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Haploidentical

Haploidentical Bone Marrow Transplantation with Post-Transplantation Cyclophosphamide Plus Thiotepa Improves Donor Engraftment in Patients with Sickle Cell Anemia: Results of an International Learning Collaborative

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A B S T R A C T

Curative therapy for individuals with severe sickle cell disease (SCD) who lack an HLA-identical sibling donor has been frustratingly elusive. In with the goal of improving engraftment while minimizing transplantation-related morbidity, a multi-institutional learning collaborative was developed in the context of a Phase II clinical trial of nonmyeloablative, related HLA-haploidentical (haplo) bone marrow transplantation (BMT) with post-transplantation cyclophosphamide. All eligible participants had hemoglobin SS, and 89% (16 of 18) had an identifiable donor. The median patient age was 20.9 years (IQR, 12.1 to 26.0 years), and the most common indication for transplantation was overt stroke (in 69%; 11 of 16). In the first 3 patients, the conditioning regimen consisted of antithymocyte globulin, fludarabine, cyclophosphamide, and low-dose total body irradiation. Graft-versus-host disease (GVHD) prophylaxis included post-transplantation cyclophosphamide, mycophenolate mofetil, and sirolimus. Primary graft rejection occurred in 2 of the 3 patients (67%), which triggered the study-stopping rule. To reduce graft rejection risk, thiotepa was added to the conditioning regimen, and then 15 patients (including 2 with previous graft rejection) underwent haplo-BMT with this thiotepa-augmented conditioning regimen. At a median follow-up of 13.3 months (interquartile range [IQR], 3.8 to 23.1 months), 93% (14 of 15) had >95% stable donor engraftment at 6 months, with 100% overall survival. The median time to neutrophil engraftment (>500) was 22 days (IQR, 19 to 27 days), and that for platelet engraftment (>50 x $10^{9/L}$) was 28 days (IQR, 27 days to not reached). Two patients had grade III-IV acute GVHD, 1 patient had mild chronic GVHD, and 86% of patients (6 of 7) were off immunosuppression therapy by 1-year post-transplantation. Our data suggest that haplo-BMT with post-transplantation cyclophosphamide and thiotepa improves donor engraftment without significantly increasing morbidity or mortality and could dramatically expand curative options for individuals with SCD.

INTRODUCTION

Sickle cell disease (SCD) has evolved from a life-threatening condition in early childhood to a chronic disease in adults [1,2]. Improved supportive care [3], use of hydroxyurea therapy [4], and regular blood transfusion therapy for primary [5] and secondary [6] stroke prevention have resulted in improved childhood clinical outcomes and survival. Approximately 99% of all children born with SCD are expected to live to adulthood [7,8]. The most feared complication in children with SCD is stroke [9]. Unfortunately, regular blood transfusion therapy for secondary prevention of stroke is palliative [10,11].

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Approximately 45% of children with stroke receiving regular blood transfusions will have recurrence (overt or silent stroke) within approximately 5 years [10]. Adults with SCD are plagued with age-dependent chronic organ dysfunction [12], which can be cumulative [13,14], impacting quality of life [15] and resulting in earlier death [16].

The use of myeloablative, HLA-identical sibling donor hematopoietic stem cell transplantation (HSCT) as a curative therapy for children with symptomatic SCD is well established [17–20]. However, this myeloablative approach is deemed too toxic in adults [21,22], in whom the risks of short and long-term complications, including a transplantation-related mortality of 5%, a rate of acute graft-versus-host disease (GVHD) of 10% to 20%, and a 5-year probability of chronic GVHD of 14% to 22% are prohibitive [18,23,24]. Nonmyeloablative HLA-identical sibling donor transplantation using $CD34^+$-mobilized peripheral blood stem cells in adults appears promising [24,25], but this approach is of limited applicability owing mainly to the very small pool of HLA-matched sibling donors for eligible recipients (approximately 10% to 15%) [24,26]. Unrelated HLA-matched donors also have limited availability for individuals with SCD [27], and results using this approach have not been encouraging [28–30].

To address both the limitations in donor availability and the unacceptable toxicity of myeloablative conditioning regimens in adults with SCD, nonmyeloablative strategies for related haploidentical HLA-matched donors have been considered. Initial attempts at expanding the donor pool, including the use of T cell-depleted ($CD34^+$-selected) related haploidentical peripheral blood stem cells in recipients with SCD [31], resulted in a transplantation-related mortality of 25% and event-free survival (EFS) and disease recurrence of 38%. The use of $CD3^-$/$CD19^-$-depleted haploidentical peripheral blood stem cell grafts in individuals with severe SCD [32] at a median follow-up of 26 months showed stable engraftment, a 22% incidence of significant neurologic events, 4% transplantation-related mortality, and a 56% cumulative incidence of grade I-II acute GVHD. Three patients with stable mixed chimerism in the peripheral blood showed complete engraftment in line-specific chimerism analyses of red cell precursors (CD235a, glycophorin A) in the bone marrow, with no SCD-related complications. Similarly, the use of ex vivo depletion of T cells ($CD3^+$ or α/β T cells) and B cells ($CD19^+$) from haploidentical peripheral blood stem cell grafts (lymphocyte subset responsible for GVHD occurrence) in children with nonmalignant disorders [33] showed an EFS of 91%, a graft rejection rate of 17%, a 13% incidence of acute GVHD, and 9.3% transplantation-related mortality.

Nonmyeloablative, T cell-replete, related haploidentical bone marrow transplantation (haplo-BMT) with post-transplantation cyclophosphamide (PTCy) has emerged as a viable alternative to overcoming the 2 major obstacles to the use of HSCT as a curative modality for SCD: limited donor availability and regimen-related toxicity [34]. However, an initial feasibility trial using this approach was associated with an unacceptable graft rejection rate of 43%, albeit with no transplantation-related mortality.

In an effort to decrease graft rejection without increasing transplantation-related mortality in recipients of haplo-BMT, we assembled a 3-site international, multi-institutional learning collaborative in 2013. The primary objective of this 3-site collaborative effort was to provide sufficient evidence, if available, for a definitive multi-institutional Phase II study of haplo-BMT with PTCy in children and adults with severe SCD.

METHODS

Study Design

We performed this prospective Phase II trial of nonmyeloablative haplo-BMT in patients with severe SCD in the context of a multi-institutional learning collaborative. The primary endpoint was 1-year EFS. Primary or secondary graft rejection, stroke, and death were considered events. Secondary endpoints included overall survival (OS), hematopoietic cell recovery, acute and chronic GVHD, infections, hepatic sinusoidal syndrome, interstitial pneumonitis, seizures, posterior reversible encephalopathy syndrome, and health-related quality of life. The trial (ClinicalTrials.gov identifier NCT01850108) was approved by the Institutional Review Board at each participating site and opened in October 2013.

Learning Collaborative Model and Study Setting

We used a health care quality improvement methodology developed by the Institute for Healthcare Improvement [35]. This short-term learning system brings together several teams of individuals from hospitals or clinics to solve a clinical problem. Team collaboration using the learning collaborative model has achieved good results in other clinical areas [36,37]. We created a multidisciplinary haplo-BMT consortium for SCD comprising 3 clinical sites in France, the United Kingdom, and the United States. The learning collaborative had a common Data Safety Monitoring Committee, whose members included a statistician, adult and pediatric transplant physicians, and pediatric hematologists. Project oversight was reviewed twice during the first year by an advisory board. The 3 sites enrolled participants, and the following strategy was undertaken in the learning collaborative: (1) use of common eligibility criteria, (2) monthly telephone conferences, (3) collection of minimal and accurate data, (4) common objectives and stopping rules, and (5) a spirit of collaboration. The main objective was to reduce the graft rejection rate using nonmyeloablative haplo-BMT as a curative modality for children and adults with severe SCD. The study-stopping rules with Data Safety Monitoring Committee oversight required a pause in the study and protocol modifications for a mortality rate of >20%, graft rejection, or severe chronic GVHD in the first 5 enrolled participants.

