3 (15)
Indications for transplant	
TRV ≥ 2.7 m/s	3 (15)
Stroke or silent infarct	7 (35)
Acute chest syndrome	6 (30)
Bone necrosis	7 (35)
Sickle nephropaty	2 (10)
Alloimmunization	3 (15)
Serum ferritin ≥1000 ng/dL	2 (10)
Donor status[a]	
Non-Hgb S trait donor	10 (50)
Hgb S trait donor	10 (50)
Comorbidity score (Sorror)	
High	2 (10)
Intermediate	4 (20)
Low	14 (70)

SCD sickle cell disease, *TRV* tricuspid regurgitant jet velocity, *Hgb* hemoglobin

[a] Donor source is HLA-matched related donors in all transplants

Statistics

Baseline categorical variables are listed in Table 1. The OS and disease-free survival (DFS) were estimated using by Kaplan–Meier analysis. OS was defined as the time from transplant to death, with surviving patients censored at last follow-up. DFS was defined as the time from transplantation to disease relapse and/or death. Probabilities of DFS and OS were calculated as described previously [15]. NRM was defined as death from any cause other than disease progression or relapse. Cumulative incidences of NRM and graft rejection were calculated, with graft rejection and death as competing events, respectively [16]. The

cumulative incidence rates of aGVHD and cGVHD were calculated with graft rejection or death without graft rejection and GVHD as competing events [17]. Mann–Whitney *U*-test was used for the comparison of groups without a normal distribution for clinical and hematological parameters. Variables included in the final models were significant at $p = 0.05$. Statistical analysis was performed using the SPSS software (ver. 17.0; SPSS Inc., Chicago, IL, USA) and *P*-values based on Gray's test were estimated using the NCSS software (ver. 11.0.9; NCSS, Kaysville, UT, USA).

Results

Table 1 lists clinical characteristics.

A total of 20 patients were included in this study, of whom 7 (35%) were female. The median age of our patient cohort was 33 years (range: 20–45 years), with three patients aged >41 years. Sixteen patients (80%) had initiated treatment at our center, with the remaining 4 (20%) having been referred from another center. Mutational analyses revealed 17 patients (85%) to be homozygous for Hgb S, and 3 patients (15%) positive for Hgb S-β thalassemia. Major indications for HSCT included acute chest syndrome, stroke, or silent infarct. Although serum ferritin levels >1000 ng/mL were evident in 2 (10%) patients, iron deposition was not detected in the liver or the heart. Red cell allo-immunization was observed in 3 (15%) patients, characterized primarily by Cc, Ee, and Kell antibodies. Cross incompatibility was detected in 2 (10%) patients. Major blood group incompatibility was detected in 1 (5%) patient, with minor blood group incompatibility present in 4 (20%) additional cases. Full HLA-matched siblings were selected as donors for 18 (90%) patients; the remaining 2 patients received donations from HLA-matched parents. While half of all donors were non-Hgb S trait donors, the remaining 10 donors were positive for the Hgb S trait. Among patients, 14 (70%) had low co-morbidity scores (Table 1).

Two allo-immunized patients did not undergo red cell exchange due to incompatibility, instead undergoing one volume of plasma exchange for removal of allo-antibodies. Crisis-like pain was reported in four cases as a result of dexamethasone treatment, which had been administered to suppress inflammation during conditioning. For all subsequent patients, treatment was delayed until after red cell exchange, thereby allowing the procedure to be completed without incident.

The conditioning regiment was well tolerated by all patients. Elevated serum amino transferase and bilirubin levels ≤3-fold that of normal values was the most significant finding related to drug toxicity. Sufficient numbers of stem cells (>5×10^6 CD34 cells/kg patient weight) were readily collected from Hgb S trait donors. Two donors required two sessions of stem cell collection.

