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Increasing the dose of total body irradiation to decrease graft failure associated with HLA-haploidentical transplantation for patients with severe hemoglobinopathies. A single institution prospective clinical trial.

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Abstract

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Author contributions:

RAB, RJJ, and JB-M developed the protocol. RAB, KRC, CJG, and JB-M enrolled patients. JB-M, collected the data and was the PI of the study. All authors analyzed and interpreted data, wrote or critically reviewed the manuscript, and agreed upon the final version.

Data sharing

Patient-level data will not be shared.

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Background: While severe hemoglobinopathies can be cured with allogeneic blood or marrow transplantation (BMT), the availability of matched donors and toxicities can be problematic. We previously found that nonmyeloablative haploidentical related BMT (haploBMT) with post-transplant cyclophosphamide (PTCy) expanded the donor pool while limiting graft-versus host disease. However, graft failure, albeit with full host hematopoietic recovery, occurred in 50% of patients. Here, we studied whether increasing total body irradiation (TBI) from 200 to 400cGy would improve engraftment while maintaining the safety profile.

Methods: The study is closed to accrual and this is the primary analysis. Twelve consecutive sickle cell disease and five β-thalassemia patients received anti-thymocyte globulin (rabbit) 0.5 mg/kg on day −9 and 2 mg/kg on days −8 and −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 400 cGy on day −1. Unmanipulated bone marrow was collected and infused on day 0. Graft-versus host disease prophylaxis consisted of PTCy 50 mg/kg/day on days +3 and +4 post transplant; sirolimus to maintain a level of 5–15 ng/dL for one year, and mycophenolate mofetil 15mg/kg per dose (maximum 1 gram) every 8 hours (until day 35) were started on day 5. The primary objective was evaluation of engraftment by intention to treat. Trial registration number: ClinicalTrials.gov NCT00489281.

Findings: With a median age of 16 (range 6–31 IQR 7.5, 27.5) years, there was 1 graft failure; 13 patients achieved full donor chimerism and 3 exhibited mixed donor-host chimerism. Five patients developed acute graft-versus host disease, and three chronic, all with complete resolution. All patients are alive with a median follow-up of 705 (range 355–1294; IQR 398, 943) days. Only one of the 16 engrafted patients is transfusion dependent. Fourteen have discontinued immunosuppression.

Interpretation: Increasing TBI to 400cGy significantly reduced graft failure rates while maintaining the safety of haploBMT with PTCy in this small study. These results suggest that engraftment after haploBMT for hemoglobinopathies is possible and primary graft failure, the main problem previously reported, has been solved. Therefore, this curative approach should no longer be restricted to patients with HLA-matched donors.

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Introduction

Sickle cell disease and β-thalassemia major are severe hemolytic anemias caused by mutations in the β-globin gene. In sickle cell disease, an amino acid substitution in β-globin (β6 $^{glu->Val}$ β^S) results in polymerization and red cell sickling that leads to hemolytic anemia, vaso-occlusive crises, strokes, and other end organ damage.(1) In 2010, there were more than 300,000 newborns with sickle cell disease.(2) For adults with sickle cell disease, the average annual cost of medical care exceeds 35,000.00 US dollars.(3) Most adults and many children develop a chronic debilitating condition, leading to high rates of disability and unemployment.(4) The median survival for sickle cell patients in the United States is 40 years(5). Patients with thalassemia require life-long blood transfusions, which predisposes them to iron overload and associated organ-specific complications, along with an increased risk for transfusion-related viral infections including hepatitis B and C.

Allogeneic blood or marrow transplantation (alloBMT) is the only cure for patients with sickle cell disease and thalassemia.(6, 7) Historically, alloBMT for severe-hemoglobinopathies required myeloablative conditioning to enable engraftment. Some adult patients with sickle cell disease have generally been excluded from myeloablative BMT trials because of anticipated excess morbidity and mortality resulting from accumulated disease-related end-organ damage. Finding a suitable donor has also been a challenge. HLA-matched sibling donors are available in less than 10% of potential alloBMT recipients with sickle cell disease.(8) Less than a quarter of African-Americans have HLA-matches in unrelated registries.(9, 10) Accordingly, broader application of alloBMT in severe β -hemoglobinopathies is dependent on novel strategies that address the issues of donor availability as well as limit the toxicity from myeloablative conditioning regimens and graft-versus host disease.

Recently, we showed encouraging results with non-myeloablative alloBMT utilizing related haploidentical donors and post-transplant cyclophosphamide (PTCy).(11) PTCy results in low rates of graft-versus host disease,(12–15) post-BMT lymphoproliferative disorder,(16) and donor derived malignancies.(17) However, despite cures in the majority of patients and low toxicity, the graft failure rate, albeit all with full host recovery, was 50% (one patient suffered secondary graft failure after the manuscript was published). In an attempt to decrease graft failure, we hypothesized that increasing the dose of total body irradiation (TBI) from 200 to 400 cGy before BMT would increase engraftment without increasing transplant-related morbidity and mortality.(18) Such an advance would be impactful; a safe, affordable, life-changing, curative, therapeutic option could be available to essentially any patient in need while limiting late effects including infertility in long-term survivors.