Participants

Eligible participants included those with severe SCD, including hemoglobin (Hb)SS, $HbS\beta^0$ and $HbS\beta^+$ thalassemia, HbSC, HbSE, HbSD, HbSO-Arab, and HbS-HPFH; those with good baseline performance status (Eastern Cooperative Oncology Group score 0 or 1; Karnofsky Performance Scale score ≥70), age 1 to 70 years; and those without an HLA-matched sibling donor but with a suitable and available first-degree haploidentical donor. Individuals who had undergone previous haplo-BMT with graft rejection were eligible. Informed consent was obtained from parents or from participants aged >16 years, and assent was obtained from younger participants before enrollment, in accordance with the Declaration of Helsinki. Participants were referred for transplantation by independent hematologists and were fully informed about other available treatment options for their severe SCD. The risks and benefits of haplo-BMT were discussed extensively with each participant and his or her caregivers before signing informed consent. Disease severity was determined based on the presence of at least 1 of the following: (1) stroke or cerebrovascular event lasting >24 hours; (2) evidence of cerebrovascular disease, confirmed by magnetic resonance angiography despite regular transfusion therapy for at least 12 months; (3) silent cerebral infarct, defined as an abnormal magnetic resonance imaging (MRI) of the brain (signal abnormality of at least 3 mm in 1 dimension and visible in 2 planes on T2-weighted or fluid-attenuated inversion recovery images), with associated evidence of vasculopathy or progression based on local interpretation; (4) recurrent acute chest syndrome despite hydroxyurea therapy; (5) stage I or II chronic lung disease [15]; and (6) recurrent vaso-occlusive pain episodes (>2 per year for the previous 2 years) despite receipt of hydroxyurea or regular blood transfusion therapy, and other complications published as indications for transplantation in SCD [17–19]. Adequate pretransplantation organ function was required, including a left ventricular shortening fraction >26% and absence of liver cirrhosis (Ishak stage ≥5).

Treatment

All 3 sites used the same Johns Hopkins haplo-BMT platform (ClinicalTrials.gov identifier NCT00489281). The HbS level was maintained at ≤35% at least 7 days before the start of conditioning, and iron chelation and hydroxyurea therapy were discontinued at least 24 hours before the start of conditioning. The conditioning regimen consisted of Thymoglobulin (0.5 mg/kg on day -9, 2 mg/kg on days -8 and -7; total dose, 4.5 mg/kg), fludarabine (30 mg/m^2 on days -6 to -2; total dose, 150 mg/m^2), cyclophosphamide (14.5 mg/kg on days -6 and -5; total dose, 29 mg/kg), and total body irradiation (200 cGy) on day -1. GVHD prophylaxis included Cy 50 mg/kg on days +3 and +4, mycophenolate mofetil on days +5 to +35, and sirolimus at a target of 5 to 15 ng/mL for 1 year. In the learning collaborative, there were differing opinions regarding the optimal conditioning regimen to address the initial high graft rejection rate [34] (Figure 1).

Donors and Grafts

Bone marrow stem cells were harvested from consenting first-degree relatives who shared at least 1 HLA haplotype with the patient, did not have SCD or another hemoglobinopathy, and were in good health. Potential donors were initially typed at the HLA-A, -B, -C, and -DRB1 loci at an intermediate- or high-resolution level. HLA-DRB1 and -DQB1 alleles were typed at a high-resolution level. Haplotypes were determined based on family studies whenever possible [38]. All donors (except for 3 in the thiotepa group) were primed with filgrastim (GCSF) 10 μg/kg/day for 5 consecutive days (days -5 to -1). The target total nucleated cell (TNC) dose was 8 to 16 $\times$ 10^8 cells/kg of recipient ideal body weight, with the volume not to exceed 20 mL/kg of donor weight once the minimal target dose was reached, infused on day 0. Donors with sickle cell trait were not excluded. When more than 1 donor was available, the selection priority was as follows: donor age <40 years, donor with the fewest HLA allele mismatches and major/minor ABO mismatches, cytomegalovirus (CMV) mismatched donor-recipient pairs, and those with the fewest donor-specific anti-HLA antibodies. Bone marrow was unmanipulated except in major ABO-incompatible grafts, and graft volumes >20 mL/recipient kg were plasma-depleted.

Supportive Care

Supportive care recommendations included the administration of GCSF based on institutional standards; weekly surveillance for CMV, adenovirus, and Epstein-Barr virus (EBV) reactivation; prompt treatment of viral reactivations or infections; strict blood pressure control; seizure prophylaxis for the duration of immunosuppressive therapy; maintenance of platelet count >50 $\times$ 10^9/L to mitigate against the risk of intracranial hemorrhage [17]; antifungal prophylaxis through day +100; and prompt treatment of other suspected infections according to institutional standards.

Outcome Measurements

Chimerism studies was measured by polymerase chain reaction analysis of variable number of nucleotide tandem repeats unique to donors or recipients of total peripheral blood and isolated $CD3^+$ T cells on days +30, +60, +180, and +365 ($\pm$7 days) and then annually for 2 years. Primary graft rejection was defined as the presence of <5% donor cells as assessed by peripheral blood chimerism (any lineage) by day +60. Secondary graft rejection was defined as the presence of <5% donor cells as assessed by peripheral blood chimerism (of any lineage), with previous evidence of $\geq$5% donor cells. OS was defined as the time from transplantation to death and was censored at the last follow-up. Neutrophil recovery defined as the first of 3 days when the neutrophil count was $\geq$.5 $\times$ 10^9/L. Platelet recovery was defined as a platelet count $\geq$50 $\times$ 10^9/L in the first of 7 days post-transplantation without a platelet transfusion. GVHD severity was graded using the established National Institutes of Health's consensus criteria [36]. Transplantation-related mortality was defined as death due to any transplantation-related cause other than disease recurrence.

Statistical Analysis

Baseline patient characteristics were summarized with median and interquartile range (IQR) for continuous variables and with frequency and proportion for categorical variables [36]. When there are only 3 data entries, each value is listed instead of the data summary. EFS, OS, and other time-to-event data were estimated using the Kaplan-Meier method, and median survival was estimated and reported with a 95% confidence interval. Because there was no competing risk, cumulative incidence was computed using the Kaplan-Meier estimator. Data were collected using Excel (Microsoft, Redmond, WA) spreadsheets, checked by the different principal investigators from enrolling sites. All data analysis, including data cleaning, was done using R version 3.4 (R Foundation for Statistical Computing, Vienna, Austria). The original study-stopping rule was based on a Bayesian beta-binomial model with a beta (.2, .8) distribution as the prior. The data were to be reviewed after every 5 participants, and stopping boundaries were set up so that the probability of stopping for safety review was small if the true toxicity probability was 5%. Graft rejections occurred in 2 of the first 3 patients. Although the boundary was not crossed, the data monitoring committee requested a safety review and accrual halt at that point.