Table 2 Transplant outcome of 20 adult SCD patients

Characteristics	No. (%) of patients
Neu. engrafment at 30 days	20 (100)
Plt. engraftmant at 30 days	NA[a]
OS, at 1 year	20 (100)
DFS, at 1 year	20 (100)
Cumulative incidence of NRM, at 1 year	0
Cumulative incidence of graft rejection at 1 year	0
Cumulative incidence of graft rejection at 2 year	0
Cumulative incidence of aGVHD at 100 days	
Grade II–IV	1 (5)
Grade III–IV	0
Cumulative incidence of aGVHD at 180 days	
Grade II–IV	1 (5)
Grade III–IV	0
Cumulative incidence of cGVHD at 1 year	
As a whole	0
Extensive	0
Cease of IS at 1 years	12 (92)[b]
Infection at 1 year	
Bacterial	6 (30)
CMV	5 (25)
BK virus	3 (15)
Herpes zoster	1 (5)

SCD sickle cell disease, *Neu* neutrophil, *Plt.* platelet, *OS* overall survival, *DFS* disease free-survival, *NRM* non-relapse mortality, *aGVHD* acute graft-versus-host disease, *cGVHD* chronic graft-versus-host disease, *IS* immunosuppressive, *CMV* cytomegalovirus, *NA* not applicable

[a] Platelet engraftment time was not estimated as values decreased <50×10^9/L in no cases

[b] Calculated based on 13 patients who completed 1 year follow-up period

One to three units (mean 2.2 units) of packaged red cell suspension was required in 13 (65%) patients during the pre-engraftment period. The remaining 7 patients (35%) received 1–6 units (mean 2.9 units) of concentrated platelet transfusions to maintain platelet counts ≥50,000/μL.

Table 2 shows transplant outcomes.

Mean neutrophil engraftment time was 18 days (range: 13–22 days); platelet engraftment time could not be evaluated as platelet count did not decrease <30,000/μL during the transplant procedure. Mean duration of hospital stay was 34.6 days (range: 30–52 days). Average follow-up duration was 13.8 months (range: 0.3–50 months), with > 1 year follow-up reported in 13 (65%) patients. One-year OS and DFS were 100% (Fig. 1a, b). Cumulative incidence of grade

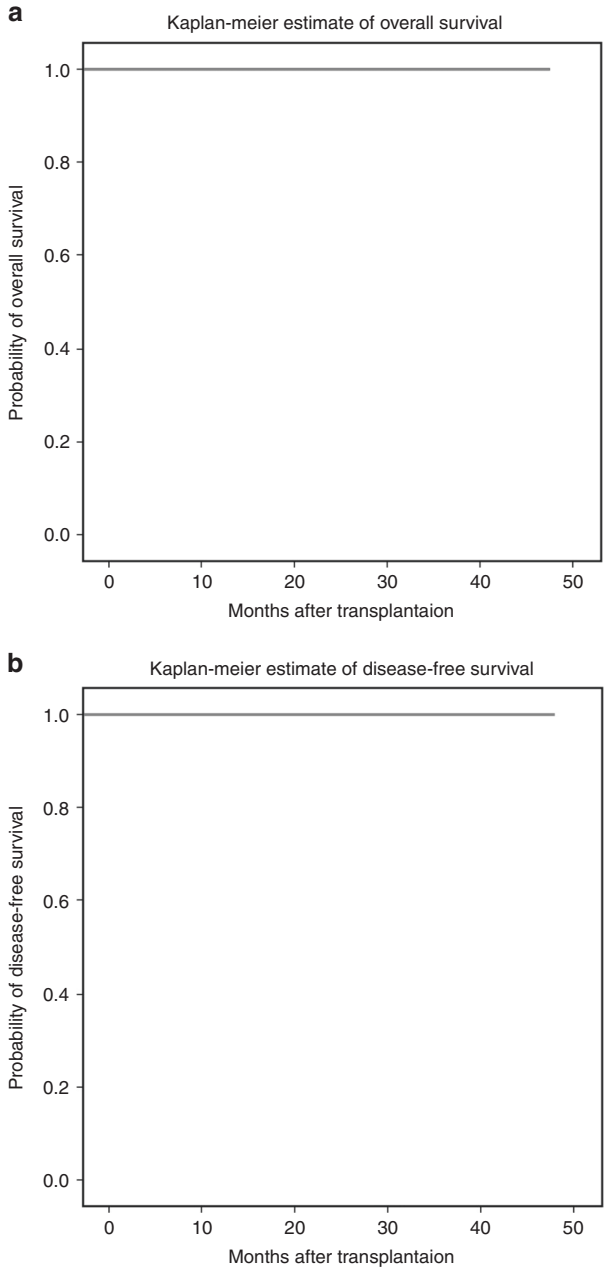

Fig. 1 Overall survival and disease-free survival curves. **a** Kaplan–Meier estimates of overall survival following allogeneic transplantation, **b** Kaplan–Meier estimates of disease-free survival following allogeneic transplantation. (Color figure online)

