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Nonmyeloablative Matched Sibling Donor Hematopoietic Cell Transplantation in Children and Adolescents with Sickle Cell Disease

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A B S T R A C T

Sickle cell disease is a potentially debilitating hemoglobinopathy associated with early mortality. The only established curative therapy is hematopoietic cell transplantation (HCT) with a matched sibling donor. The National Institutes of Health nonmyeloablative regimen of alemtuzumab/300 cGy total body irradiation and prolonged sirolimus exposure for graft-versus-host disease (GVHD) prophylaxis was administered to 16 children and adolescents. Infused products were unmanipulated granulocyte colony stimulating factor mobilized peripheral blood stem cells. All patients achieved mixed donor-recipient engraftment with no cases of secondary graft failure to date. Two patients have donor myeloid chimerism in the range of 30% to 40%. No sickling crises post-HCT have been observed. Event-free and overall survival rates are 100% with median follow-up of 19.5 months. No cases of GVHD have been observed. Sirolimus weaning was possible in all but one eligible patient to date. Ongoing follow-up and a larger prospective clinical trial are required to determine the long-term safety and efficacy of this regimen in children.

INTRODUCTION

Hematopoietic cell transplantation (HCT) is the only established curative therapy for sickle cell disease (SCD). Rates of disease-free survival exceed 95% with a matched sibling donor (MSD) using myeloablative conditioning (MAC) [1]. However, busulfan-based conditioning regimens are associated with substantial morbidity and high rates of infertility, particularly in adolescent females; recipients older than 16 years of age have poorer outcomes [1,2]. Graft-versus-host disease (GVHD) remains a challenge and a major reason not all children and adolescents with an MSD are offered HCT [1,2].

Cure of nonmalignant phenotypes can be achieved with minimal mixed donor chimerism (MC), allowing for a reduction in the exposure to myeloablative alkylating agents and total body irradiation (TBI) [3,4]. As a result, reduced-intensity conditioning and nonmyeloablative regimens have been developed to reduce the acute and late toxicities of HCT, particularly for children with comorbidities and for young adult patients [5,6]. Regimens that allow for preservation of gonadal function are a priority for patients and HCT physicians, although fertility outcomes after reduced-intensity conditioning HCT have not been well described [6,7].

A non-MAC regimen developed at the National Institutes of Health (NIH) has achieved high rates of successful donor hematopoietic cell engraftment resulting in cure of the sickle cell phenotype in adolescents and adults [8,9]. A disease-free survival of 87% was achieved, with only one death in a patient with secondary graft failure. The University of Illinois at Chicago added to these results with cure achieved in 12 of 13 subjects, no deaths, and one failure in a recipient with a history of sirolimus nonadherence [10]. Importantly, no patient in these two studies experienced acute or chronic GVHD [9,10]. Several pregnancies have been described in female recipients, and no cases of treatment-related malignancy have been reported (M. M. Hsieh, NIH, e-mail communication, May 7, 2018) [9].

Based on these results and family advocacy, the Alberta Children's Hospital/University of Calgary has offered both MAC and the alemtuzumab/low-dose TBI conditioning regimen (NIH regimen) for adolescents and children with SCD with HLA MSDs.

MATERIALS AND METHODS

Study Design

A retrospective cohort analysis of all patients with SCD who have undergone a 10 of 10 MSD HCT at the Alberta Children's Hospital using the NIH nonmyeloablative protocol was undertaken. The analysis includes all such

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patients transplanted between June 2013 and December 2017. The analysis of this retrospective cohort was approved by the Conjoint Health Research Ethics Board at the University of Calgary. All patients and/or guardians signed institutional review board-approved informed consent/assent documents.

Patients

Patients 18 years of age or younger with SCD and an MSD were eligible for transplant (age limit as per institutional policy). Our referring comprehensive SCD clinics discuss the option of HLA typing with all patients, and all patients with an HLA-matched sibling are offered an HCT consultation. Families were historically only offered MAC and were required to meet the Seattle Consensus Criteria due to the body of published literature in children. Over time, with emerging literature describing very low rates of morbidity and mortality with MSD HCT, children with SCD and an MSD were offered HCT consultation. The NIH protocol was used initially in a high-risk patient at our center with neurovascular disease with success. As a result, following extensive discussion at our institution, it was decided that if patients and parents were provided balanced information, then it was reasonable to offer the NIH protocol as an alternative to MAC, and both MAC and the NIH protocol were presented to families. This decision was informed by the impressive safety and efficacy described in adolescents and adults with SCD using the NIH protocol and experience with various combinations of alemtuzumab and low-dose TBI in other regimens for nonmalignant diseases in children. Additionally, other published regimens for SCD have higher rates of GVHD than the NIH regimen, an important cause of morbidity and mortality in HCT for SCD [1,2,5].