Methods

Study design and participants.

The protocol, ClinicalTrials.gov NCT00489281, whose 200 cGy cohort was previously published,(11) was amended to use 400cGy TBI as approved by the Johns Hopkins Institutional Review Board (IRB). All patients gave IRB-approved informed consent before BMT. A total of 11 (65%) patients received treatment on trial after informed consent was granted in accordance with the Declaration of Helsinki. An additional 6 (35%) patients consented to BMT, but received identical treatment outside of the study as a result of insurance coverage that precluded treatment on a clinical trial. Permission to include these 6 patients in the analysis of outcomes was granted by the Johns Hopkins IRB. Patients aged 2–70 years and receiving their first BMT were eligible. Additional eligibility criteria included good performance status (ECOG 0 or 1; Karnofsky and Lansky 70–100), ability to sign consent (or assent if minors), and the presence of an HLA haploidentical relative willing to donate. Eligible diagnoses included: sickle cell anemia (Hb SS), Hb S/ β° thalassemia, Hb S/ β+ thalassemia, Hb SC disease, Hb SE disease, Hb SD disease, Hb SO-Arab disease, or Hb S/hereditary persistence of fetal hemoglobin, β thalassemia major. In addition, subjects also had to have at least one of the following hemoglobinopathy-related complications already published as indications for BMT in these patients:(6, 8, 19) stroke, magnetic resonance imaging changes indicative of brain parenchymal damage, magnetic resonance angiogram

evidence of cerebrovascular disease, abnormal transcranial Doppler velocity, acute chest syndrome requiring exchange transfusion or hospitalization, recurrent vaso-occlusive pain crisis (more than two per year for the last two years), stage I or II sickle lung disease,(20) sickle retinopathy, osteonecrosis, red cell alloimmunization (more than two antibodies) during long-term transfusion, constellation of dactylitis in the first year of life and a baseline hematocrit of 21% and leukocytosis (>13.4 × 10^3mm^3) in the absence of infection during the second year of life, history of invasive pneumococcal disease, pitted red blood cell count >3.5% during the first year of life, or transfusion dependence. The coverage decision by the Centers for Medicare and Medicaid Services to only provide payment of alloBMT for patients with sickle cell disease on a clinical trial that had a comparison arm with non-BMT patients, prompted the closure of this trial in 2017.

Procedures

Donors and grafts.—Relatives who shared at least one HLA haplotype with the patient, did not have sickle cell disease or thalassemia major or intermedia, and were in good health were allowed to serve as donors(11). Donors with sickle cell trait and thalassemia minor were not excluded from bone marrow donation. When more than one donor was available, the donor was selected based on younger age, ABO matching, and CMV serology. If donor anti-HLA antibodies were detected, the next best related match was chosen. Donor bone marrow was harvested with a target yield of 4 × 10^8 nucleated cells/kg recipient ideal body weight and infused on day 0. The marrow was unmanipulated except that major incompatible ABO grafts had red blood cells depleted by buffy coat preparation and minor ABO incompatible grafts had plasma removed.

HLA Typing.—HLA phenotyping was performed as previously described.(11, 12) Potential family members were initially typed at the HLA-A, HLA-B, and HLA-DRB1 loci at an intermediate resolution level. Family members selected as donors were then further typed at the HLA-C locus at an intermediate resolution level. DRB1 and DQB1 alleles were typed at a high-resolution level. As needed, recipients and potential donors were typed at a high-resolution level for HLA-Cw alleles. Haplotypes were determined based on family studies whenever possible.

Conditioning regimen and graft-versus host disease prophylaxis.—Patients received intravenous anti-thymocyte globulin (rabbit) (Sanofi-Genzyme, Massachusetts USA) 0.5 mg/kg on day −9 and 2 mg/kg on days −8 and −7; intravenous fludarabine (Actaris Group, New Jersey, USA) 30 mg/m^2 on days −6 to −2, intravenous cyclophosphamide (Sandoz, New Jersey, USA) 14.5 mg/kg on days −6 and −5, and total body irradiation 400 cGy on day −1. A steroid taper was given to prevent reactions to anti-thymocyte globulin as follows: methylprednisolone 1mg/kg intravenously 1 hour prior anti-thymocyte globulin on days −9 to −7. This dose could be repeated once 3 hours after the first dose. On day −6 and −5, methylprednisolone 0.75 mg/kg/ IV as a single dose; on days −4 and −3, methylprednisolone 0.5 mg/kg/ IV as a single dose; on day −2 methylprednisolone 0.25 mg/kg/ IV as a single dose. Unmanipulated bone marrow was collected and infused on day 0. graft-versus host disease prophylaxis consisted of intravenous cyclophosphamide 50 mg/kg/day on days +3 and +4, and oral sirolimus (Pfizer, New York, USA) to maintain a

level of 5–15 ng/dL for at least one year +/− 14 days (or more if mixed chimeras at the judgment of treating physician) as well as oral mycophenolate mofetil (Genentech, South San Francisco, USA) 15mg/kg per dose (maximum 1 gram) every 8 hours (until day 35) that were started on day +5. Patients were allowed to take generic sirolimus and mycophenolate mofetil depending on their pharmacy supply and their insurance coverage.