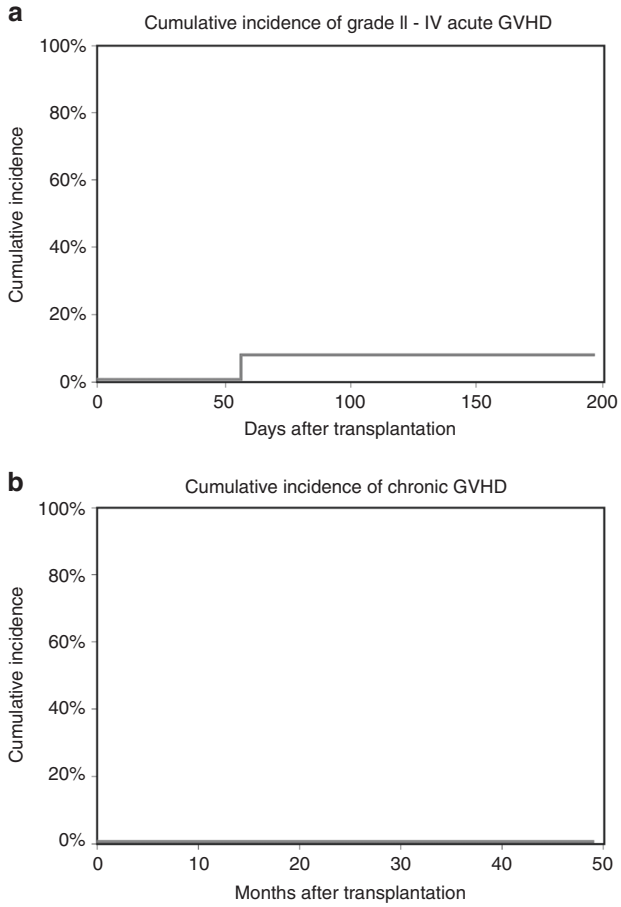

Fig. 2 Cumulative incidence of GVHD curves. **a** Cumulative incidence of grade II–IV aGVHD. **b** Cumulative incidence of cGVHD as a whole. (Color figure online)

II–IV aGVHD was calculated as 5% at days 100 and 180, with no cases of grade III–IV aGVHD observed at days 100 or 180. Cumulative incidence of cGVHD at 1 year was 0% (Fig. 2a, b). No cases of death or graft rejection were reported at 1 year. While full donor chimerism was observed in two patients, exhibiting 93% and >97% donor engraftment by day 100 of posttransplantation, full donor chimerism (total cell) occurred within 180 days in all patients. Chimerism was seen to sustain in all and immunosuppressive therapy was successfully discontinued in 11 of the 12 patients (91.7%) who completed 1 year follow-up. All blood count and hemoglobin electrophoresis values of four patients who completed 2 years after transplant were found within normal ranges.

Table 3 shows hematological values after transplant.

Mean Hgb values of 13.2 ± 1.6 g/dL were observed at day 100 posttransplantation and remained at these levels thereafter. Hgb S ratios fell to <5% by day 100 among patients who underwent transplantation from healthy donors; this ratio was <40% in patients who underwent transplant from Hgb S donors. In contrast, Hgb A ratios increased inversely proportionally to the reduction in Hgb S in both groups. While mean leukocyte and platelet values did not change after day 100, significant reductions in both total bilirubin and ferritin levels were observed at subsequent time points ($P = 0.0001$ and $P = 0.01$, respectively).

Table 4 lists frequency of painful crisis, hospitalization rate, narcotic use, and self-reported SF-36 QoL scores before and after HSCT among 20 SCD patients.

Table 3 Hematological parameters before and after hematopoietic stem cell transplantation among 20 SCD patients