RESULTS

Participant and Donor Characteristics

Participant and donor characteristics are summarized in Tables 1 and 2. Sixteen participants received 18 haplo-BMTs, and all had HbSS. After the stopping rule was met (2 of 3 engraftment failures in the first three trial participants), to improve the graft rejection rate, thiotepa (10 mg/kg/day on day -7) was added to the conditioning regimen following a protocol amendment [39] (Figure 1). Fifteen participants underwent haplo-BMT with thiotepa, including 2 participants who developed graft rejection following initial haplo-BMT without thiotepa. One participant was treated according to protocol but could not sign informed consent, because his health insurance did not include a clinical trial benefit. This participant was included in the analysis of this case series with permission from the Vanderbilt University Institutional Review Board.

The median age of haplo-BMT recipients was 22.1 years (IQR, 16.5 to 36.5 years) in those not receiving thiotepa and 20.4 years (IQR, 12.1 to 26.0 years) in those receiving thiotepa. Seven recipients were aged <18 years at the time of transplantation, and 11 were male. The main indications for transplantation included acute chest syndrome (in 13 of 16 recipients), cerebrovascular disease (overt stroke and silent cerebral infarcts, in 12 of 16), and acute vaso-occlusive pain episodes despite hydroxyurea therapy (in 9 of 16). Most participants had more than one indication for transplantation. Thirteen patients were receiving regular blood transfusions, and 5 were receiving hydroxyurea therapy before transplantation. The estimated median pretransplantation liver iron concentration, as measured on T2*-weighted MRI and/or FerriScan, was 7.8 mg Fe/g (IQR, 2.3 to 29 mg Fe/g) in haplo-BMT recipients not receiving thiotepa and 7.0 mg Fe/g (IQR, 4.0 to 23 mg Fe/g) in those receiving thiotepa (P = 1.0).

The donors included 12 parents (8 mothers and 4 fathers) and 6 siblings, all but 2 of whom had sickle cell trait. None of the donors with sickle cell trait experienced significant toxicity with the use of GCSF as a priming agent. Donor-specific anti-HLA antibodies were assayed in all recipients; 3 (patients 2, 11, and 15)

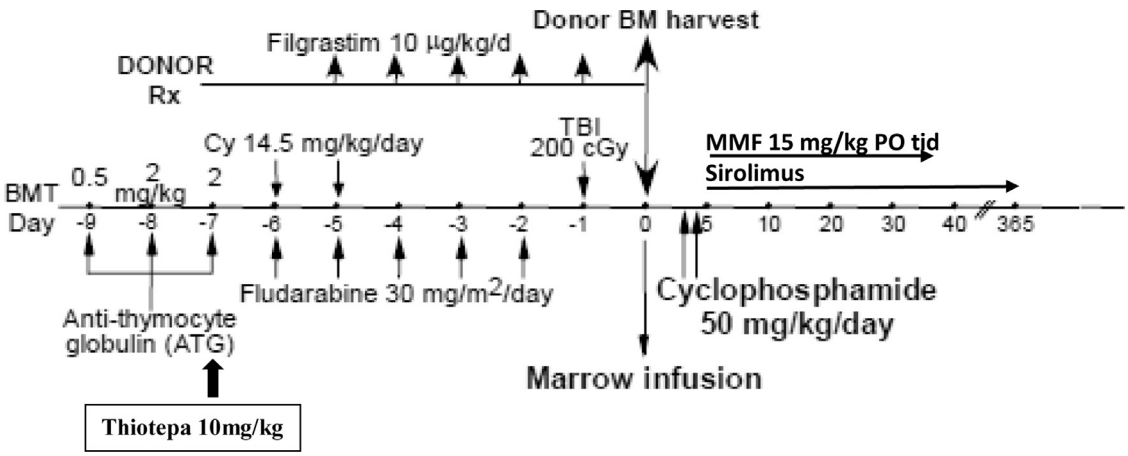

Figure 1. Conditioning schema for haploidentical BMT with PTCy plus thiotepa.

Table 1
Donor and Recipient Baseline Characteristics

Characteristic	Haplo-BMT without Thiotepa (n = 3)	Haplo-BMT with Thiotepa (n = 15)	All Haplo-BMT (n = 18)*
Age, yr, median (IQR)	(10.8, 22.1, 50.9)	20.4 (12.1-26.0)	20.9 (12.1-26.0)
Age <18 yr, n	1	6	7
Age >18 yr, n	2	9	11
Male sex, n (%)	(3) 100	(11) 73	(11) 61
SCD phenotype (HbSS), n	3	15	18
Total follow-up after study entry, mo, mean (SD)	ND	13.0 (6.0-29.7)	16.7 (6.5-30.8)
Donor, n			
Mother	1	7	8
Father	1	3	4
Sibling	1	5	6
Sickle cell trait (AS)	2	14	16
Indication for transplantation, n†			
Stroke (overt and silent infarcts)	3	9	12
Moyamoya disease	2	7	9
Abnormal TCD velocity (≥200 m/s)	1	3	4
Acute chest syndrome	3	10	13
Vaso-occlusive pain crisis	1	8	9
RBC alloimmunization	1	5	6
Sickle cell nephropathy	1	0	1
Transfusional iron overload, n	3	14	17
Serum ferritin, ng/mL, median (IQR)	(1129, 1500, 1984)	2362 (1069-4892)	2081 (834-3898)
Liver iron concentration, mg Fe/g, median (IQR)‡	2.3, 7.8, 29.4	7.0 (4.0-22.9)	7.40 (3.88-23.09)
Disease-modifying therapy before transplantation, n§			
Regular blood transfusion	3	10	13
Hydroxyurea	0	5	5
Donor-recipient sex match, n			
Sex-matched transplant	0	9	9
Female donor, male recipient	3	3	6
Male donor, female recipient	0	2	2
CMV serostatus of donor and recipient, n			
CMV-seronegative donor and recipient	1	3	4
CMV-seropositive donor and recipient	2	8	10
CMV-seronegative donor and CMV- seropositive recipient	0	3	3
CMV-seropositive donor and CMV-seronegative recipient	0	1	1
ABO incompatibility, n			
None	2	9	11
Minor	1	6	7
Major	0	0	0
Donor-specific antibody, n	0	3	3
Cell dose, median (range)			
TNC, $\times 10^8$/kg	5.5, 7.6, 7.8	7.7 (5.9-10.5)	7.65 (5.85-9.73)
$CD34^+$ cells, $\times 10^6$/kg	2.3 (2.0-3.0)	3.4 (2.6-5.0)	3.00 (2.35-4.80)

ND indicates not determined; TCD, transcranial Doppler ultrasound.
* Two patients underwent 2 transplantations, the second after an initial graft rejection with thiotepa.
† Some patients had more than 1 indication.
‡ As determined by T2*-weighted MRI [62].
§ Some patients were receiving chronic RBC exchange and hydroxyurea.

had donor specific anti-HLA antibodies with a mean fluorescence intensity (MFI) >3000 [36,40]. In the 3 recipients who did not receive thiotepa, TNC doses were 7.8, 7.6, and 5.5×10^8 cells/kg, and $CD34^+$ cell doses were 3.0, 2.0, and 2.03×10^6 cells/kg, respectively. In the recipients who received thiotepa, the median TNC dose was 7.7×10^8 cells/kg (IQR, 5.9 to 10.4×10^8 cells/kg), and the median $CD34^+$cell dose was 3.4×10^6 cells/kg (IQR, 2.5 to 4.9×10^6 cells/kg) ($P = .64$ and .23, respectively).

