

Fludarabine-Based Reduced-Intensity Conditioning Regimen for Hematopoietic Stem Cell Transplantation in a Pediatric Patient with Sickle Cell Disease: A Case Report

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ABSTRACT

Reduced-intensity conditioning (RIC) regimens have the potential to decrease toxicities related to hematopoietic stem cell transplantation (HCT) in patients with sickle cell disease (SCD). While initial results may have been acceptable in adults and young adults, there are no well-established strategies in children with SCD. Here, it is described the clinical course of two children with symptomatic SCD who have successfully undergone HSCT using Fludarabine-based conditioning.

Keywords: Reduced-intensity conditioning; Children; Nonmalignant disorders; Sickle cell disease

INTRODUCTION

Hematopoietic stem cell transplantation (HSCT) is an established curative treatment for sickle cell disease (SCD). Rates of disease-free survival exceed 95% with a matched sibling donor (MSD) using myeloablative conditioning (MAC)¹. While often successful, many children with SCD have significant comorbidities at the time of HSCT, and these standard myeloablative preparative regimens are associated with notable toxicity and a relatively high incidence of transplant mortality, as well as long-term sequelae. Some groups have described reduced-intensity conditioning (RIC) strategies. While initial results may have been acceptable in adults and young adults, there are not well-established strategies in children with SCD. A reduced dose of busulfan in combination with fludarabine has also been used as a RIC regimen in adults and children with malignant and

nonmalignant diseases undergoing HSCT. Here, it is described the clinical course of two children with symptomatic SCD who have successfully undergone HSCT between 2018 and 2020 using high-dose fludarabine (180 mg/m²), serotherapy and low dose of busulfan.

Case presentation

Patient 1 (P1) is a 10-year-old boy complicated by stroke receiving monthly red blood cell (RBC) transfusions, hydroxyurea, and oral iron chelation. Meanwhile, He began experiencing episodic right upper quadrant pain and persistent jaundice. He was found to have persistent hyperbilirubinemia (total serum bilirubin of 61.29 mg/dL, direct serum bilirubin of 45 mg/dL), normal alkaline phosphatase (125 U/l), aspartate transaminase (AST; 26 U/l), and alanine transaminase (ALT; 54 U/l). Iron studies showed ferritin levels 1,191 ng/ml. A liver

ultrasound, magnetic resonance imaging, and computed tomography scans were negative for ductal dilation and obstructive hepatopathy. Other causes of chronic liver disease (CLD) were excluded. A liver biopsy showed variable stages of fibrosis, sinusoidal congestion, regenerative nodules and moderate iron deposits were observed within the parenchyma and in Kupffer cells.

Patient 2 (P2) is a 5-year-old boy who commenced hydroxyurea prophylaxis at age 4. Despite excellent hydroxyurea adherence, he had recurrent hospitalizations due to frequent vaso occlusive crises (VOCs), multiple episodes of pneumonia and, transcranial Doppler ultrasound altered needing exchange transfusions. He developed iron overload as a consequence of the frequent requirement of blood transfusions.

Donors and graft

Donors were both HLA and ABO-identical. One of them had a sickle cell trait (P1) with 30–36% sickle-cell hemoglobin (HbS). No Cytomegalovirus (CMV) IgG mismatch was present. The graft source was unmanipulated peripheral blood stem cells mobilized with granulocyte colony-stimulating factor (G-CSF) application at a daily dose of 10 µg/kg for 5 days. Pretransplant features and graft characteristics are summarized in Table 1.

Transplantation Procedures

Conditioning regimen

RIC conditioning regimen consisted of six doses of intravenous fludarabine 30 mg/m² one dose per day on days –8 to –3. Low-dose iv. Busulfan (12.8 mg/kg) was administered four times daily (2 h infusions) in P1 and twice daily over 3 h in P2, dosed according to published weight-based recommendations from d-5 to d-2.³ Serotherapy consisted of intravenous rabbit anti-thymocyte globulin (rATG), (Sanofi-Genzyme) 2.5 mg/kg, one dose per day on days –5 to –3⁴. Graft-versus-host disease (GvHD) prophylaxis consisted of calcineurin inhibitors (cyclosporin), until day 180, combined with mycophenolate mofetil (1200 mg/m² per day, twice a day from day 0 until day 60). Antimicrobial prophylaxis was administered with trimethoprim-sulfamethoxazole, acyclovir, and

Anidulafungina in P1 and voriconazole in P2. Both patients received anticonvulsant prophylaxis with lorazepam. Both patients had the following vaccinations: BCG vaccine at birth, Hepatitis B, Diphtheria, Pertussis, and Tetanus (DPT), Haemophilus influenzae type b (Hib), Polio, Rotavirus, Pneumococcus, Seasonal influenza, and measles, rubella, and mumps (MMR). Lorazepam was used to prevent seizures. Cytomegalovirus infection was managed with a preemptive approach, based on weekly viral load monitoring up to day +100. Granulocyte colony-stimulating factor was given s.c. from day +12 until neutrophil engraftment. Hydroxyurea was discontinued at the beginning of conditioning. No antithrombotic prophylaxis was given to either of these two patients.