All patients met institutional standards for HCT for malignant and nonmalignant diseases, including age-appropriate cardiac, respiratory, hepatic, and renal evaluations. Patients with major ABO-incompatible donors were excluded due to concerns regarding pure RBC aplasia as per the NIH publications [8,9]. HLA antibodies are not routinely tested at our center when the donor is a 10 of 10 HLA match.

Donor Selection

Donor selection was based on medium-resolution HLA A, B, C, DQ and high-resolution DR histocompatibility. Siblings who were 10 of 10 HLA matched with either normal hemoglobin or sickle cell trait (based on hemoglobin electrophoresis) were considered appropriate for donation. Extended RBC phenotyping comparing donor to recipient antigens was not routinely performed. Donors for recipients with RBC antibodies underwent testing for the specific antigen of the recipient antibody. Donors who would require a blood-primed apheresis circuit (usually less than 25 kg at our institution) were deferred until this weight requirement was met to avoid blood product exposure. If donors did not meet the weight requirement for apheresis, MAC HCT was offered with bone marrow harvest, but all families elected to wait until the donor met this weight requirement. Donors underwent a comprehensive medical and psychosocial clearance process as per international and institutional standards of care [11,12]. Donors were not considered research subjects; peripheral blood stem cell donations by minors is routine at our institution and other centers internationally with appropriate safeguards. The donor evaluation included an assessment by a physician other than the HCT physician for the recipient (typically a community pediatrician) and an extensive determination of willingness and psychological suitability to donate with a social worker and child psychologist as per American Academy of Pediatrics guidelines [12].

Treatment

Hydroxyurea (HU) was recommended to all patients as per routine SCD best practice guidelines. For those not already on HU, a minimum of 4 to 6 months of exposure to reduce marrow cellularity was recommended. Hemoglobin S (Hb S) was reduced to 30% or less within 4 weeks prior to HCT using either automated RBC exchange transfusion or chronic simple transfusions.

The conditioning regimen consisted of alemtuzumab 0.2 mg/kg/dose that was administered subcutaneously daily for 5 days (day -7 to day -3, inclusive). A single fraction of 300 cGy of TBI was given on day -2. Testicular shielding was applied to all male patients during radiation. Fertility preservation referrals were made as indicated.

GVHD prophylaxis consisted of sirolimus dosed to achieve a trough level of 8 to 12 ng/mL for the first 3 to 6 months post-HCT, followed by 6 to 8 ng/mL for the remainder of the first year. In the absence of GVHD, patients were eligible for sirolimus weaning at 1 year post-transplant if donor T-cell chimerism reached 50% or greater [8,9].

Similar to the NIH adult protocol, donors received 5 days of granulocyte colony stimulating factor for peripheral blood stem cell mobilization. The target collection was 10×10^6 or more of $CD34^+$ cells per kilogram of recipient weight. All peripheral blood stem cell products were infused fresh without manipulation or T-cell depletion.

Patients were eligible for early discharge post-HCT prior to neutrophil engraftment if clinically stable, if in close proximity to the hospital, and if reliability for follow-up had been established. Clinic visits were scheduled at least twice a week following early discharge (pre-engraftment).

Institutional protocols for routine SCD HCT supportive care were followed as per published standards. No seizure prophylaxis was given for any patient after consultation with our neurology service, as the risk of posterior reversible encephalopathy syndrome with sirolimus was considered to be much lower than that with calcineurin inhibitors. Platelet counts were maintained greater than 50×10^9/L, hemoglobin levels were maintained between 90 and 110 g/L, magnesium levels were kept in the normal range, and blood pressure values were monitored to keep values less than the 95th centile for age and sex. Prophylaxis for *Pneumocystis jiroveci* was given to all patients until 2 months after completion of sirolimus wean. All patients were maintained on oral penicillin at least until the pneumococcal immunization series was initiated at 6 months and completed at 8 months post-HCT. No other prophylactic antibiotics were given, even if patients were eligible for early discharge. Immune reconstitution was monitored as per institutional standards, including quantification of naive and mature T and B cells, NK cell enumeration, and Ig levels.

Outcomes

Primary outcomes were overall survival (OS) and event-free survival (EFS). Events were defined as death or treatment failure. Treatment failure was defined as a clinical manifestation of SCD or graft failure. Failure to initiate sirolimus wean by 2 years post-HCT or a failure to complete sirolimus weaning by 2.5 years post-HCT were initially considered events, but the definition was revised after peer review because such outcomes are not universally described as events. As a result, these outcomes were described separately from events as secondary endpoints and reported as immune suppression/GVHD-free EFS (IS/GVHD-free EFS). Other secondary endpoints included hematopoietic recovery, levels of donor chimerism, Hb S levels, rates of acute and chronic GVHD, immune reconstitution, infectious complications, and rates of grade 3 or 4 toxicities using the Common Terminology Criteria for Adverse Events Version 4. Standard neutrophil and platelet engraftment definitions were used.

