Bone Marrow Transplantation
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CORRESPONDENCE

Haploidentical hematopoietic stem cell transplant for patients with sickle cell disease using thiotepa, fludarabine, thymoglobulin, low dose cyclophosphamide, 200 cGy tbi and post transplant cyclophosphamide

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Sickle cell disease (SCD) is a very common hemoglobinopathy worldwide and is the most common genetic diseases in the US. There is significant morbidity and mortality associated with the disease. Complications include recurrent vaso-occlusive pain crises, stroke, renal, and pulmonary dysfunction. Affected patients have poor quality of life, frequent hospital admissions and reduced life expectancy. Effective treatments associated with reduced complications include chronic red blood cell transfusions and hydroxyurea therapy. Stroke is the most devastating complication and despite chronic blood transfusions almost half of the patients will suffer a recurrent stroke or new silent cerebral infarcts [1]. The only curative therapy is allogeneic hematopoietic stem cell transplant (HSCT) [2–4]. Toxicities related to preparative regimen and lack of HLA identical siblings are the major limitations of transplant for patients with SCD. Additionally, identifying fully matched related or unrelated donor for African-American patients can be difficult [5, 6]. HLA-haploidentical transplant can expand the possibility of identifying suitable donors for patients with sickle cell disease [7].

The Hopkins group reported on 17 patients with sickle cell disease who underwent reduced intensity preparative regimen with post-transplant cyclophosphamide [7]. Six of the 17 patients (35.3%) experienced graft failure. Recently Fitzhugh et al. reported on using post-transplant cyclophosphamide as graft versus host disease (GVHD) prophylaxis post haploidentical peripheral blood stem cell transplant [8]. The authors described their experience with a reduced intensity transplant using alemtuzumab and 400 cGy total body irradiation as a preparative regimen. Only 50% of the patients in the last cohort were disease free, but none of the patients were off immune suppression because of continued mixed chimerism. In an effort to improve engraftment, we developed an institutional protocol to intensify the myeloablation by adding thiotepa at a dose of 10 mg/kg to the previously published Hopkins preparative regimen. We report on the first four consecutive patients transplanted using this approach with at least 1 year follow-up.

Between August of 2014 and August of 2016 four consecutive patients underwent haploidentical HSCT on an institutional study. This study is approved by the institutional IRB. All patients had at least one haploidentical unaffected donor. Organ function eligibility included a performance status (Karnofsky or Lansky) of ≥70, glomerular filtration rate of >60 ml/min/1.73 m^2, FEV1 >80% predicted and left ventricular ejection fraction of >50%. Patients were offered fertility preservation and all female recipients had menstrual suppression therapy. The preparative regimen consisted of antithymocyte globulin (rabbit ATG) 0.5 mg/kg on day −9 and 2 mg/kg on days −8 and −7, thiotepa 10 mg/kg on day −7, fludarabine 30 mg/m^2 on days −6 to −2, cyclophosphamide 14.5 mg/kg on days −6 and −5, and total body irradiation 200 Gy on day −1. Unmanipulated and T-cell replete hematopoietic stem cells were infused on day 0. GVHD prophylaxis consisted of cyclophosphamide 50 mg/kg/dose on days +3 and +4, and sirolimus to maintain a level of 8 to 12 ng/dL and mycophenolate mofetil (15 mg/kg/dose, maximum 1000 mg) every 8 h (until day 30) were started on day +5. Mycophenelate was stopped at day 30 and sirolimus was tapered starting 9 months post-transplant with a plan to stop by 12 months if there was no evidence of GVHD. GCSF primed bone marrow (5 mcg/kg of GCSF for