Transplant-associated complications

The conditioning regimen was well tolerated, even by P1 with substantial pretransplant comorbidities. Both patients experienced oral mucositis (WHO grade I). The need for transfusions was low with eight units of packed erythrocytes and four units of platelets in P1 and two units of packed erythrocytes in P2. No Cytomegalovirus (CMV) reactivation was observed. P1 developed neurologic toxicity at day +8 post- HSCT. Manifestations included sudden onset seizures and altered sensorium in the presence of hypertension. Posterior reversible encephalopathy syndrome (PRES) was discarded. He required temporary use of amlodipine to maintain blood pressure below the 95th centile for age. Central nervous system examinations were stable following control of hypertension. Also, P1 had upper respiratory tract infections with Mycoplasma and respiratory syncytial virus at days +51 post- HSCT that resolved without medication. There were no other viral infections and no fungal. No acute organ toxicity grade 3 was noted attributable to the conditioning regimen. No patient developed hepatic sinusoidal obstruction syndrome (VOD). None of the patients experienced acute or chronic GvHD. There was no transplant-related mortality (TRM).

Engraftment and chimaerism

Neutrophil engraftment with an absolute neutrophil count >500 per/L was reached after day +22 (P1) and day +13 (P2). Chimerism was analyzed by short

tandem repeats of STRs. All patients had mixed chimerism in peripheral blood on day+28 with 93–82% donor cells. However, mean whole blood donor chimerism of 100 % was achieved (Table 1).

Table 1: Patients and donor characteristics

	Patient 1 (P1)	Patient 2 (P2)
Type of sickle Hb	HbSS	HbSS
Age at HSCT (years)	10	5
Patient pretransplant morbidity and indications for HSCT	Recurrent VOC, Stroke, ACS, Liver fibrosis, Cholestasis: BT: 51.38 mg/d BD: 42 mg/d	Abnormal TCD, recurrent VOC
Medical management before HSCT	Chronic RBC transfusions, Hydroxyurea, iron chelation	Chronic RBC transfusions, Hydroxyurea, exchange transfusions, iron chelation
CD34 Dose Infused (106/kg)	4.9	6.1
Donor Hb electrophoresis	HbAS	Hb AA
Donor gender	Male	Female
Donor age (years)	12	17
Days to ANC recovery (>0.5 x 10 ⁹ /L)	22	13
Days to platelet recovery (>50x 10 ⁹ /L)	22	14
Donor Myeloid Chimerism at Day 30	93 %	82%
Donor Myeloid Chimerism at Day 100	N/A	91%
Donor Myeloid Chimerism at Day 300	96 %	100%
HbS last follow-up (%)	30	0
Status and follow-Up (days)	Cured/903	Cured/348

Abbreviations: Hb, hemoglobin; HBSS, homozygous sickle cell anemia; HSCT, hematopoietic stem cell transplantation; VOC, vaso-occlusive crises; ACS, acute chest syndrome; BT, total bilirubin; BD, direct bilirubin; TCD, transcranial doppler ultrasonography; RBC, red blood cells; HbAS, Hb S trait; Hb AA, normal electrophoresis.

DISCUSSION

In the past decade, some studies have investigated the effect of RIC/reduced-toxicity regimens in pediatric patients with SCD (Table 2). Despite excellent overall outcomes as compared to MAC regimens, certain problems such as primary/secondary graft failure and GvHD were seen. Low intensity conditioning with low dose TBI and alemtuzumab has emerged as an effective and safe regimen for adults and young adults^{3,9}. However, there is a need for prospective studies including a large number of pediatric patients to broaden its application in children. On the other hand, King et al.⁶ reported the results of 52 children between 0.8 and 20.3 years of age with hemoglobinopathies (43 with SCD and nine with thalassemia) underwent HCT using RIC with alemtuzumab, fludarabine and melphalan between March 2003 and May 2014. It was shown that the overall and event-free survival were 93% and 90.7% for SCD at a median of 3.42 (range, 0.75 - 11.83) years and mortality associated with transplant-related complications was noted in three (5.7%) recipients, all 17–18 years of age. Acute and chronic

GVHD was noted in 23% and 13%, respectively. Graft rejection was limited to the single umbilical cord blood recipient who had prompt autologous hematopoietic recovery. Fourteen (27%) had mixed chimerism at 1 year and beyond; all had discontinued immunosuppression between 4 and 12 months from transplant with no subsequent consequence on GVHD or rejection. Madden et al.¹⁰ reported similar rates of GVHD and high rates of graft failure in a late follow-up beyond 2 years after HCT in 43 children with non-malignant disorders undergoing HCT with a RIC regimen (alemtuzumab, fludarabine, and melphalan). However, only ten patients with hemoglobinopathies were included in the study. For this reason, no conclusions can be drawn in that population. On the other hand, Bhatia et al. reported encouraging results with a RIC regimen using BU 12.8–16 mg/kg, fludarabine 180 mg/m², alemtuzumab 54 mg/m² (BFA) before HLA-matched sibling donor transplantation in pediatric recipients with symptomatic SCD with adequate organ function. Two-year event-free survival (EFS) and overall survival (OS) were both 100%. Acute

GvHD rates fluctuating from 0% to 17% and the incidence of chronic GvHD were 11%⁷. These findings support the use of RIC for pediatric patients with hemoglobinopathy undergoing matched sibling marrow transplantation.