Statistical Analysis

Pre-HCT, HCT, and post-HCT characteristics were summarized with means and SDs, as well as medians and interquartile ranges (IQRs) for numerical/continuous variables. For categorical variables, frequencies and percentages are presented. The initiation of sirolimus weaning and completion of weaning was analyzed using the Kaplan-Meier method, with censoring as required based on duration of follow-up.

RESULTS

Patient, Donor, and Graft Characteristics

Recipient characteristics for the 16 transplants meeting eligibility criteria for analysis within the study period are summarized in Table 1. All patients had symptomatic SCD. The median age at transplantation was 12 years (range, 3 to 18 years). HU was taken regularly prior to HCT referral by 13 of 16 patients for sickle cell supportive care, with a median duration of 36 months (range, 1 to 168 months). Three patients who underwent transplant had RBC antibodies pre-HCT (patient 3, indeterminate antigen; patient 5, anti-Lewis and anti-Jkb; patient 8, anti-C and anti-E). Three patients were receiving a chronic transfusion program with a mean duration of 54.50 (SD, 76.11) months. No patient had significant iron overload pre-HCT, which was determined by ferritin and T2* magnetic resonance imaging as recommended by the hemoglobinopathy service; none had liver dysfunction. No grade 3 or 4 baseline cardiac, respiratory, or renal dysfunction was present.

Three patients with an HLA-matched sibling were not offered HCT due to major ABO incompatibility. There were no failed donor stem cell mobilizations and no adverse events for Hb AA or AS donors with the use of granulocyte colony stimulating factor. The ages of the donors ranged from 6 to 20 years. The median dose of $CD34^+$ cells collected was 11.65×10^6 CD34 cells/kg (IQR, 8.45 to 13.48). All donors had ABO-compatible RBCs with their recipient siblings, with exception of one minor ABO incompatibility. No donor had RBC antigens to which recipients had documented antibodies. All collections were completed in 1 day, with exception of a single 2-day collection. To safely undergo a second day of collection, this donor

Table 1
Recipient Characteristics

Patient	Age (Years)	Sex	Sickle Genotype	Type of Crises Pre-HCT	Chronic Transfusion (Yes/No)	Ferritin Level Prior to HCT (μg/L)	# Crises/ Year on HU
1	8	Female	SS	Recurrent VOC	No	102	0
2	18	Female	SS	Recurrent VOC	No	206	1
3	17	Female	SS	Recurrent VOC/ACS/Splenic sequestration	No	83	1
4	10	Female	SS	Recurrent VOC/Splenic sequestration	No	182	0
5	5	Female	SS	Recurrent VOC/Splenic sequestration	No	369	0
6	6	Male	SS	Recurrent VOC	No	103	No HU
7	17	Female	SS	Recurrent VOC/ACS/Splenic sequestration	No	200	0
8	17	Male	SS	Recurrent VOC/ACS	No	176	3
9	13	Female	SS	Recurrent VOC/ACS/Splenic sequestration/Minimal retinal ischemia	No	182	3
10	4	Female	SS	Recurrent VOC/ACS	No	106	0.3
11	12	Male	SS	Recurrent VOC/ACS	No	79	0.2
12	17	Female	SS	Recurrent VOC/Stroke/Sepsis/Hepatic encephalopathy	Yes (Deferasirox)	1468	0.14
13	10	Female	SS	Recurrent VOC/abnormal TCD	Yes (Deferasirox)	2024	No HU
14	3	Female	SS	Recurrent VOC/ACS/Splenic sequestration/ Dactylitis/Hyperhemolysis	Yes (Deferasirox)	2128	0
15	12	Male	SS	Recurrent VOC/Osteomyelitis	No	165	No HU
16	14	Male	SS	Recurrent VOC	No	156	1

VOC indicates vaso-occlusive crisis; ACS, acute chest crisis; TCD, transcranial doppler ultrasonography.

required one platelet transfusion. All donors remain clinically well with ongoing follow-up as per institutional practice.

OS, Events, and Treatment Failure

All patients are alive at median follow-up 19.5 months post-transplant (IQR, 15.2 to 27.8). The OS and EFS were 100% and 100%, respectively. There were no cases of primary or secondary graft failure, with no sickling events post-HCT (i.e. no pain or other crises or need for opioids for vaso-occlusion). Fourteen patients have been assessed in a comprehensive hemoglobinopathy clinic post-HCT to screen for SCD morbidity with no evidence of recurrence of SCD-related pathologies. All patients are followed in comprehensive HCT short-term and long-term follow-up clinics. Such clinics routinely assess immune reconstitution and review infectious complications as per institutional standards.