Outcomes.

Primary endpoint.—The primary endpoint of this study was to evaluate engraftment. Patients had chimerism studies on peripheral blood on days 30, 60, 180, 360, yearly thereafter and as clinically indicated. Chimerism was measured by PCR analysis of variable number of nucleotide tandem repeats unique to donors or recipients on total peripheral blood and isolated CD3+ cells. Graft failure was defined as undetectable DNA of donor origin on at least 2 occasions no less than 1 week apart.

Secondary endpoint.—Secondary endpoint was the description of the toxicities of transplantation in this population.

Statistical analysis.

The primary objective of this phase II clinical trial was to obtain a risk-stratified estimates of two-year progression-free survival with a precision of +/− 20% (95% confidence bound). To obtain this precision, it was necessary to accrue at least 50 patients. The hypothesis of this study was that non-myeloablative conditioning with high-dose posttransplant cyclophosphamide would increase the number of sickle cell disease patients eligible for allogeneic transplantation by allowing the safe and effective use of related haploidentical donors. Given the decision by the Centers for Medicare and Medicaid Services mentioned, the study was closed to accrual before reaching the target enrolment, therefore, no attempt was made to perform the planned analysis given the early close of the study. Instead, a description of the results obtained was performed.

Results

Patients and donors.

Seventeen consecutive patients were enrolled between September 24, 2014 2014 and August 1, 2017: 12 (71%) with sickle cell disease and 5 (29%) with β-thalassemia major (full patient characteristics on Table 1). Ten patients (59%) with sickle cell disease patients had previously received hydroxyurea. Data are current as of August 4, 2018. The median age was 16 (range 6–31; IQR 7.7, 27.5). The median follow up is 705 days (range 355–1294; IQR 398, 943). Two (12%) patients received the graft from a major ABO mismatched donor (B to O and A to O), 5 (29%) from a minor ABO mismatched donor, and 10 (59%) were ABO matched. All the donors were related haploidentical family members, and included 7 (41%) siblings, 5 (29%) mothers, 4 fathers (24%), and 1 aunt (6%).(21)

Engraftment.

Of the 17 patients, 1 (6%) experienced primary graft failure with recovery of host hematopoiesis; 13 (76%) patients achieved full donor chimerism and three (18%) achieved

mixed donor-host chimerism. The patient who rejected the graft fully reconstituted host hematopoiesis by day 70. Graft characteristics and chimerism are shown in Table 2. The median time to count recovery was 28 (IQR 22.5, 31.5) and 26 (IQR 15, 34) days respectively.

Graft-versus host disease and BMT related toxicities.

Toxicities were graded per the Common Terminology Criteria for Adverse Events version 5.0 when indicated, as appropriate. Five (29%) patients experienced grade II-IV acute graft-versus host disease, including 4 (23%) with maximal grade II and 1 (6%) with grade III; chronic graft-versus host disease (2 mild, 1 moderate per NIH Consensus) developed in 3 (18%) patients (Table 2). As of their last visit to Johns Hopkins, graft-versus host disease resolved in all cases and all patients are off systemic graft-versus host disease therapy. Sickle cell disease pain crisis after anti-thymocyte globulin was seen in all sickle cell disease patients, 1 (6%) patient developed sirolimus-induced diabetes (grade 3), and 1 (6%) had BK virus hemorrhagic cystitis (grade 3). All patients developed peri-transplant fever. One patient (6%) developed engraftment syndrome, idiopathic pneumonia syndrome, candidemia (grade 3), pneumonia (grade 3), EBV reactivation treated with IVIgG (grade 2), adenovirus, and CMV reactivation (grade 2) in the setting of grade III graft-versus host disease. One patient (6%) with Meniere's had worsening of his symptoms after exposure to antibiotics (tinnitus grade 2). Patient 1 developed an abnormal karyotype in host cells at 22 months after BMT, although her marrow morphology and flow cytometry continue to show no abnormalities. Her bone marrow karyotype at 36 months after BMT was 46,XX,del(13)(q12q14)[2]/46,sl,del(1)(q32q42),ins(4;?)(q13;?)[4]/46,sl,t(3;6)(q12;p21),add(5)(q34),a dd(20)(q11.2)[2]/46,XX[2]//46,XY[2]. There were no pathologic mutations on a next generation sequencing (NGS) panel. This patient was on hydroxyurea for 3 years before BMT.