Thiotepa Improves Donor Engraftment

The addition of thiotepa to the conditioning regimen resulted in prompt and sustained engraftment. Two of the 3 initial participants (patients 1 and 2), who received initial haplo-BMT without thiotepa as part of their conditioning, developed primary graft rejection at day +30 and day +39 post-transplantation, respectively. Both underwent a second haplo-BMT with thiotepa, patient 1 from the same donor and patient 2 from the other parent, at 15 and 44 months after the initial graft rejection, respectively (Table 3). In the 15 patients who received thiotepa, 14 (93%) achieved durable donor engraftment. In the 3 patients who did not receive thiotepa, neutrophil recovery was observed at days +32, +34, and +40, and platelet recovery $\geq 50 \times 10^9$/L was observed at days +24, +27, and +100. In the patients who received thiotepa, neutrophil recovery occurred at a median of 22 days (IQR, 18 to 27 days), and platelet recovery occurred at a median of 28 days (IQR, 27 to 42 days) (Figures 2 and 3). Early T cell chimerism was seen in the thiotepa recipients, with no graft rejection in 14 of the 15 patients with >90% donor T cells at day +60 post-transplantation. Immunosuppression therapy was discontinued in most engrafted patients 86% (6/7) at day +365 post-transplantation, except in patients 6 and 14, owing to grade I and IV gut GVHD, respectively (Table 4).

None of the haplo-BMT recipients who did not receive thiotepa had donor-specific anti-HLA antibodies. Three patients who received thiotepa had significant donor-specific anti-HLA antibodies. Patient 11, who received a female-to-female transplant, had a high level of donor-specific anti-

Table 2
Baseline Characteristics of Patients with Severe SCD Who Underwent Haplo-BMT with PTCy with or without Thiotepa in the Conditioning Regimen

BMT ID*	Recipient ID	Age, yr	Sex	SCD Type	Indication for Transplantation	Disease- Modifying Therapy	Liver Iron Concentration, mg Fe/g†
Recipients without thiotepa in conditioning regimen							
1	1	11	M	SS	ACS at age 2 yr; abnormal TCD at age 6 and 7 yr (normal, age 8 yr on RBT). SCI, high-grade stenosis in bilateral A1 segments at age 6 yr; frontal lobe and occipital lobe infarcts at age 7 yr. CNS event: vision loss, dysconjugate gaze, right temporal lobe and right thalamus infarcts at age 8 yr; new stroke at age 9 yr (multiple acute infarctions bilaterally both supratentorial and infratentorial in the distribution of the vertebrobasilar system despite RBT; Moyamoya disease; anti-Jka antibody (RBC alloimmunization)	RBT	7.8
2	2	22	M	SS	Multiple overt strokes initially at age 5 yr and subsequently at age 7, 13, and 16 yr despite RBT. Others: SCI, h/o ACS, right-sided moyamoya disease with associated cerebral infarcts, multifocal right-sided vessel occlusions on MRI	RBT	29.4
3	3	51	M	SS	Recurrent VOC and hospitalization (more than 2/yr), failed hydroxycarbamide, h/o ACS, and SCI	RBT	2.3
Recipients with thiotepa in conditioning regimen							
4	4	22	M	SS	Sickle cell retinopathy, recurrent ACS, RBC alloimmunization, and recurrent VOC despite hydroxyurea therapy	Hydroxyurea	1.3
5	5	26	F	SS	History of overt stroke at age 11 yr; progressive SCI, ACS, and recurrent VOC unresponsive to hydroxyurea and RBT; transfusional iron overload	RBT	23.3
6	6	20	F	SS	History of overt stroke at age 5 yr with paraparesis, visual and cognitive impairment, recurrent seizures; SCI on RBT; transfusional iron overload	RBT	17.4
7	7	8	M	SS	Progressive bilateral watershed strokes at age 3 yr (right->left) with cortical involvement on right; bilateral frontal lobe injury; secondary seizures, moyamoya disease s/p revascularization surgeries at age 5 yr and h/o ACS	RBT	4.12
8	1	12	M	SS	Graft failure after previous transplantation without thiotepa (delay of 15 mo)	RBT	3.8
9	2	26	M	SS	Graft failure after previous transplantation without thiotepa (delay of 44 mo)	RBT	22.46
10	8	40	F	SS	History of hemorrhagic stroke (right medial thalamic hemorrhage) with intracranial internal carotid occlusion at age 25 yr; moyamoya disease; RBC alloimmunization and transfusional iron overload; sickle cell retinopathy	RBT	43.63
11	9	40	F	SS	Recurrent VOC unresponsive to hydroxyurea (>6/yr), ACS transfusional iron overload and SCI	Hydroxyurea	55.38
12	10	33	M	SS	History of overt stroke at age 2 yr with left-sided hemiparesis; secondary seizures and recurrent seizures; nonruptured left supraclinoid ICA cerebral aneurysm of 4.5 mm (age 30 yr at diagnosis, s/p coil embolization); moyamoya disease; s/p right bypass 18 mo before haploidentical BMT	Hydroxyurea	7.0
13	11	8	M	SS	Acute stroke at age 4 yr (anterior division left MCA, cortical, and old left subcortical stroke); at age 6 yr, new right ACA stroke seen on MRI, chronic at time of MRI; at age 8 yr, new (chronic) stroke w/o symptoms seen on MRI, right centrum semiovale despite RBT; moyamoya disease; stroke and progressive SCI	RBT	1.0
14	12	21	M	SS	Severe macrovasculopathy: right ICA occlusion, left cervical ICA stenosis Multiple SCI, recurrent priapism, 1 episode of ACS and recurrent VOC despite hydroxyurea therapy	Hydroxyurea; RBT monthly for 3 mo before haploidentical BMT	ND
15	13	20	F	SS	2 episodes of ACS (1 with hospitalization in intensive care) and recurrent VOC despite hydroxyurea therapy	Hydroxyurea; RBT monthly for 3 mo before haploidentical BMT	ND
16	14	12	F	SS	SCI, abnormal TCD, recurrent VOC and ACS	RBT	6.3
17	15	7	F	SS	SCI, abnormal TCD, recurrent VOC and ACS	RBT	4.4
18	16	14	M	SS	Stroke, SCI, bilateral stenosis (terminal ICA and supraclinoid)	RBT	1.4

F indicates female; M, male; SS, HbSS; ACS, acute chest syndrome; VOC, vaso-occlusive crisis; RBT, regular blood transfusion; CNS, central nervous system; ICA, internal carotid artery.

* Transplantation location (BMT ID): 1-13, United States; 14 and 15, France; 16-18, United Kingdom.

† Calculations based on Wood et al. [62].

Table 3
Donor and Recipient Characteristics in Haplo-BMT with PTCy with or without Thiotepa in the Conditioning Regimen

BMT ID*	Recipient ID	Donor	Recipient Sex	Recipient CMV Serostatus	Donor CMV Serostatus	ABO Incompatibility	Donor Haplotype	Anti-HLA Donor-Specific Antibody	TNC Dose, $\times 10^8$ cells/kg	$CD34^R$ Cell Dose, $\times 10^6$ cells/kg
Patients without thiotepa in conditioning										
1	1	Mother	Male	Positive	Positive	None	5/10	Negative	7.83	3.03
2	2	Mother	Male	Positive	Positive	None	6/10	Negative	7.63	1.98
3	3	Sister	Male	Negative	Negative	Minor	6/10	Negative	5.48	2.3
Patients with thiotepa in conditioning										
4	4	Half-sister	Male	Negative	Positive	None	5/10	Negative	5.95	6.07
5	5	Mother	Female	Negative	Negative	Minor	5/10	Negative	4.85	1.65
6	6	Father	Female	Positive	Negative	None	5/10	Negative	6.18	1.67
7	7	Mother	Male	Negative	Positive	Minor	5/10	Negative	13.51	3.44
8	1	Mother	Male	Positive	Positive	None	5/10	Negative	7.98	2.95
9	2	Father	Male	Positive	Positive	Minor	5/10	Positive†	4.14	4.84
10	8	Sister	Female	Negative	Negative	Minor	7/10	Negative	4.92	3.69
11	9	Sister	Female	Positive	Positive	None	5/10	Negative	7.7	1.85
12	10	Half-brother	Male	Positive	Positive	None	5/10	Negative	7.97	2.55
13	11	Mother	Female	Negative	Positive	None	5/10	Positive‡	10.59	2.65
14	12	Brother	Male	Positive	Positive	Minor	5/10	Negative	11	5.06
15	13	Mother	Female	Positive	Positive	None	5/10	Negative	13	4.82
16	14	Mother	Female	Positive	Positive	Minor	5/10	Negative	5.96	3.05
17	15	Father	Female	Positive	Positive	None	7/10	Positive§	10.26	13.71
18	16	Father	Male	Positive	Positive	None	5/10	Negative	5.83	6.1