Twelve of 16 patients discontinued sirolimus with a median follow-up of 11 months following discontinuation of the drug (range, 1 to 40 months). No patient required sirolimus to be reinstituted. Three patients were not eligible for weaning, because they were under 1 year post-HCT at the time of data collection. One patient had not initiated weaning at 18 months post-HCT, with T-cell chimerism averaging 30%. One patient did not complete sirolimus weaning by 2.5 years—weaning began at 22 months; however, the wean was stopped due to dropping T-cell chimerism. With recovery of donor T cells, weaning was reinitiated at 30 months post-HCT and completed by 32 months. It is noteworthy that this patient had a history of poor adherence to sirolimus. This patient had stable MC 13 months after discontinuation of sirolimus.

Nine patients initiated sirolimus weaning at 1 year, with an additional three patients weaning by 2 years post-HCT. Median duration of sirolimus exposure for patients with at least 1 year of follow-up was 14 months (range, 14 to 32 months).

Engraftment/Donor Chimerism/Immune Reconstitution

All patients developed grade 4 neutropenia. Neutrophil engraftment occurred at a median of 22 days (IQR, 20.5 to 25). Platelet engraftment occurred at a median of 16.5 days (IQR, 0.00 to 19.25). Eleven patients had platelet counts less than 50 10^9/L requiring platelet transfusion(s), and nine patients required packed RBC transfusion(s) for a hemoglobin level less than 90 g/L. The median number of transfusions required was one for platelets (IQR, 0 to 2); 8 patients required one packed RBC transfusion, and one patient required two such transfusions (IQR, 0 to 1.00).

All patients had evidence of donor myeloid chimerism by 30 days post-HCT. All patients achieved stable MC sufficient to maintain Hb S levels below 50%. Donor myeloid and T-cell chimerism over time are shown in Figures 1 and 2, respectively. The cumulative incidence of achieving donor $CD3^+$ chimerism of 50% or greater (criteria to wean immune suppression) is presented in Figure 3. T-cell recovery rose over the second half of the first year post-HCT, consistent with previous alemtuzumab data [13]. Kinetics of recovery of $CD4^+$ T cells over time is shown in Figure 4. Levels of donor myeloid engraftment were stable in all patients after discontinuation of sirolimus. Table 2 summarizes engraftment at last follow-up. No patient required intravenous Ig replacement. Hb S levels reflected donor Hb S status, as presented in Figure 5.

Safety

All recipients tolerated the conditioning regimen well, with no grade 3 or 4 reactions to alemtuzumab. The median length of hospital stay was 7 days from stem cell infusion (range, 4 to 19 days). As experience with the HCT regimen increased, patients were discharged prior to engraftment with close follow-up in the outpatient setting, which accounts for the variability in duration of hospitalization. This practice change was made with increasing experience with the protocol and the relatively small number of interventions with blood products, magnesium, and antihypertensives observed. Clinical follow-up—including laboratory and blood pressure monitoring—were performed at least twice a week in the ambulatory clinic. Early discharge was considered only if the patient was medically suitable,

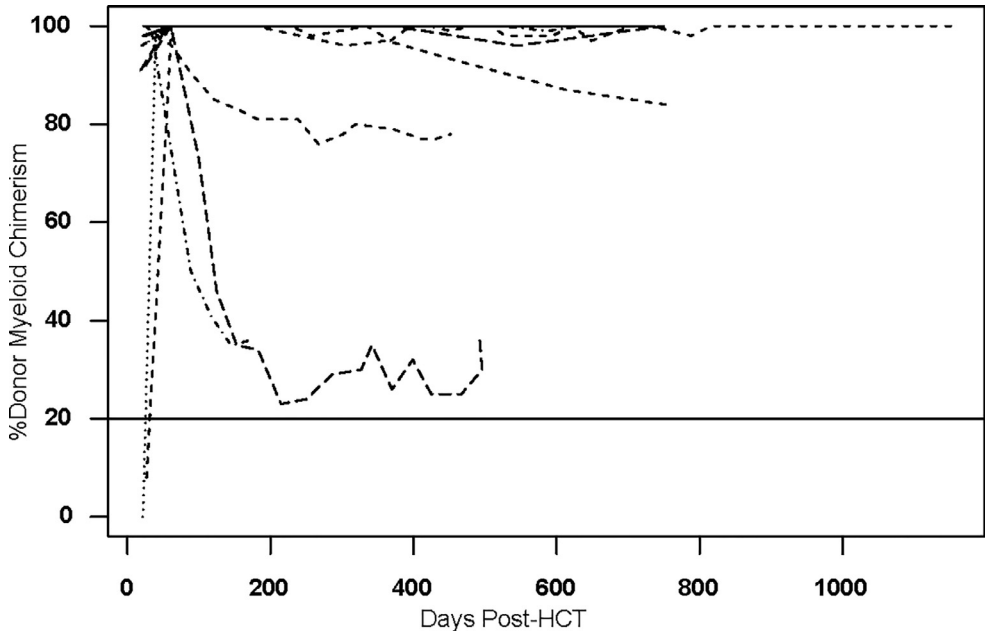

Figure 1. Donor myeloid chimerism post-HCT.