	Pretransplant[a], *n*: 20	100 days, *n*: 20	180 days, *n*: 16	1 year, *n*: 12	*P*[b]
Hemoglobin, g/dL	8.9 ± 1.1	13.2 ± 1.6	13.2 ± 1.4	13.7 ± 1.6	0.001
Donor source					
Non-Hgb S trait donor					
Hgb S (%)	84.8 ± 5.6	0	0	0	
Hgb A (%)	0.7 ± 0.3	61.9 ± 12.7	96.9 ± 0.2	97.2 ± 0.3	0.018
Hgb S trait donor					
Hgb S (%)	84.8 ± 5.6	31.3 ± 4.2	35.6 ± 5.0	35.4 ± 5.1	0.018
Hgb A (%)	0.7 ± 0.3	64.7 ± 4.5	60.4 ± 4.7	60.4 ± 5.1	0.018
Leukocyte, $\times 10^{-9}$/L	11.9 ± 5.7	7.7 ± 2.8	8.4 ± 3.5	9.4 ± 2.5	0.108
Platelet, $\times 10^{-9}$/L	535 ± 271	333 ± 124	419 ± 88	436 ± 104	0.050
Bilurubin, mg/dL	2.8 ± 1.5	0.6 ± 0.2	0.5 ± 0.2	0.5 ± 0.2	0.001
Ferritin, ng/mL	432 ± 351	NA	NA	271 ± 282	0.010

All values are given as mean ± standard deviation

SCD sickle cell disease, *CI* confidence interval, *LDL* low density lipoprotein, *NA* not applicable

[a] Pretransplant denotes approximately 1 month before hematopoietic stem cell transplant. Hgb S or Hgb A denotes percentage of hemoglobin S or hemoglobin A of total hemoglobin in the peripheral blood

[b] *P*-values represent the comparison between pretransplant values and posttransplant 1 year values

Table 4 Painful crisis, hospitalisation rate, narcotic use, and self-reported SF-36 QoL scores before and after HSCT among 20 SCD patients

	Pretransplant[a], *n*: 20	100 days *n*: 20	180 days *n*: 16	1 year *n*: 12	*P*[b]
Painful crisis ≥2 per year, n (%)	18 (90)	0	0	0	NA
Hospitalization ≥1 per year, n (%)	18 (90)	6 (30)	4 (20)	1 (5)	NA
Narcotic need, n (%)	17 (85)	2 (10)	1 (5)	0	NA
Quality of life score					
Role physical	59.9 ± 14.2			80.2 ± 23.7	0.059
Health general	21.0 ± 22.3			71.9 ± 21.7	0.005
Bodily pain	30.6 ± 27.2			93.9 ± 14.5	0.004
Energy/vitality	51.2 ± 27.2			67.7 ± 21.3	0.061
Social functioning	38.9 ± 18.1			55.9 ± 27.1	0.062
Role emotional	42.4 ± 39.7			69.8 ± 23.1	0.095
Mental health	62.2 ± 20.2			74.2 ± 17.4	0.072

All values are given as mean ± standard deviation

HSCT hematopoietic stem cell transplantation, *NA* not applicable

[a] Pretransplant denotes approximately 1 month before hematopoietic stem cell transplant

[b] *P*-values represent the comparison between pretransplant values and posttransplant 1 year values

While 18 (90%) patients reported two or more painful crises before transplant, no painful crises were reported in any patient after transplant. Hospitalization and narcotic use also significantly decreased after transplant. SF-36 outcomes for both general health and bodily pain significantly improved at 1 year ($P = 0.005$ and $P = 0.004$, respectively).

Febrile neutropenia was observed in 8 (40%) patients. *Enterobacter faecium* and *klebsiella* spp. were detected in 2 of these patients. CMV antigenemia was detected in 5 (25%) patients, and BK virus (BKV)-related hemorrhagic cystitis was detected in 3 (15%) cases. Among these incidents, infection was detected after day 100 in two cases. Varicella zoster was detected 6 months posttransplantation in one case. Pneumonitis was observed in 3 cases (15%) and was associated with use of the immunosuppressant sirolimus. Unexpectedly, a temporary serum aminotransferase level elevation up to three times was higher than normal values around 1 month and persisted for up to 2 months in 8 (40%) patients. Liver biopsy that was performed in one patient revealed pathological evidence of drug effect. Three cases of hyperlipidemia were also reported.

While some expected granulocyte colony-stimulating factor (G-CSF) or apheresis-related side effects, including minor pain, headache, paresthesia, and fatigue, were

observed in 10 Hgb S trait patients and 10 non-Hgb S trait donors, no additional adverse events were reported. Post-procedural outpatient follow-up of donors was also uneventful.