Sickle cell disease specific outcomes.

All patients remain alive. Hematologic parameters pre and post BMT can be seen in Table 3. Eleven patients (92%) with sickle cell disease engrafted, and all but 1 (8%) are transfusion independent. The patient who still requires transfusions, Patient 35, received a major ABO mismatched graft (A to O), and continues to have high anti-A titers associated with mixed chimerism. The other recipient of a major ABO incompatible graft, Patient 43, is a full donor chimerism and as such is transfusion independent. None of the engrafted patients have been admitted to the hospital for an acute sickle cell pain crisis since undergoing alloBMT. Patient 40 still has anemia and elevated levels of Hemoglobin S as of last check-up; however, she has not experienced a sickle cell crisis since transplant (>2 years). As of their last follow-up, only one (8%) patient is still on immunosuppression.

Thalassemia major specific outcomes.

All patients were diagnosed and started on transfusion programs between the ages of 3 and 18 months, and all required chelation for transfusion-associated iron overload. Just prior to BMT, all patients were treated with oral deferasirox alone or in combination with parenteral deferoxamine to lower serum ferritin to a level around or below 1000 ng/ml. Liver biopsies completed on all patients pre-BMT confirmed evidence of hepatic iron deposition with minimal hepatocellular inflammation and portal fibrosis. All patients also received

hydroxyurea at a minimum dose of 15mg/kg/day for at least 8 weeks prior to BMT. After BMT, 4 (80%) of 5 patients are fully engrafted and 1 (20%) exhibits mixed donor-host chimerism. All 5 (100%) are transfusion independent (median time from BMT to last red cell transfusion 17, range 13–157, days (IQR 14, 119 days). Hematologic parameters before and after BMT as well as donors' (for comparison) are shown in Table 3. Three (60%) of the 5 patients are off immunosuppression at last follow-up.

Discussion

In this study, 17 patients underwent a non-myeloablative haploidentical BMT with 400cGy TBI. Transplant was well tolerated and 13 patients achieved full donor chimerism, 3 mixed chimerism, and only 1 graft failure. Of these patients, only three are still on immunosuppression. This is in sharp contrast with our previous experience utilizing lower doses of TBI(11).

AlloBMT was first shown to cure sickle cell disease in the early 1980s, but its use has been restricted by difficulties finding matched sibling donors and transplant-related morbidity and mortality especially in the setting of myeloablative conditioning. Non-myeloablative conditioning has reduced the toxicity, but even with HLA-identical donors has been associated with graft failure and universal mixed chimerism in both thalassemia and sickle cell disease patients.(22) Although resolution of symptoms related to the hemoglobinopathy can still occur with mixed chimerism, many such patients remain on long-term immunosuppression and can slowly lose chimerism over years.(23) The addition of PTCy to non-myeloablative conditioning(11, 24) has expanded the donor pool such that most sickle cell disease patients can now safely undergo alloBMT. Unfortunately, a higher rate of graft failure was observed with the HLA-haploidentical donors.(11) Here, we demonstrate that increasing the TBI dose in the conditioning regimen from 200 cGy to 400 cGy improved engraftment; only one graft failure and 3 mixed chimeras were observed in 17 hemoglobinopathy patients receiving non-myeloablative conditioning and PTCy, without increasing morbidity or mortality. Importantly, donors were secured for all 17 consecutive patients referred for alloBMT on this cohort of the trial, demonstrating that most patients with severe hemoglobinopathies referred for BMT are no longer limited by lack of donor availability.

The one engrafted patient (Patient 35) who remains transfusion dependent after receiving a major ABO incompatible allograft is the result of mixed donor-recipient chimerism and persistent host immunity producing high levels of anti-donor RBC antibody. The other recipient of a major ABO incompatible graft (Patient 43) is a full donor chimerism and transfusion independent. Of note, 2 of the sickle cell patients with mixed donor-recipient chimerism are sisters (patients 35 and 40) who both received allografts from their father. None of the 11 engrafted patients with sickle cell disease have been admitted after the alloBMT for sickle cell disease related pain crises, and all patients with β-thalassemia are transfusion independent. Moreover, only 3 engrafted patients are still on immunosuppression.