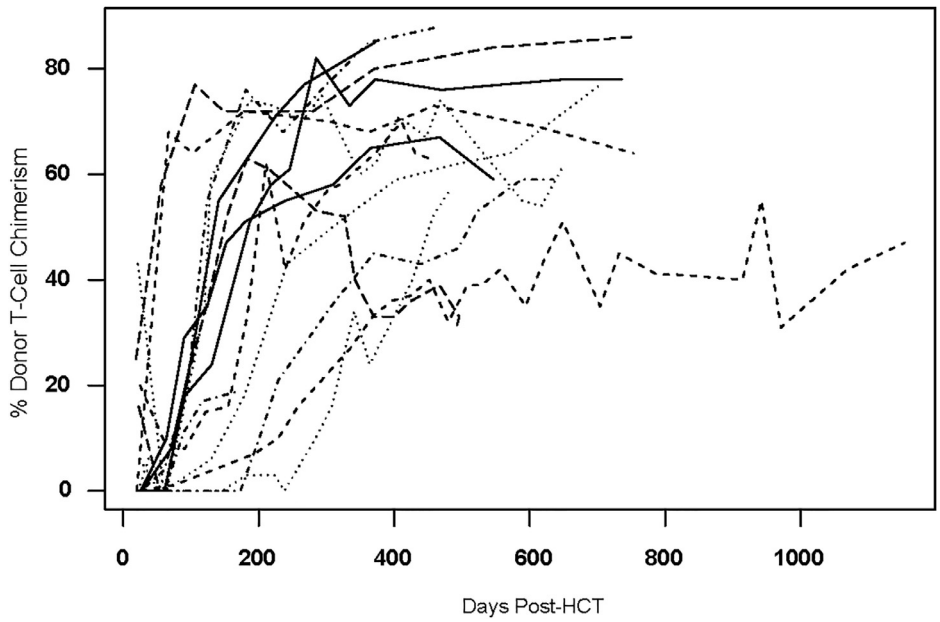

Figure 2. Donor T-cell chimerism post-HCT.

accommodations were within a 30-minute drive from the hospital, transportation was reliable, and both families and providers felt early discharge was safe. Ten patients (62%) required admission after initial discharge with a median of one admission per patient (IQR, 1 to 1.75). Reasons for readmission included fever with neutropenia, cytomegalovirus (CMV) reactivation requiring preemptive therapy, and dehydration. One patient developed septic shock due to line infection with *Acinetobacter ursingii* (grade 4 infection). No late opportunistic infections were observed with prolonged sirolimus exposure.

None of the patients developed other grade 3 or 4 toxicities, posterior reversible encephalopathy syndrome, or seizures (despite lack of prophylactic antiepileptic drugs). Two patients required temporary use of amlodipine to maintain blood pressure below the 95th centile for age. Central nervous system examinations were stable; to date, post-HCT neuroimaging is not standard at our center in the absence of clinical concerns.

There were no occurrences of CMV or Epstein-Barr virus disease or post-transplantation lymphoproliferative disorder. Five patients had Epstein-Barr virus reactivation, but none required therapy. Eight of the 16 patients had CMV reactivation, 3 of whom required preemptive therapy (valganciclovir, 1 patient; foscarnet, 1 patient; both, 1 patient). CMV pre-emptive therapy practices were based on viral load and time post-HCT as per institutional guidelines, with these guidelines having undergone revision during the period of study with variability in viral load measurement, reporting, and threshold