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5 days) was the preferred stem cell source and was used in 3 patients (patients 1, 2, and 3). Peripheral blood stem cells (mobilized with 10 mcg/kg of GCSF for 5 days) were used in one patient (patient #4) due to inability to collect bone marrow under general anesthesia. The use of GCSF primed bone marrow was based on the study by the Hopkins group and our previously reported data [7, 9]. Because all donors had sickle cell trait they were monitored daily for potential complications including excessive pain and for 4 weeks post collection All donors tolerated the GCSF therapy and collection without any complications. Supportive care included RBC transfusions within 4 weeks of conditioning with a target hemoglobin S level of <30%. Platelet transfusion thresholds were maintained at >50,000/μL and hemoglobin was kept at 10 g/dL. Neutrophil engraftment was defined as the first of 3 consecutive days during which absolute neutrophil count was ≥500/μL, and platelet recovery was defined as platelet count of 50,000 at least 1 week after the last platelet transfusion. Acute GVHD was graded based on previously reported criteria [10]. Chronic GVHD was graded based on the NIH consensus guidelines [11]. Patients were monitored weekly in the first 100 days and every other week until 1 year for cytomegalovirus (CMV) and Epstein–Barr virus (EBV). Levofloxacin prophylaxis was given until day 100 post-transplant. Donor and recipient HLA specific antibodies were measured before transplant and the least incompatible donors were chosen. All donors except for patient 4, had no recipient or donor directed HLA antibodies. The donor for patient 4 had HLA DR8 antibody that is present in the recipient.

Patient 1 is a 12-year-old boy with hemoglobin SS who had a stroke at 5 years of age and was on chronic transfusions and iron chelation therapy. He underwent a haploidentical transplant from his mother at age 11 years based on the Hopkins protocol, and he experienced graft rejection with 100% recipient chimerism at day 30 post-transplant. He was kept on chronic transfusions until the second transplant. Pre-transplant workup revealed evidence of old infarct on a brain magnetic resonance imaging (MRI), liver MRI showed moderate iron overload and serum ferritin was 4600 ng/ml. He achieved neutrophil and platelet engraftment on day +15 and +21, respectively. He received five red blood transfusion post-transplant, last was on day +14 post-transplant. Hemoglobin evaluation at 3 months, 12 months and 24 months post-transplant showed 28.5%, 36% and 37.5% hemoglobin S, respectively. Follow-up evaluation at 1 year post-transplant showed no change in the brain MRI and no change in baseline organ function compared to pre-transplant evaluation.

Patient 2 is a 12-year-old girl with hemoglobin SS disease who had eight episodes of acute chest syndrome in the 3 years prior to transplant and had an average of two admissions per month for pain crisis. Pre-transplant workup revealed silent infarcts on a brain MRI, liver MRI showed moderate iron overload and serum ferritin was 1200 ng/mL. She achieved neutrophil and platelet engraftment on day +20 and +19, respectively. She received two red blood transfusion post-transplant, last was on day +8 post-transplant. Hemoglobin evaluation at 3 months, 12 months, and 18 months post-transplant showed 29.2%, 39.6%, and 40.9% hemoglobin S, respectively. Follow-up evaluation at 1 year post-transplant showed no change in the brain MRI and no change in baseline organ function compared to pre-transplant evaluation.

Patient 3 is a 23-year-old female with hemoglobin SS disease who had history of acute chest syndrome twice in the year prior to transplant, and had recurrent pain crisis requiring hospital admission 2–3 times per year. Pre-transplant workup revealed normal brain MRI, liver MRI showed no evidence of iron overload and serum ferritin was 1300 ng/mL. She achieved neutrophil and platelet engraftment on day +24 and +33, respectively. She received eight red blood transfusion post-transplant last was on day +23 post-transplant. Post-transplant brain MRI and organ function evaluation 1 year post-transplant was not changed from pre-transplant evaluation. Hemoglobin evaluation at 3 months and 12 months post-transplant showed 28% and 33.7% hemoglobin S, respectively.

Patient 4 is a 16-year-old girl with hemoglobin SS and history of stroke at 3 years of age who was on chronic transfusions until the time of transplant. Pre-transplant workup revealed abnormal brain MRI consistent with prior stroke, liver MRI showed no evidence of iron overload and serum ferritin was 910 ng/mL. She received seven red blood transfusion post-transplant last was on day +28 post-transplant. Post-transplant brain MRI and organ function evaluation 1 year post-transplant was not changed from pre-transplant evaluation. Hemoglobin evaluation at 3 months and 12 months post-transplant showed 38.9% and 39.5% hemoglobin S, respectively.