Discussion

Reduced organ reserves, inflammation, and hyperproliferative bone marrow represent the most significant complications of allo-PSCT in adult SCD patients. Transplant protocols must therefore be designed to minimize the appearance of these issues. Here we report the outcomes of allo-PSCT performed using a Flu/Bu/ATG-based conditioning regimen (Flu 150/ Bu3.2/ATG 30/Cy29/TBI 200 cGy) for the treatment of SCD. This regimen has been successfully employed at our institution for prevention of graft loss, in combination with prophylactic use of sirolimus, and posttransplant Cy to reduce the likelihood of GVHD. Favorable outcomes have been achieved with regard to sustained grafting, survival, and transplant-related mortality, along with reductions in the incidence of aGVHD and cGVHD during the 3-year follow-up period.

The present study makes two significant contributions to the literature, reporting outcomes from a homogenous ethnic group with a relatively high mean age, as well as detailed results from patients treated using peripheral blood as the stem cell source. While heterogeneity of clinical outcomes and mortality rates in SCD are strongly influenced by ethnicity, all patients included in this study were members of a single ethnic group [3, 26, 27]. Mean age, which has been shown to be inversely correlated with patient survival, was higher than that of the 154 adult cases described in EBMT and CIBMTR prior to initiation of this study [9]. Use of peripheral blood is limited in previous studies due to the increased risk of GVHD [9, 11, 16]. Peripheral blood stem cells were used in 43 out of the 154 patients by Gluckman et al. [9], making this study the largest serial analysis to date of transplants performed using peripheral blood as the stem cell source after chemotherapy conditioning.

Satisfactory clinical outcomes have been observed in the majority of transplants conducted in pre-school-aged children for whom organ reserves were preserved [9, 11]. As of 2013, 846 of the 1000 transplants had been performed in pediatric patients who underwent myeloablative conditioning (Bu, Cy in ≥85% of cases) using full HLA-matched sibling donors. In these cases, the ratio of cumulative cGVHD was ~10% over 2 years, with 90% OS ≥90% at 5 years [9]. Current therapeutic regimens have been optimized to reduce overall toxicity, with sufficient safety and efficacy profiles for use in children [28–30]. The best example of this can be seen in a prospective, single-center prospective study by Bahatia et al. that used PSCT with Flu/BU, combined with alemtuzumab-based conditioning of bone marrow and cord blood. The authors recorded rates of total cell and erythroid cell donor chimerism in peripheral blood of 91% and 88% on days 100 and 365, respectively. Two-year OS was 100%, with an aGVHD ratio of 17% [31]. Based on these and other promising results in pediatric cases, an international panel reviewed the literature and recommended allo-HSCT from HLA-matched sibling donors for all young patients with symptomatic SCD [11].

Disease-related organ damage in SCD remains a significant concern despite use of hydroxyurea, advancements in infection prophylaxis, and other supportive treatments [32–34]. Currently, allo-PSCT from an eligible donor is the only curative treatment method available for SCD. Unfortunately, this treatment is not without significant risks, with severe cGVHD and progressive organ damage seen in some patients, particularly those who received multiple transfusions [11, 12]. These and other complications are particularly problematic in adult patients, with transplant-related mortality as high as 13% even in adolescents and young adults [28]. Significant factors include increases in inflammatory cytokines, endothelial activation, and endothelial damage; activation of histiocytes and macrophages; bone marrow hyperactivity and micro-environmental factors; increased susceptibility to infections; and hemosiderosis [2, 5, 6, 16, 29]. Together, these factors can lead to increased drug toxicity, graft insufficiency, transplant complications, and GVHD.

A recent large case series reported 1-year mortality of 4–7%, with ratios of up to 20% for aGVHD and cGVHD in transplants conducted with Flu/Bu-based conditioning [27, 35]. The influence of non-myeloablative regimens on engraftment, effectiveness, and toxicity in adults is currently being investigated as part of a series of ongoing phase I/II studies across multiple centers. Current preliminary studies indicate that this protocol may emerge as a standard treatment option, assuming that a proper conditioning regimen can be established [18, 19, 36]. Hsieh et al. reported sustained engraftment in 26/30 patients (median age 28.2 years) using a non-myeloablative conditioning regimen with alemtuzumab and low-dose TBI, with no reports of either aGVHD or cGVHD in any patients. Mobilized peripheral blood cells were used as the stem cell source. Only 15 engrafted patients discontinued immunosuppression medication with continued stable donor chimerism and no GVHD; however, 13% of the patients eventually lost the graft [18]. In the study of Saraf et al., a conditioning regimen including alemtuzumab and low-dose TBI was used for transplant of adult SCD patients using peripheral blood. While graft failure developed in 1/13 patients, immunosuppressive therapy could be discontinued

in only 4 patients who were followed up for 12–44 months [36].