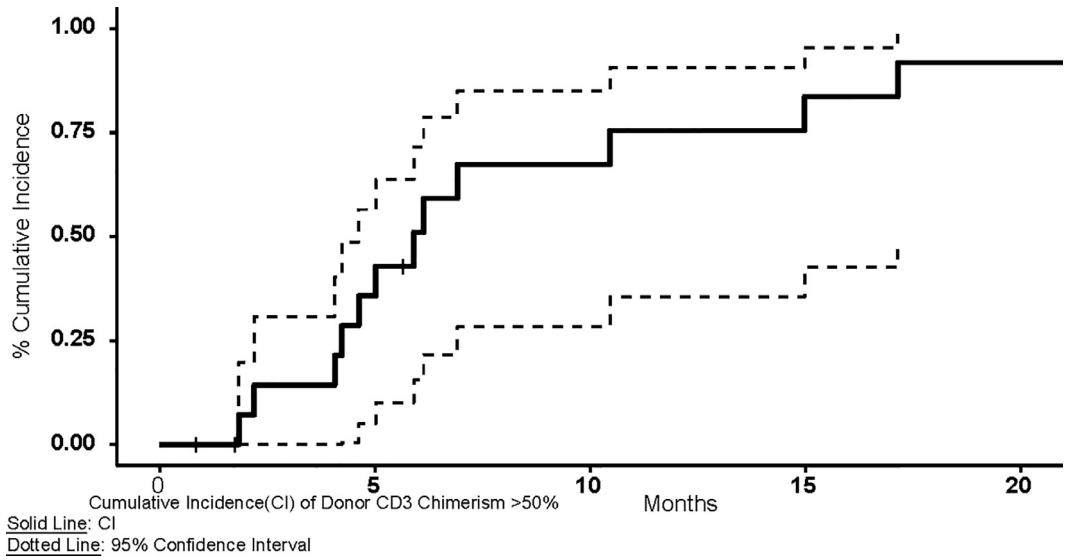

Figure 3. Cumulative incidence of donor CD3 chimerism greater than 50%. Solid line: cumulative incidence. Dotted line: 95% confidence interval.

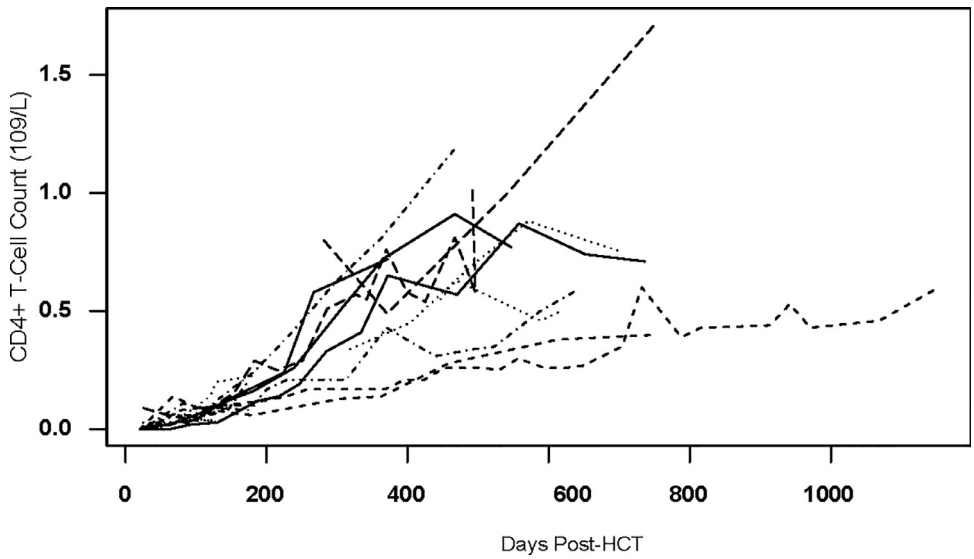

Figure 4. $CD4^+$ T-cell counts post-HCT.

for intervention over the study period. Initially, a viral load greater than 5000 IU/mL would prompt pre-emptive therapy; however, more recently, this threshold for intervention has been reduced to 1000 IU/mL.

No patient developed acute or chronic GVHD, resulting in an IST/GVHD-free EFS of 75% at 3 years (95% confidence interval, 42.6 to 100).

Long-Term Follow-Up Data

Follow-up of neuropsychology testing, pulmonary function, transcranial Doppler evaluations, and surveillance for treatment-related malignancies is ongoing. Evaluation of pneumococcal titers is not routinely available at our institution. Ongoing monitoring for late infection with encapsulated organisms is important because susceptibility to pneumococcal infection is possible even with revaccination. Access to pneumococcal titer testing is being pursued. Pre- and post-HCT magnetic resonance brain imaging is to be implemented as part of a prospective neuroimaging study.

DISCUSSION

Outcomes of myeloablative HCT for SCD using MSDs have been well established, with high rates of OS and EFS [2,14]. Reduced-intensity and nonmyeloablative regimens are particularly desirable to reduce acute and late toxicities of HCT [5-7]. It is hoped that fertility preservation is more likely with these less toxic regimens, but data confirming this hypothesis are lacking. The risks of acute and chronic GVHD remain important considerations when offering HCT to children with SCD who have an MSD [15]. Younger patients with SCD have better outcomes than adolescents over 14 years of age, and waiting until a given patient's phenotype has manifested comes with the risk of potential long-term morbidity from SCD, higher rates of transplant-related mortality, and subsequent HCT ineligibility due to performance status and comorbidities [2,5,16-19].