Cell doses are given per kilogram of ideal body weight of recipient.
* Transplantation location (BMT ID): 1-13, United States; 14 and 15, France; 16-18, United Kingdom.
† Recipients with positive cross-match against anti-A*74:01 (MFI, 2532) and-C*03:02 (MFI, 21,519); antibodies were mother-specific.
‡ Recipients with antibodies against A2 class1- A2: MFI, 19,511; relative ratio, 68.1 (consistent with high antibody levels). Weak antibodies against A74: MFI, 1494; relative ratio, 5.1.
§ Recipient with antibodies against B18 class 1; MFI, 20,624 units.

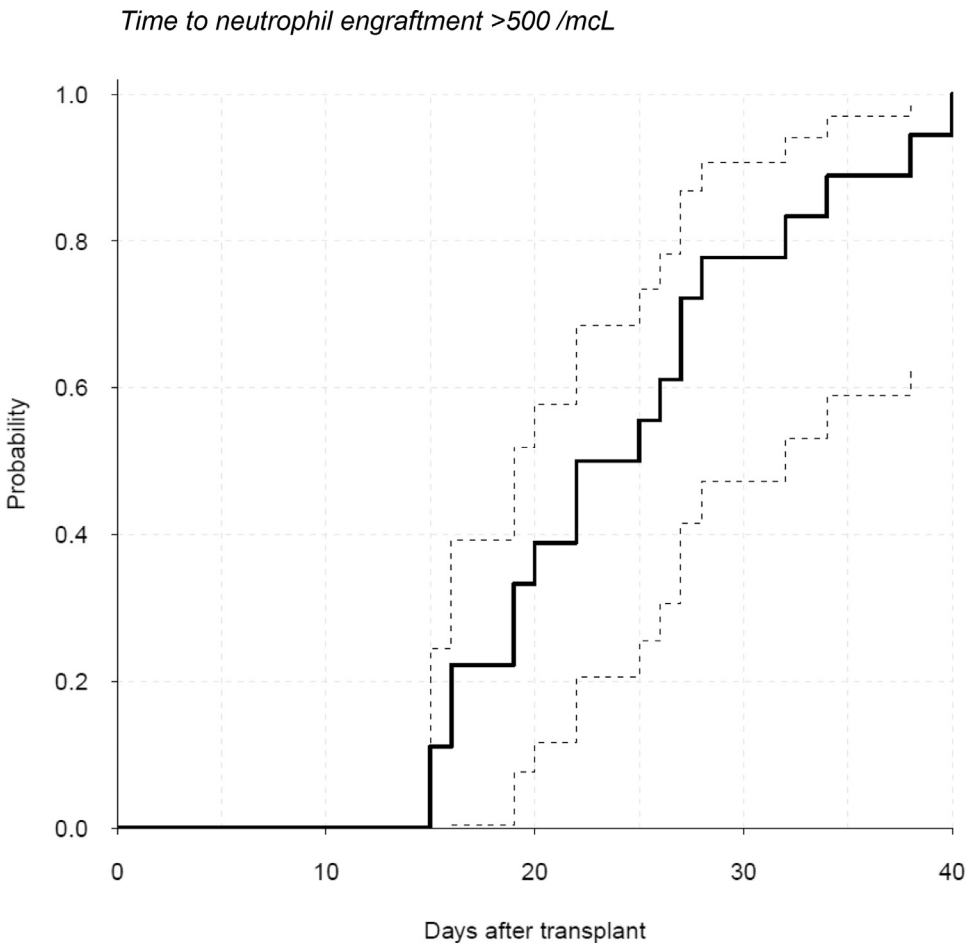

Figure 2. Neutrophil engraftment after haploidentical BMT with PTCy plus thiotepa in 15 participants with severe SCD.

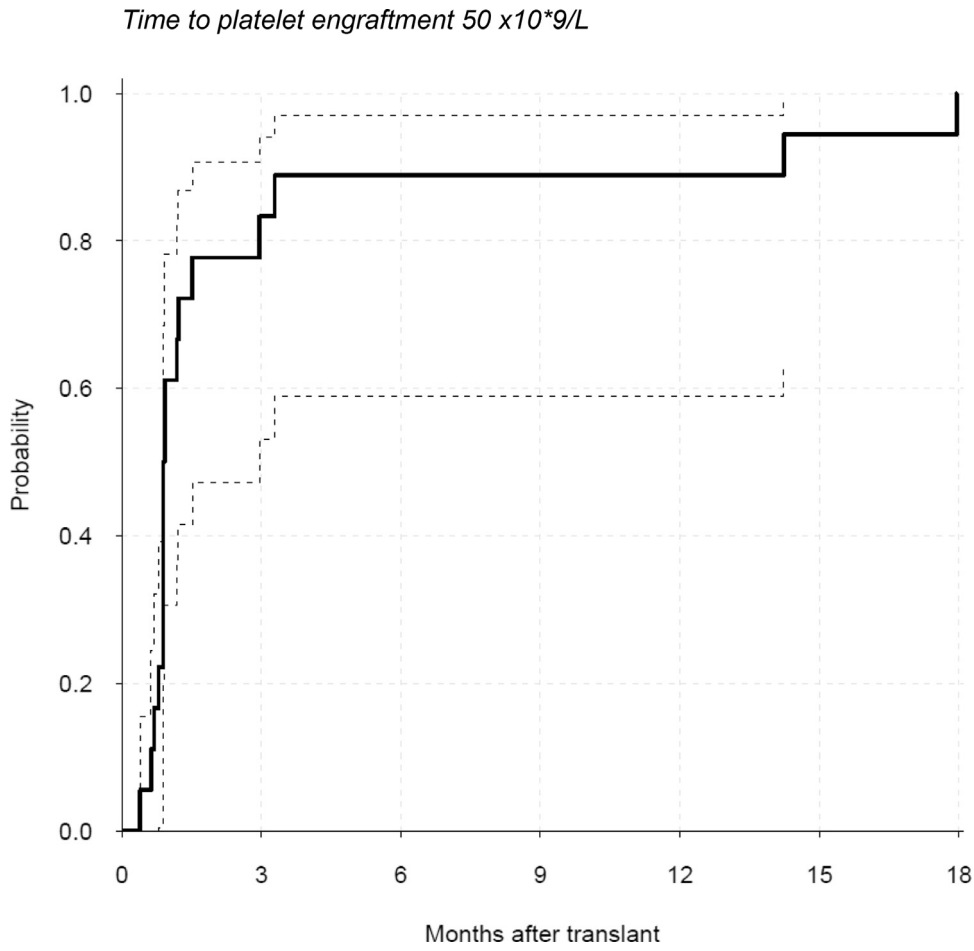

Figure 3. Platelet engraftment >50 x $10^{9/L}$ after haploidentical BMT with PTCy plus thiotepa in 15 participants with severe SCD.