An abnormal karyotype is present in host cells of one patient in the cohort (Patient 35). Not only had this patient received hydroxyurea for 3 years before BMT, but she also has mixed donor-recipient chimerism. There are a few reports of therapy-related myeloid neoplasm in sickle cell disease patient treated with long-term hydroxyurea.(25–27) Whether the addition of BMT will increase this risk in unknown.(28) Moreover, this patient currently has normal marrow morphology without any molecular abnormalities associated with therapy-related myeloid neoplasm (t-MN) on NGS testing. Importantly, clonal cytogeneic abnormalities after BMT should not be considered diagnostic of t-MN, as they can be transient or may not progress; however, while no clinical t-MN has developed in this patient, longer follow-up is needed.(29)

Several groups have attempted to improve outcomes for patients with severe hemoglobinopathies. The potential impact of our data may be best appreciated in comparison with published observations. Recent studies using alternative stem cell sources, reduced intensity conditioning platforms, or both to expand the donor pool and or limit transplant-related toxicity for patients with severe sickle cell disease and thalassemia have produced disappointing results. Graft-versus host disease, opportunistic infection and mortality following myeloablative conditioning remained problematic for children with thalassemia even when augmented graft-versus host disease prophylaxis was used(30). Utilization of haploidentical donors following myeloablative conditioning have produced mixed results. Depletion of TRCab+ and CD19+ cells from stem cell grafts reportedly improved engraftment when compared to CD34+ selected grafts but were still associated with extensive chronic graft-versus host disease, post-transplant lymphoproliferative disease, and delayed immune reconstitution(31). By contrast, use of haploidentical donors and PTCy resulted in excellent rates of engraftment and overall survival albeit in the context of full intensity conditioning and two additional blocks of pre-transplant, immunosuppressive therapy.(32) Multicenter trials using a alemtuzumab, fludarabine, and melphalan based regimen and either cord blood or bone marrow grafts from matched unrelated donors for children with sickle cell disease(33, 34) or thalassemia (35) were recently conducted. Unfortunately, outcomes were limited by unacceptably high rates of graft failure(33) mortality(34), extensive chronic graft-versus host disease and viral reactivation(34, 35). While some efforts to combine haploidentical donors and reduced intensity, nonmeyloablative, preparative regimens were plagued but suboptimal donor engraftment(11, 23), a recent study demonstrated more robust donor chimerism with augmented conditioning in a small number of patients with sickle cell disease(15).

Other potentially curative approaches (gene therapy and genome editing) for severe hemoglobinopathies are being studied. Since gene therapy and future genome editing approaches use autologous stem cells, no donor is required and the risk of graft-versus host disease is avoided. However, myeloablative conditioning is currently required for engraftment after autologous BMT with gene corrected stem cells.(36, 37) Myeloablative conditioning can be associated with substantial risk of infertility, morbidity, and mortality even in patients with moderate end organ toxicity, which will ultimately exclude many patients with sickle cell disease and thalassemia from such protocols. Further, currently gene therapy for thalassemia is not curative for most patients in that it does not eliminate

transfusion needs, and recent concerns have been raised about the safety of clinical gene editing approaches.(37, 38)

The current study has clear limitations. Due to the U.S. Government's decision on funding clinical trials for sickle cell disease patients, this line of investigation had to be stopped before it was completed limiting the total number of individuals transplanted, and the conclusions that can be drawn from smaller patient cohort. Furthermore, this was a single center study and patients with very severe co-morbidities were ineligible for enrollment. While a follow up of 705 days (IQR 398, 943) is adequate to suggest an improvement on engraftment over our previous experience, and to corroborate the low rates of acute and chronic graft-versus-host disease, it may not be enough for other complications such as secondary malignancies, infertility, or maintenance of the graft in the long term, particularly on patients that are mixed chimeras. However, at the same time, the improvement on engraftment compared to other series published, including our own, suggests this observation is an important finding.

In summary, despite several trials describing the universal availability and safety of haploBMT for hemoglobinopathies,(11, 23) recent reports on novel therapies for this disease continue to comment on the limited availability of donors and the toxicity alloBMT.(39) Our data demonstrate that increasing TBI from 200cGy to 400 cGy allows for stable engraftment and is well tolerated in hemoglobinopathy patients receiving haploBMT. Moreover, the majority develop full donor chimerism and can safely have their immunosuppression stopped after one year. Major ABO incompatible donors should be avoided when possible as mixed chimerism with persistent host anti-donor RBC antibodies can cause prolonged transfusion dependence. The American Society of Hematology (http://www.hematology.org/Research/Recommendations/Sickle-Cell/3151.aspx last accessed December 10, 2018) and National Institutes of Health (https://www.nih.gov/about-nih/who-we-are/nih-director/testimony-21st-century-cures-implementation-updates-fda-nih last accessed December 10, 2018) have recently introduced important initiatives to cure sickle cell disease. The encouraging results of this study warrants further investigation to determine if the curative potential of alloBMT can extend beyond the traditionally small fraction of patients with severe hemoglobinopathies with matched donors and fit enough to receive myeloablative conditioning.