Based on compelling data from the NIH, University of Illinois, and local patient/family advocacy, we adopted this very low intensity nonmyeloablative regimen for children with SCD. This protocol was initially used for one patient with

Table 2
Engraftment at Last Chimerism Evaluation

Patient	Donor Hb Genotype	Donor ABO Compatibility	CD34 Dose Infused (10^6/kg)	Time from Day 0 to Chimerism Testing (Days)	Time Off Sirolimus (Days)	Hb (g/L)	% Hb S	% Donor T Cell Chimerism	% Donor Myeloid Chimerism
1	AA	Compatible	11	736	403	120	0	78	100
2	AS	Compatible	6.5	452	204	126	34	63	78
3	AS	Compatible	15	482	30	129	28	57	100
4	AA	Compatible	9.3	468	147	112	0	88	100
5	AS	Compatible	12.3	492	N/A	126	40	31	37
6	AS	Compatible	16	547	227	129	39	59	100
7	AA	Compatible	8	1153	310	133	0	47	100
8	AS	Compatible	13	651	304	143	36	62	100
9	AA	Compatible	8.6	635	140	153	0	59	100
10	AS	Compatible	14	750	920	129	34	86	100
11	AS	Compatible	13.3	372	339	135	40	85	100
12	AS	Compatible	7.1	753	777	140	40	64	84
13	AA	Minor	12.9	704	1198	140	0	77	100
14	AA	Compatible	15	173	N/A	103	3.8	19	36
15	AS	Compatible	6.1	54	N/A	122	25	0	100
16	AA	Compatible	8.7	26	N/A	108	8.4	4	100

Hb indicates hemoglobin.

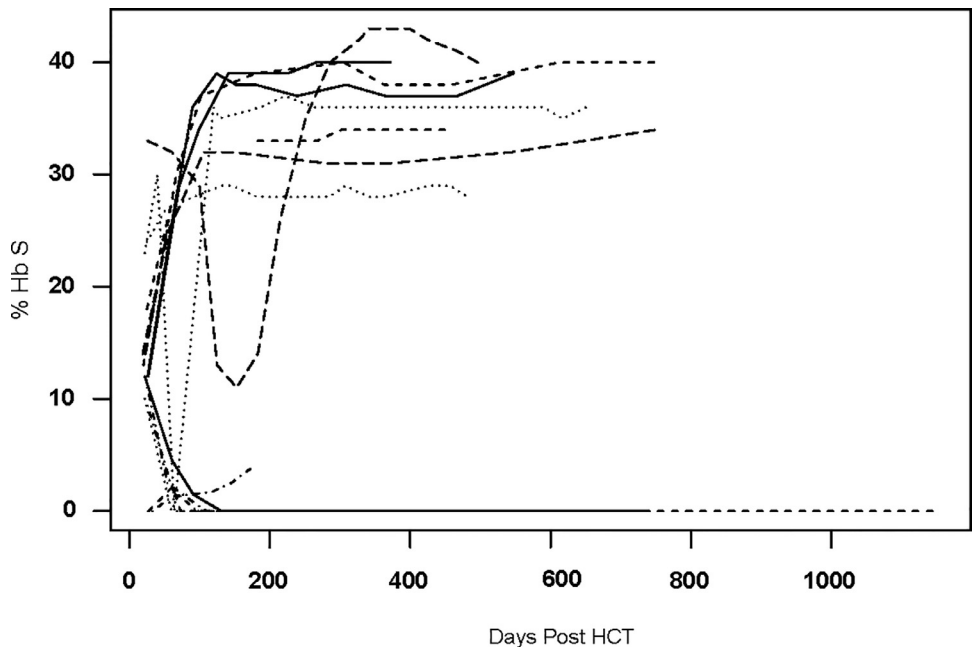

Figure 5. Percentage Hb S post-HCT.

neurovascular disease who sought an outside opinion and requested a less intensive HCT approach. When the NIH protocol was first offered at our center, the growth of our HCT program for SCD was not anticipated. As a result, this protocol was not offered on a prospective clinical trial. Based on a favorable experience with our first case and compelling published data in young adult patients 17 years of age and older—notably a much lower risk of GVHD compared to other published regimens—our group felt that with a balanced and informed discussion with patients and families of the risks and benefits of MAC and the NIH protocol, it was reasonable to offer either approach to younger patients.

When our data are combined with the adult literature, GVHD has not been observed in more than 60 MSD HCTs with this regimen [9,10,20]. The absence of GVHD may be due to the timing of alemtuzumab administration in combination with the T-cell MC. With all patients surviving in our study, an HCT approach with no GVHD risk makes transplantation an attractive option for children with SCD and an MSD. However, longer follow-up is required to exclude late graft failure events that are well described in HCT for SCD, which is particularly relevant to this population with MC. Our estimates of OS and EFS are projections using statistical modeling, as the median duration of follow-up is less than 2 years. Two publications

suggest that 20% to 25% donor myeloid engraftment can be curative for SCD, and with two of our patients having 36% and 37% donor myeloid engraftment, the long-term maintenance of donor-derived erythropoiesis for these patients is uncertain at present [3,21]. It must also be noted that our sample size is small and our analysis retrospective. This approach requires further validation in this population. With established disease-modifying agents such as HU, which clearly reduce sickling crises in most patients, it is acknowledged that most children survive to adulthood and with improved quality of life.