HLA antibodies against maternal HLA class I (A2 MFI = 19,511 units). She developed acute submandibular swelling and angioedema on day +21 attributed to sirolimus and was treated with methylprednisolone (2 mg/kg/day), but developed primary graft rejection. Patient 2 had significant maternal-specific donor-specific anti-HLA antibodies after initial graft rejection with his mother as the initial bone marrow donor. After desensitization [36], he underwent a second haplo-BMT with thiotepa from his father (with more permissive donor-specific anti-HLA antibodies), and then engrafted durably. Patient 15, who received a male-to-female transplant with her father as the donor (7/10 match), had significant donor-specific anti-HLA antibodies against HLA class I (B18 MFI = 20,624 units) and also engrafted durably (Table 3). All patients with graft rejection recovered host hematopoiesis without marrow aplasia.

Excellent EFS and OS

At the time of this report, all 16 patients were alive, at a median follow-up of 16.7 months (IQR, 6.5 to 30.8 months). Patient 3, who did not receive thiotepa, engrafted durably and was event-free for 1461 days until the end of the follow-up. Among the 15 patients who received thiotepa, 1 (patient 11) experienced graft rejection on day +31 after sirolimus was stopped for what was believed to be allergic reaction to therapy. The remaining 14 patients did not have any qualifying event (graft rejection, stroke, or death) at a median follow-up of 13.0 months (IQR, 6.0 to 29.7 months) (Figure 4).

Outcome of SCD-Related Symptoms in Engrafted Patients

All engrafted patients showed evidence of SCD phenotype reversal. Among engrafted patients whose donors had sickle cell trait, HbS was <50% and was undetectable in patient 8 with an HbAA donor at 6 months post-transplantation. Anemia and markers of hemolysis improved among the engrafted patients, and all became transfusion-independent before day +60, with no clinical evidence of new cerebrovascular events, acute chest syndrome, or priapism (Table 5). Data were collected from the first enrollment to March 31, 2018.

Despite no genetic evidence of HbSS, 33% (3 of 9; patients 2, 8, and 9) of the patients who were opioid-dependent (defined as daily opiate use for >3 months) pretransplantation had persistent chronic pain post-transplantation requiring daily opioid use, a well-known phenomenon in adults who undergo transplantation for SCD [34,41]. These patients were weaned off opiates using a multidisciplinary approach [41]. The other engrafted patients did not experience any acute vaso-occlusive pain episodes post-transplantation.

GVHD

No patient in the non-thiotepa cohort developed acute or chronic GVHD. Five of the 15 patients in the thiotepa cohort (33%) developed acute GVHD (grade I-II, n = 2; grade III-IV, n = 2), and patient 6 had de novo moderate chronic GVHD. Patient 6, who had biopsy-proven grade I acute gut GVHD, required a delay in discontinuation of immunosuppressive therapy until day +833, and patient 14, with grade IV acute gut

Table 4
Transplantation Outcomes in Recipients of Haplo-BMT with PTCy and Thiotepa

BMT ID*	Recipient ID	Time to Neutrophil Engraftment, d†	Time to Platelet Engraftment, d‡	Donor Chimerism at 6 mo Post-BMT, %		End of Immune Suppression, day	Days Post-BMT	Last HbS, %	Acute GVHD Grade	Chronic GVHD	Viral Reactivation	Other Severe Complications
				$CD3^+$	$CD33^+$							
4	4	38	32	100	100	+365	675	18.1	None	No	None	None
5	5	26	37	100	100	+365	960	38.9	III	No	EBV, HSV	MMF gastritis
6	6	22	90	100	100	+833	1037	40.8	None	Moderate	EBV, BK	Gastrointestinal bleed
7	7	25	21	100	100	+365	961	32.4	None	No	CMV, EBV	None
8	1	15	21	100	100	+365	1325	28.3	II	No	None	Typhilitis
9	2	20	46	100	100	NR	340	26.5	None	No	CMV	VOD
10	8	28	12	100	100	NR	274	0	None	No	None	None
11	9	27	12	100	100	NR	331	38.7	None	No	None	None
12	10	19	20	100	100	NR	232	5.5	None	No	EBV, HHV-6	Encephalopathy
13	11	16	NR	0	0	+100§	547	NA	None	No	None	PRES
14	12	22	19	100	100	+365	1204	40	None	No	EBV	None
15	13	27	12	99	99	+365	996	31	I	No	EBV	None
16	14	16	28	100	100	NR	288	18.9	IV	No	CMV, HHV-6	PRES
17	15	15	27	99	99	NR	239	24	I	No	Adenovirus, HSV	IFI chest
18	16	19	28	100	100	NR	155	6.6	None	No	None	None

HSV, herpes simplex virus; VOD, veno-occlusive disease of the liver; PRES, posterior reversible encephalopathy syndrome; MMF, mycophenolate mofetil; NR, not reached; IFI, Invasive fungal infection

* Transplantation location (BMT ID): 1-13, United States; 14 and 15, France; 16-18, United Kingdom.

† Neutrophil engraftment (absolute neutrophil count >500).

‡ Platelet engraftment (platelets >20,000).

§ Immunosuppression stopped after primary graft failure.

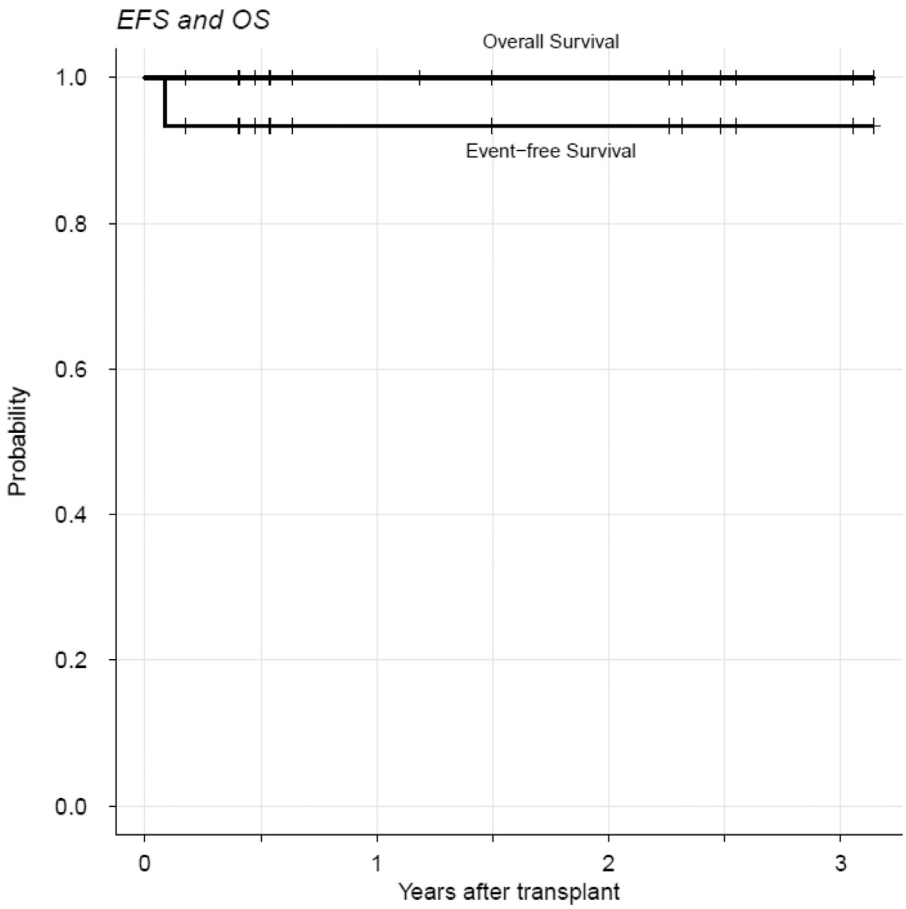

Figure 4. Probabilities of vent-free survival (EFS) and Overall survival (OS) after haploidentical BMT with PTCy plus thiotepa in 15 participants with severe SCD.