In light of these data, the components of the transplant regimen, and the reasons underlying each, were carefully determined in this study. Very low doses of Bu and TBI 200 cGy were used to facilitate engraftment and prevent graft loss. Peripheral blood, which contains ~10-fold more T-lymphocytes than bone marrow and is known to facilitate engraftment, was used as a stem cell source. rATG-F, which has a longer half-life than thymoglobulin (4–10 days), was used for prevention of cGVHD and to facilitate engraftment [37]. Sirolimus was preferred to cyclosporine due to its superior nephrotoxicity profile [16, 38]. While sirolimus does not block T-cell activation, it does inhibit T-cell proliferation. The drug has been shown to induce anergy in activated, but not proliferative, T cells and facilitates cell differentiation. High-dose Cy posttransplantation is being used for prevention of cGVHD in HLA-unmatched transplants [16, 39]. This procedure has been shown to eliminate proliferative allo-reactive T cells, while preserving resting, non-reactive T cells. Infection risk also decreases through preservation of virus-specific T cells [40]. Posttransplant Cy was reported to be used in allo-transplants in adult SCD patients and HLA-matched sibling donors by Bolanos-Meade et al. [41]. Use of posttransplant Cy together with sirolimus has been shown to result in an additive effect [39], mediated through increased regulatory T populations.

The successful outcomes of allo-immunized patients with major blood group incompatibility and cross incompatibility represent one of the most significant results from this study. Major blood group incompatibility and alloimmunization did not negatively affect transplant outcomes, with autoreactive antibodies disappearing posttransplant. Pretransplant plasma exchange and avoiding from unnecessary transfusions may have also contributed this.

Low-dose steroids used after cell infusion have been reported to play a role in the suppression of inflammatory cytokines and may help prevent serious immune events (GVHD, early graft loss, etc.) [16].

While posttransplant CMV, herpes, and BKV reactivation were encountered, no viral-associated mortality was observed. In cases of CMV antigenemia, patient symptoms were easily managed using the well-established protocols. The most important factor for easy control of viral reactivation may include these patients not receiving chemotherapy before transplant, an important difference versus malignant patients, allowing for better immune construction overall. The temporary elevation of serum aminotransferase levels, which was proven to be drug related, was considered to result from inflammatory reactions and transaminitis.

Given the genetic basis SCD, assessment of Hgb S trait donors has taken on increased importance. While G-CSF-related severe pain was reported as a potential complication in Hgb S trait donors, G-CSF has been used safely in both related and unrelated Hgb S trait donors [16, 42, 43]. It has been hypothesized that ethnicity may play a role in adverse G-CSF reactions. Among successful trials, no unexpected side effects developed in Hgb S trait donors, with all immune mobilization procedures performed without incident.

As part of a holistic approach to patient care, disease perception, feelings, and QoL should be considered in medical efforts aimed at curing SCD. Here QoL scores were found to have significantly improved after transplant. Assesment of QoL before transplant and during 1 year after transplant could have yielded better information. However, we considered that these assessments could have influenced from the heterogenous nature of the disease and the variable course of the transplant process. Therefore, we performed only two QoL assessments. Limited published studies of QoL post-PSCT for SCD have described similar improvements in QoL over time [44].

The most significant limitations to this study were the use of a single medical center and the limited number of patients. Although the safety and effectiveness of low-dose TBI have been shown in benign disease, its long-term effects on fertility and organ damage should be evaluated [18]. Support has previously been provided by fertility treatment centers with respect to preservation of fertile capacity, through preservation of sperm, ovum, and zygote [16, 45]. Further studies conducted with similar protocols are certainly required.

Conclusion

Here we have shown that PSCT using TBI in combination with a Flu/Bu/ATG-based conditioning regimen, followed by posttransplant Cy and sirolimus for GVHD prophylaxis, could enable safe and sustained engraftment in adult SCD patients. The outcomes presented here support the opinion that allo-PSCT from HLA-matched donors, performed before organ damage has developed, is a viable option for adult SCD patients. Furthermore, by using peripheral blood as the preferred stem cell source, not excluding blood group incompatibility or donor Hgb S trait status will significantly enhance the potential donor pool. Further studies are required for assessment of long-term effects.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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