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Research in context

Evidence before this study

Allogeneic blood or marrow transplantation is a curative therapy available for patients with severe haemoglobinopathies (i.e. thalassaemia and sickle cell disease). Unfortunately, the vast majority of patients who need a transplant lack a matched donor and/or cannot withstand myeloablative conditioning. More than 95% of patients will have an unaffected related haploidentical donors, i.e., siblings, parents, and even second degree relatives such as aunts and uncles, but high rates of mortality related to graft-versus-host disease have limited the use of such donors historically. However, improved graft versus host preventive strategies such as post-transplant cyclophosphamide are now associated with very low rates of graft versus host disease even with mismatched donors. In fact, allogeneic transplantation using reduced intensity conditioning with post-transplant cyclophosphamide has been associated with very low rates of graft versus host disease and transplant-related mortality following related haploidentical donor transplantation for more than a decade. The limitation has been a 30–40% risk of graft loss that has been non-fatal because of uniform host recovery.

We searched MEDLINE for articles published in English until October 19, 2018. The terms searched were “haploidentical transplantation for sickle cell disease” and “ haploidentical transplantation for thalassemia”. Haploidentical BMT has been attempted, but the published studies are small and plagued by high rates of graft failure and transplant-related toxicities.

Added value of this study

We show for the first time that haploidentical bone marrow transplantation results in a high percentage (> 90%) of donor engraftment with minimal risk of GVHD. Importantly, most patients achieve full donor chimerism and are able to discontinue immunosuppression. We have now solved the problem of graft loss. The increase of TBI from 200 to 400 cGy on day −1 improved engraftment without increasing toxicity.

Implications of all the available evidence

Curative allogeneic bone marrow transplantation should no longer be considered a therapy that is available to a small fraction of patients with severe hemoglobinopathies - i.e., those with matched donors and fit enough to receive myeloablative conditioning. The availability of haploidentical family donors, the high engraftment rate, and low toxicity, raises the bar for other exciting potential curative options (gene therapy and genome editing) that require currently myeloablative conditioning.

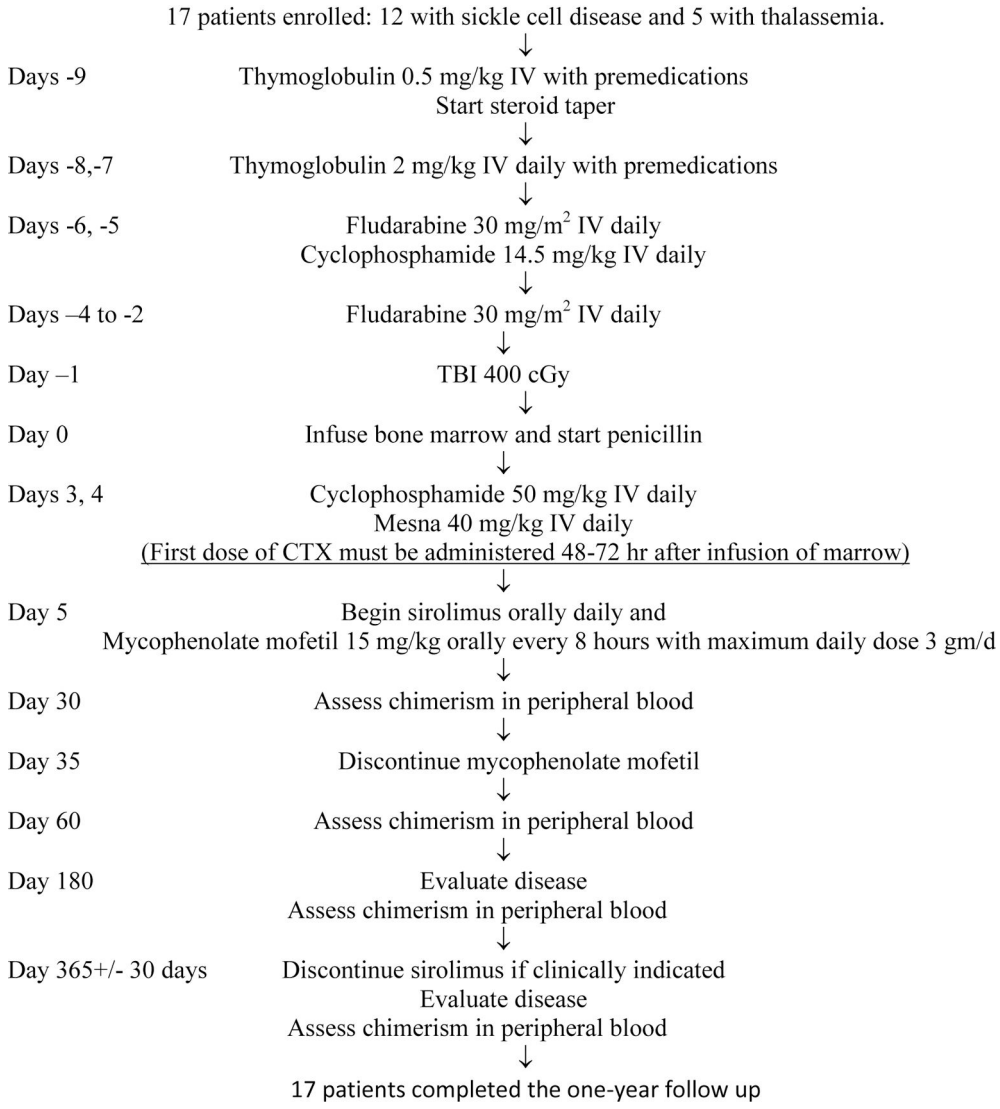

Figure 1.
Study schema

Table 1.