GVHD, required treatment with mesenchymal stem cells, extracorporeal photopheresis, and monoclonal antibodies (basiliximab and infliximab) (Table 4). At day +90 post-transplantation, estimated incidence of acute GVHD was 17% (95% confidence interval, 0 to 32%). No association was found between cell dose and presence of GvHD (*P*-values = 0.63, 0.47) (Figure 5).

Other Complications Associated with the Procedure

The main infectious complication was asymptomatic viral reactivation, occurring in 9 patients. Six patients developed EBV reactivation, which was treated with rituximab in 1 patient, but no post-transplantation lymphoproliferative disease was reported. Three patients developed CMV reactivation and were treated with ganciclovir, foscarnet, or both. Other viral infections included 1 case each of adenovirus respiratory infection and BK virus cystitis and 2 cases each of oral herpes simplex virus infection and human herpesvirus 6 (HHV-6) viremia (with 1 case complicated by HHV-6 encephalitis). Patient 2 developed veno-occlusive disease of the liver on day +15 after a second haplo-BMT for graft rejection. Patients 11 and 14 developed posterior reversible encephalopathy syndrome despite the use of prophylaxis, and patient 11 developed a new cerebral infarct detected on follow-up brain MRI after primary graft rejection. There was 1 case each of presumed invasive fungal infection, mycophenolate mofetil-induced gastritis, duodenal ulcer-related gastrointestinal bleeding, and typhilitis, all of which fully resolved (Table 4).

DISCUSSION

Until recently, 2 major obstacles have limited the use of HSCT as a curative option for children and adults with SCD: a limited donor pool [26,27] and the infeasibility of myeloablative conditioning in adults with SCD [21]. However, the use of nonmyeloablative related haplo-BMT has significantly increased the likelihood of a possible cure in both children and adults with SCD. Previously, the rate-limiting factors in the use of related haplo-BMT were a poor donor engraftment rate (60%) and a study conducted at only single institution, limiting the generalizability of results [34]. Our present study provides preliminary evidence that the addition of thiotepa alone to the Johns Hopkins conditioning regimen for haplo-BMT with PTCy improves donor engraftment without increasing morbidity or mortality. In our Phase II feasibility trial, 2 of the initial 3 patients who underwent haplo-BMT without thiotepa developed primary graft rejection, prompting a change in the protocol. However, 93% (14 of 15) of the patients who received thiotepa had durable engraftment (>90% donor hematopoiesis and T cell lymphopoiesis by day +60), with no phenotypic evidence of SCD based on hemoglobin analysis and 100% OS over a period of 13.0 months (IQR, 6.0 to 29.7 months).

A key finding in this study is the high donor availability. All 18 patients with SCD referred for haplo-BMT had at least 1 identifiable eligible family donor. However, 2 patients did not proceed to transplantation, owing to prohibitive donor-specific anti-HLA antibodies in 1 and concern about health risks of identified family donor in the other. Thus, 89% (16 of 18) of eligible patients with SCD were able to proceed to transplantation. This high donor availability for haplo-BMT changes the

Table 5
Hematologic Parameters before and after Haplo-BMT with PTCy Plus Thiotepa in the Conditioning Regimen

BMT ID*	Recipient ID	Donor Phenotype	Recipient Phenotype Pretransplantation, %†			Recipient Phenotype at 6 Mo Post-BMT, %			Hb, g/dL		LDH, U/L		Reticulocyte Count, %‡		Bilirubin, mg/dL	
			HbA	HbS	Hb Fetal	HbA	HbS	Hb Fetal	Pre-BMT	Post-BMT	Pre-BMT	Post-BMT	Pre-BMT	Post-BMT	Pre-BMT	Post-BMT
4	4	AS	19.5	67.4	9.5	76.1	18.8	1.9	9.0	10.0	287	-	13.3	2.4	5.5	.3
5	5	AS	63.9	28.9	4.6	76.4	19.4	<1.0	8.5	9.4	388	170	12.9	2.8	1.8	.2
6	6	AS	75.9	16.5	5	93.8	2.5	<1.0	10.5	13.7	807	203	5.2	<1.0	1.4	.4
7	7	AS	48.1	34.9	13.7	73.4	21.9	1	11.0	10.2	411	196	2.1	.7	.9	.3
8	1	AS	47.6	45.7	3.4	65.3	28.3	2.8	9.1	10.0	497	-	14.7	4.4	1.2	.4
9	2	AS	63.4	32	1.8	83.7	12.1	1.1	9.0	10.0	699	346	25	-	8.8	.3
10	8	AA	71.1	25.1	<1.0	96	0	<1.0	7.9	9.3	371	NA	2.7	.3	1.6	.2
11	9	AS	29.6	60.6	6.5	63.8	31.6	<1.0	5.2	10.2	1075	501	38.6	1.5	1.8	.4
12	10	AS	<1.0	89.5	4.7	90.5	5.5	<1.0	6.4	10.9	844	663	13.2	.9	2.4	.2
13	11	AS	-	88.8	-	77.5	13.7	6.1	8.2	9.5	782	194	-	-	1.9	.6
14	12	AS	70	25	5	NA	37	NA	10.9	17.6	281	130	32.1	.6	4.4	1.1
15	13	AS	75	21	4	NA	35	NA	12.4	12.6	399	210	27	.6	1.5	1.9
16	14	AS	71.3	14.2	.7	49.7	40.9	.6	7.8	11.7	563	346	1.04	2.3	3.27	.35
17	15	AS	75.6	9.3	.7	63	24	.5	12.9	10.5	377	-	1.4	4.1	2.81	.23
18	16	AS	64.7	20.8	1.5	75.8	6.6	1.2	8.8	11.8	540	361	15.5	.6	10.7	.58

Comparison of hemoglobin variants A, S, and fetal between donors and recipients at the 6-month post-transplantation visit. In patients 2-6, pretransplantation Hb values reflect transfusions received within 30 days of this testing. Also shown are comparisons of absolute reticulocyte numbers (reference, .5-1.8), hemoglobin (lower limit of normal, 12 g/dL), LDH (reference, 125-220 U/L), and total bilirubin (reference, .2-1.2 mg/dL).
LDH indicates lactate dehydrogenase.

* Transplantation location (BMT ID): 1-13, United States; 14 and 15, France; 16-18, United Kingdom.

† Baseline HbS level during supportive care before exchange transfusion before the start of conditioning for BMT.

‡ Reticulocyte value obtained at ~100 days post-BMT.