RIC using intravenous busulfan, fludarabine and rabbit rATG has been widely used for children with nonmalignant disorders¹¹⁻¹³. Jacobs et al. (2003) examined the use of a RIC regimen of fludarabine, busulfan (6,4 mg/kg given in eight doses on days -5 to -4), and rATG in 13 children with non-malignant diseases (including three patients with SCD)¹⁴. However, graft failure or chimerism loss was a significant issue. Afterward, Gungor et al, optimize this protocol, demonstrating that busulfan could be adjusted within a submyeloablative target range of 45–65 mg/L × h achieving sustained myeloid (≥90%) engraftment. This approach has shown excellent engraftment and low toxicity features in high-risk pediatric and adult patients with chronic granulomatous disease (CGD)¹⁵. Furthermore, it has been used with remarkable outcomes in other benign disorders such as hemophagocytic lymphohistiocytosis HLH¹⁶.

CONCLUSION

In conclusion, this transplantation approach produced donor cell engraftment with low rates of GvHD and transplanted related mortality (TRM) in all described patients, resulting in a cure in all of them. Because of its favorable toxicity and efficacy profile, patients with other malignant and non-malignant diseases have benefited from this reduced-intensity conditioning regime before. While this approach has the potential for major therapeutic benefits, RIC-HSCT for hemoglobinopathies remains experimental and should be performed in the context of well-constructed clinical trials in centers with expertise in SCD. If the results are confirmed in larger patient cohorts, these observations could be helpful for conditioning approaches in children with SCD with widespread comorbidities.

Abbreviations

SCD: Sickle cell disease

HSCT: Hematopoietic stem cell transplantation

MSD: matched sibling donor

RIC: reduced-intensity conditioning

P1: Patient 1

P2: Patient 2

rATG: rabbit anti-thymocyte globulin

GvHD: Graft Versus Host Disease.

TRM: transplant-related mortality.

cGvHD: chronic graft-versus-host disease

BU: Busulfan

HLA: human leukocyte antigen

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CONFLICT OF INTEREST

The author declares no competing financial interests. Author has nothing to disclose.

Ethical approval

The study was approved by the Institutional Ethics Committee in accordance with the Declaration of Helsinki. This work was performed at Hospital Pablo Tobón Uribe.

Table 2: HSCT studies with Reduced or Low Intensity Conditioning HCT for children with SCD in the last decade (n>5 patients)

Author/year	Matthes-Martin, 2013 (4)	Bhatia, 2014 (5)	King et al. 2015, (6)	Guilcher, 2019 (7)	Ngwube A, 2020 (8)
n	8	18	43	16	14
Age at transplant: median (range)	9 (2.1 - 17)	8.9 (2.3–20.2)	11.5 (0.8 -20.3)	12 (3 -18)	13 (7-21)
Donor type	MSD	MSD	MSD	MSD	MSD*/URD
Graft source	BM/CB	BM/CB	BM/CB	PBSC	BM/CB
Conditioning regimen	Flu/Mel/TT or TLI/ATG or Alemtuzumab	BU/Flu/Alemtuzumab	Flu/Mel/Alemtuzumab	TBI(300 cGy)/Alemtuzumab	Flu/Mel/Alemtuzumab+thiotepa(UD)
GvHdprophylaxis	CsA or TAC/MMF	TAC/MMF	CsA or TAC+/- MTX or MMF +/- PRED	Sirolimus	Abatacept/TAC and MTX
OS (%)	100%	100%	93%	100%	100%
aGvHD (%) (n)	0	17%	23%	0	7% (III- IV)-28% (I-II)
cGvHD (%) (n)	0	11%	13%	0	57%
Graft Rejection	0	0	0	0	7,1%
Neutrophil engraftment: median day (range)	19 (17– 27)	16 (0–41)	13 (5–21)	22 (20.5-25)	14(10-24)
DFS	100%	100%	90.7%	100%	92.9%
TRM	0	0	5.7%	0	0

Abbreviations: MSD, matched sibling donor; URD, unrelated donors; BM, Bone marrow; CB, cord blood; PBSC, Peripheral Blood Stem Cell; Flu, fludarabine; Mel, melphalan; TT, Thiotepa; TLI, total lymphoid irradiation; ATG, anti-thymocyte globulin; Bu, busulfan; TBI, total body irradiation; CsA, cyclosporine A, TAC, tacrolimus; MMF, mycophenolat emofeti; OS, overall survival; aGvHD, acute graft-versus-host disease; cGVHD, chronic graft-versus-host disease; DFS, disease-free survival; TRM, transplant related mortality

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