Demographics.

ID	Diagnosis	Age	Gender	Ethnicity	Donor ABO/Rh	Recipient ABO/Rh	Donor gender	Donor relationship	BMT Indication*
35	SS/B^0 thalassemia	20	F	Arab	A+	O+	M	Father	Osteonecrosis, pain,
36	SS	6	F	AA	O+	O+	F	Mother	Pain, abnormal TCD
37	SS	30	F	AA	O+	A+	M	Father	Pain, TIA x 3, TD
38	SS	31	M	AA	O+	B+	M	Brother	Osteonecrosis, pain,
39	SS	30	M	AA	O+	O+	M	Brother	Osteonecrosis, pain, ACS
40	SS/α-thalassemia	26	F	Arab	A+	A+	M	Father	Pain, stroke
43	SS	27	F	AA	B+	O+	F	Sister	Pain, ACS
47	SS	28	F	AA	O+	O−	F	Aunt	Pain, ACS
48	SS	26	F	AA	O+	O+	F	Mother	Pain, ACS, osteonecrosis
50	SS	13	M	AA	B+	AB+	F	Sister	Pain, ACS
51	SS	13	F	AA	O+	O+	F	Mother	Pain, ACS
52	SS	16	M	AA	O+	A+	M	Brother	Stroke
42	B-Thal	7	F	Arab	O+	O+	F	Mother	TD
44	B-Thal	6	F	Arab	O+	O+	M	Father	TD
45	B-Thal	7	F	Arab	O+	O+	F	Mother	TD
46	B-Thal	8	M	Arab	A+	A+	M	Brother	TD
49	B-Thal/Hb D Los Angeles	16	F	Arab	O+	B+	F	Sister	TD

SS: sickle cell disease hemoglobin SS, B-Thal: B-thalassemia major, Hb: Hemoglobin, M: male, F: female, AA: African-American, TCD: transcranial Doppler velocity, TIA: transient ischaemic attack, ACS: Acute chest syndrome, TD: Transfusion dependence. Patients 35 and 40 are siblings and were transplanted form the same donor.

*See Materials and Methods for study entry requirements.

Table 2.

Graft and engraftment information.

Chimerism expressed as percent of donor DNA obtained in peripheral blood samples.

ID	CD3 dose infused/kg	Nucleated cells infused/kg	CD34 dose infused/kg	Day 30 chimerism unsorted	Day 30 T cell chimerism	Most recent chimerism unsorted	Most recent CD3+chimerism	Days to most recent chimerism	Rejection	On immunosuppression
35	7.50E+07	5.80E+08	6.70E+06	>95	59	17	81	1255	No	No
36	6.70E+07	5.10E+08	8.90E+06	100	100	100	100	365	No	No
37	4.00E+07	4.60E+08	2.90E+06	100	88	100	100	412	No	No
38	3.40E+07	4.80E+08	4.00E+06	100	>95	100	100	350	No	Unknown
39	2.50E+07	3.80E+08	1.00E+06	59	0	0	0	62	Yes	Rejected
40	3.90E+07	5.90E+08	2.00E+06	42	11	20	70	762	No	Yes
43	2.80E+07	2.20E+08	3.30E+06	>95	81	100	100	365	No	No
47	3.10E+07	4.10E+08	4.00E+06	100	>95	100	100	384	No	Yes
48	4.50E+07	5.10E+08	4.70E+06	100	>95	100	100	305	No	Yes
50	5.52E+07	4.35E+08	4.09E+06	100	92	100	100	364	No	Yes
51	4.51E+07	5.07E+08	5.58E+06	100	>95	100	100	352	No	Yes
52	2.88E+07	4.51E+08	2.53E+06	>95	>95	100	100	214	No	Yes
42	4.80E+07	7.30E+08	2.00E+06	85	10	22	13	709	No	Yes
44	9.80E+07	7.50E+08	8.80E+06	100	76	100	100	680	No	No
45	2.80E+07	1.10E+09	3.10E+06	100	100	100	100	463	No	Yes
46	1.10E+08	1.50E+09	1.80E+07	100	100	100	100	359	No	No
49	1.04E+08	7.27E+08	5.45E+06	100	>95	100	100	409	No	Yes
Median	4.50E+07	5.10E+08	4.00E+06	100	81	100	100	365		
IQR	2.99E+07, 7.10E+07	4.43E+08, 7.285E+08	2.715E+06, 6.14E+06	95, 100	67.5, 95	61, 100	90.5, 100	351, 571.5		

Table 3A.