A criticism of the NIH regimen is that some adult patients are not eligible for weaning of sirolimus by 1 year post-HCT [8-10]. Only 4 of 13 of the adult patients in the cohort published by Saraf et al. met this metric, although 50% of the NIH cohort were able to successfully wean sirolimus [9,10,22]. The median duration of immune suppression administration was 2.1 years in the NIH study [9]. It is estimated that less than 10% of patients receiving either myeloablative or less intensive conditioning regimens remain on immune suppression beyond 2 years post-HCT [22]. Only one of the children in our cohort could not wean sirolimus as per our established targets (with a history of nonadherence); this patient initiated weaning before 2 years, but the wean was not completed until 2.6 years post-HCT. There was no patient unable to be weaned, and nine patients were eligible for weaning at 1 year post-HCT. The requirement of 50% donor T-cell chimerism for eligibility for sirolimus weaning has been based on an amendment to the original protocol at the NIH [8,9]. It is possible that this somewhat arbitrary threshold might be further reduced to decrease the duration of sirolimus exposure, a question that might be answered with future research. No significant sirolimus toxicities were noted, but adherence to this medication for 1 or more years is not without challenges and risk of adverse effects.

The NIH regimen does not include cytotoxic chemotherapeutic agents and might be offered to most patients with an MSD due to tolerability. The dose of radiation allows for a greater likelihood of fertility preservation in females compared to myeloablative regimens, and males can be offered sperm banking or gonadal shielding, as was the case at our institution. The NIH reports five pregnancies in three females and seven pregnancies from three males with this regimen, with one male patient requiring gonadotropin therapy (M. M. Hsieh, NIH, e-mail communication May 7, 2018) [23]. More data to substantiate rates of fertility preservation will be important in the future.

TBI is associated with therapy-induced malignancy risk [24,25]. Such risk warrants appropriate caution with the use of this conditioning modality, and understandably many regimens for nonmalignant diseases do not include TBI for this reason. As an example, the risk of non-squamous cell carcinoma of the skin is higher in patients who receive TBI at younger ages [24]. Other reports have indicated that older age at HCT or the development of chronic GVHD are important risk factors for skin cancer [24-26]. No second malignancies were reported in one series of 205 patients who underwent HCT with MAC and TBI doses under 5 Gy [27]. However, the risk of malignancy with low-dose TBI has not been well defined, and late effects from low-dose TBI are not limited to malignancy and include thyroiditis and gonadal toxicity [6,9]. Surveillance for treatment-induced malignancy and other radiation-associated late effects with the use of low-dose TBI into survivorship is imperative. Appropriate counseling regarding these important potential late effects is required, especially because it will take many years to truly define the risk.

The low rate of acute toxicities noted in the first patients to undergo HCT in our cohort, combined with infrequent and predictable transfusion requirements, allowed for early discharge for patients with suitable family support. Future initiatives include the administration of the conditioning and stem cell infusions in our clinic, making this HCT procedure completely outpatient based. With significant average lifetime healthcare costs for a person with SCD, a cost analysis of this outpatient approach is imperative [28,29].

Limitations of our study include its retrospective design, small sample size, and the potential for selection bias. Our center offers HCT to all patients with an MSD resulting in variability in disease severity in this cohort. Longer follow-up is needed to substantiate these results and to monitor for late effects. It should be noted that our original protocol for this retrospective analysis included failure to initiate sirolimus weaning by 2 years or failure to complete sirolimus wean by 2.5 years as events. If these events are included, the 3-year EFS is 74% (equivalent to the IS/GVHD-free EFS). These two criteria for events were removed after strong recommendations from colleagues to use a more universally applied definition of EFS for HCT for SCD, with the IS/GVHD-free EFS reported separately.

In summary, the NIH MSD protocol can cure children with SCD and an MSD with no documented cases of GVHD. Acute toxicities were minimal, and outpatient post-HCT care was safe and feasible. All children were eligible for sirolimus wean and maintained stable donor engraftment on discontinuation of immune suppression. Ongoing follow-up to ensure graft stability is imperative. Longer follow-up is necessary to determine fertility outcomes and malignancy risk in this young population. Validation of this approach in a larger cohort and application to recipients with an MSD and major ABO incompatibility are the focus of two registered active clinical trials. The authors acknowledge that novel approaches to HCT should be undertaken in a study setting to establish validated outcomes. The position and timing of HCT compared to best supportive care for SCD is in constant evolution, particularly with novel disease-modifying agents approved for clinical use and under development.

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