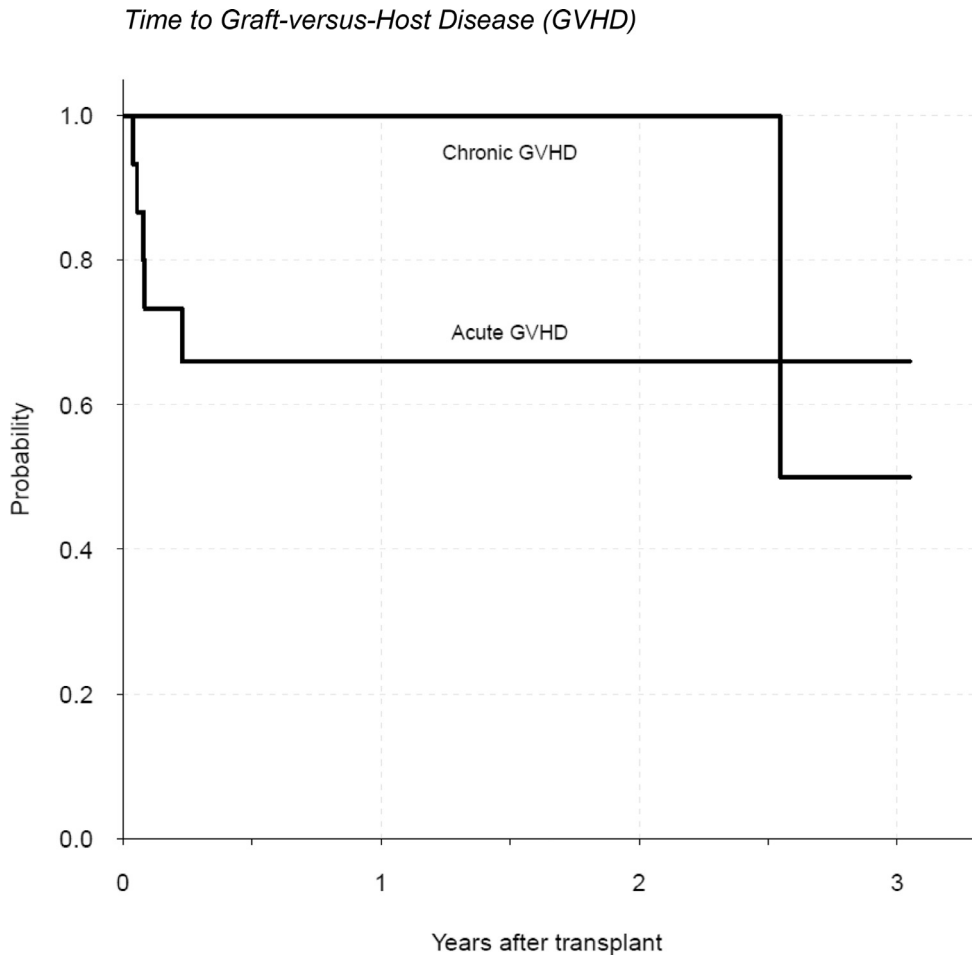

Figure 5. Probabilities of acute and chronic GVHD after haploidentical BMT with PTCy plus thiotepa for severe SCD.

landscape for the use of HSCT as a curative modality in individuals with SCD. Another key finding is the observation that most of the donors, who had sickle cell trait, had no complications from GCSF use for stem cell mobilization, reinforcing the idea that GCSF mobilization is safe in individuals with sickle cell trait [42,43]. The optimal dose of GCSF used in bone marrow stem cell mobilization remains unsettled [44-46]. GCSF-primed bone marrow allografts have been shown to improve engraftment and are associated with lower rates of overall chronic GVHD and comparable OS [47]. In our study, 20% (3 of 15) of the patients in the thiotepa cohort developed grade II-IV acute GVHD, but none developed long-term chronic GVHD, not even any of those who discontinued immunosuppression therapy at 1 year as per the study protocol. We could not correlate the presence of GVHD with TNC dose, although $CD3^+$ cell dose was measured in only a few donor grafts.

The present study is the largest and first international multicenter study using a learning collaborative model [35-37] for haplo-BMT in patients with SCD across the age spectrum. Thiotepa (N,N′,N″-triethylenethiophosphoramide) and its major metabolite (Tepa) are trifunctional alkylating agents used extensively in conditioning for allogeneic HSCT as an engraftment-enhancing agent [39,48], associated with rapid engraftment and relatively low transplantation-related mortality [49]. In the present study, 61% of the patients (11 of 18) were aged >18 years, and some had multiple overlapping comorbidities, including stroke, moyamoya disease, cerebral vasculopathy, alloimmunization, recurrent priapism, acute chest syndrome, and vaso-occlusive pain episodes. We used an incremental approach to adding therapy to improve engraftment in the collaborative consortium, identifying the optimal conditioning regimen that achieves this primary objective while minimizing toxicity. Despite initially varying opinions among the investigators regarding the addition of 1 drug (thiotepa) versus 3 drugs (hydroxyurea, azathioprine for preconditioning, and thiotepa) to the Johns Hopkins conditioning regimen, in the end we were able to harmonize a single protocol across 3 clinical sites in the United States, the United Kingdom, and France.

Our protocol of T cell-replete haplo-BMT with PTCy and thiotepa appears to be more effective than current T cell-depleted haploidentical peripheral blood stem cell transplantation protocols in improving donor engraftment and preventing chronic GVHD [31-33].

Our relative small sample size of 18 participants limits ability to present conclusive results, 87% (6 of 7) of the successfully engrafted patients were able to discontinue immunosuppression therapy at 1 year in accordance with protocol. Other attempts to improve this haploidentical platform with PTCy for SCD are described in Supplementary Data 1 [31,34,50-54].

The increased incidence of viral reactivation had no impact on engraftment. Asymptomatic viral reactivation, mainly CMV (20%; 3 of 15) and EBV (40%; 6 of 15), occurred in 15 patients (60%) by day +100. Similarly increased viral reactivation rates

were seen after T cell-replete [55] and T cell-depleted [56] haploidentical peripheral blood stem cell transplantation with PTCy in individuals with hematologic malignancies and SCD [33,57,58]. This high rate of early viral reactivation or infection following T cell-replete haploidentical HSCT with PTCy has been attributed to a low number of adoptively transferred memory T cells and reconstitution of naive and nonactivated memory cells later in the post-transplantation period, protecting recipients from late infectious complications [59,60]. In the present study, despite evidence of early immune reconstitution (Supplementary Data 2), the high rate of viral reactivation necessitates frequent monitoring, optimization of antimicrobial strategies, and preemptive therapy.

Limitations of this Phase II study include its nonrandomly allocated study design. Nonetheless, we were able to systematically evaluate 16 patients over a 3-year course using this learning collaborative model without external funding. Certain aspects of the supportive care were institutionally based; however, we have no evidence that institutional preferences influenced the primary outcome (engraftment rate). Although there was no central decision making support, team members from the 3 clinical sites discussed the challenging cases and issues at least once a month via conference calls and met in person at an annual hematology meeting. We did not have a central neuroradiology review for imaging studies of the brain, and without central adjudication, central nervous system imaging assessments are likely to be inconsistent and unreliable [61]. However, the main objective of the learning collaborative was to assess engraftment in a systematic fashion and accelerate enrollment in this protocol.

We have provided preliminary evidence indicating that the addition of thiotepa in T cell-replete haplo-BMT with post-transplantation cyclophosphamide improves donor engraftment without significantly increasing morbidity or mortality in individuals with SCD. The excellent EFS and OS makes this approach suitable for individuals who develop severe SCD despite good supportive care (ie, hydroxyurea and regular blood transfusion therapy). Our results provide justification for the currently open National Institutes of Health-funded Bone Marrow Transplant-Clinical Trial Network Phase II trial (BMT CTN 1507) investigating engraftment rates and end-organ progression (lung, kidney, and brain). If these results are further confirmed in a larger cohort with a longer follow-up period, then ultimately a Phase III trial can be offered to determine whether the long-term benefits of related haplo-BMT with post-transplantation cyclophosphamide plus thiotepa are superior to optimal medical management for children with stroke and high-risk adults with severe SCD.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.11.027.

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