Hematologic parameters for patients with sickle cell disease.

ID	Bilirubin pre-BMT (mg/dL)	Most Recent Bilirubin (mg/dL)	Absolute reticulocytes pre-BMT (1000xmm^3)	Most Recent absolute reticulocytes (1000xmm^3)	Hb (g/dL) pre-BMT	Most Recent Hb (g/dL)	Donor Hb (g/dL)	LDH (U/L) pre-BMT	Most Recent LDH (U/L)	Donor Hb S (%)	Most Recent Hb S (%)	Most Recent MCV (fL)	Donor MCV (fL)	Transfusion Independence
35	1.6	1.2	133.2	193.4	12.5	9.4	16.2	556	223	31.8	33.5	86.5	77.8	No
36	1.3	0.2	467.8	51.4	8.2	12.4	12.5	566	425	38.9	37.2	80.6	85.2	Yes
37	7.3	0.2	556.4	80.8	7.4	10.4	12.8	495	220	33.7	35.1	89.6	87.3	Yes
38	2.3	0.3	56.7	127	9.3	16.8	15.7	212	411	38.3	37.1	81.2	82.4	Yes
39	3.5	2	302	302	7.5	9.5	15.8	523	353	37.7	<5	89.8	85.2	No
40	0.4	0.3	100.8	42.7	8.3	8.7	16.2	155	209	32	76.2	60	77.8	Yes
43	2.5	0.5	273.4	87.7	9.9	13.4	12.7	297	158	31.9	33	87.6	81.1	Yes
47	4.4	0.2	798.8	175.4	7.3	10.5	13.8	632	332	N/A	0	92.7	92.3	Yes
48	0.4	0.2	200.1	64.6	9	11	12.8	302	290	37.4	37.8	83.9	83.8	Yes
50	1.8	0.2	167	54.8	9.3	12.6	11.7	N/A	208	31.5	30.2	71.5	77.1	Yes
51	3.3	0.3	542.4	144.3	8.3	13.9	12.6	346	229	35.2	35.9	81.6	84	Yes
52	5.7	0.6	234.8	65.2	10.9	14.7	13.1	428	419	N/A	<5	99.8	91.6	Yes
Median	2.4	0.3	254.1	80.8	8.65	11.7	12.95	428	229	34.45	35.5	85.2	83.9	
IQR	1.375, 4.175	0.2, 0.575	141.65, 523.75	57.25, 167.625	7.675, 9.75	9.725, 13.775	12.625, 15.775	297, 556	211.75, 396.5	31.875, 37.85	11.3, 37.175	80.75, 89.75	78.625, 86.775	

Table 3B.

Hematologic parameters for patients with B-Thalassemia.

ID	Bilirubin pre-BMT (mg/dL)	Most Recent Bilirubin (mg/dL)	Absolute Reticulocytes pre-BMT (1000xmm^3)	Most Recent absolute reticulocytes (1000xmm^3)	Hb (g/dL) pre-BMT	Most Recent Hb (g/dL)	Donor Hb (g/dL)	LDH (U/L) pre-BMT	Most Recent LDH (U/L)	Most Recent MCV (fL)	Donor MCV (fL)	Ferritin pre-BMT (ng/mL)	Most Recent Ferritin (ng/mL)	Transfusion Independence
42	0.7	0.2	23.3	44.4	9.5	8.7	10.2	193	274	64.9	62	626	429	Yes
44	0.4	0.3	6.1	61.1	7	10.8	12.1	174	189	56.4	58.4	370	188	Yes
45	0.5	0.2	5.9	120.4	10.4	9	10	301	269	57.8	61.4	1331	356	Yes
46	1.3	0.5	11.6	82.3	11.1	8.9	12.4	143	251	53.7	55.9	881	702	Yes
49	0.9	0.4	23.3	87.6	6.9	12.7	11.4	N/A	203	82.1	83.5	743	1011	Yes
Median	0.7	0.3	11.6	82.3	9.5	9	11.4	183.5	251	57.8	61.4	743	429	
IQR	0.45, 1.1	0.2, 0.45	6, 23.3	52.75, 104	6.95, 10.75	8.8, 11.75	10.1, 12.25	150.75 274	196, 271.5	55.05, 73.5	57.15, 72.75	498, 1106	272, 856.5	

Hb: hemoglobin, LDH: lactic dehydrogenase, MCV: mean corpuscular volume, BMT: bone marrow transplant. Pre-BMT values were transfused, *comparison vs donor.