Patient characteristics are presented in Table 1. All patients tolerated the preparative regimen without any complications except for patient 3 who developed moderate sinusoidal obstructive syndrome that resolved with supportive care. Three patients developed asymptomatic CMV viremia that resolved with antiviral therapy. All patients achieved neutrophil engraftment between day +15 and +24 and platelet engraftment +19 and +33. Peripheral blood chimerism was first measured at day 30 and all patients had 100% donor chimerism in CD3 and CD33. Grade 2 acute GVHD developed in all 4 patients (upper gut in 3 and skin in 1). All patients responded to corticosteroid therapy at a dose of 2 mg/kg for 2 weeks followed by a 4 week taper.

Table 1 Patient characteristics

UPN	Age at SCT (years)/Sex	Indication	Donor	Donor age (years)	HLA match	Stem cell source	Recipient/ Donor ABO	Recipient/ Donor CMV	Donor specific HLA antibody	Recipient specific HLA antibody
1	12.1/M	Stroke	Mother	33	5/10	G-BM	O^+/A^+	+/+	Negative	Negative
2	12.4/F	Acute chest and recurrent pain crisis	Father	31	5/10	G-BM	O^+/A^+	+/−	Negative	Negative
3	23.5/F	Acute chest and recurrent pain crisis	Sister	19	5/10	G-BM	A^+/B^+	+/−	Negative	Negative
4	16.2/F	Stroke	Mother	38	5/10	PBSC	O^+/O^+	−/+	Negative	Positive

Table 2 Patient outcomes

UPN	Nucleated cell dose/kg	CD34 dose/kg	ANC engraftment (Day)	Platelet engraftment (Day)	Acute GVHD grade	Chronic GVHD	Follow up (months)	Immune suppression	Engraftment CD3 (% donor)	Engraftment CD33 (% donor)	Serum Hb g/dl*	HB S%*
1	7.98×10^8	2.95×10^6	15	21	2	None	34	Off	100	100	14.1	37.5
2	9.72×10^8	3.51×10^6	20	19	2	None	18	Off	100	100	12.1	39.7
3	6.80×10^8	5.58×10^6	24	33	2	None	14	Off	100	100	12	33.7
4	3.40×10^8	5.07×10^6	24	32	2	None	13	Off	100	100	13.5	38.9

* Values at time of last follow-up

None of the patients developed chronic GVHD. All patients are off immune suppression between 2 and 23 months and have maintained a 100% donor chimerism in the CD3 and CD33 cells. At the time of last follow-up Hb electrophoresis was consistent with sickle cell trait (similar to donors) in all patients and hemoglobin ranged from 12–14.1 g/dL (Table 2). No patient experienced any complications associated with sickle cell disease following transplant. Although we have observed high rate of acute GVHD in our patients none of the patients developed chronic GVHD. The risk of developing chronic GVHD should be assessed using a larger cohort of patient.

In a recent report presented at the American Society of Hematology (ASH) in 2016, Dhedin et al. [12] reported on 34 patients who underwent haploidentical transplant for sickle cell disease using similar preparative regimen. As compared to our current study the treatment was not uniform and some patients received a 3-month therapy with azathioprine and hydroxyurea prior to transplant. The overall OS and EFS for all 34 patients were 86.4% and 81.8%, respectively, with two patients experiencing transplant related mortality and 2 patients experiencing secondary graft failure. Seven patients received similar preparative regimen to our patients and those patients had 100% EFS at 1 year. Our preliminary results are encouraging and support the hypothesis that intensifying the preparative regime with the addition of thiotepa to the previously published Hopkins protocol can result in rapid and continued full donor engraftment with acceptable toxicities among children and young adults who are in good performance status and have adequate organ function. Unlike prior published reports all patients are able to taper off immune suppression and maintain full donor chimerism.

Author contributions

The study was designed by HF, all authors participated in the conduct of the study and reviewed and approved the paper.

Compliance with ethical standards

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

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